NMDA Receptors Contribute to Primary Visceral Afferent Transmission in the Nucleus of the Solitary Tract

MARIÀ LUZ AYLWIN,1 JOHN M. HOROWITZ,2 AND ANN C. BONHAM1
1 Department of Internal Medicine, Division of Cardiovascular Medicine and 2 Department of Neurobiology, Physiology and Behavior, University of California, Davis, California 95616

Aylwin, Maria Luz, John M. Horowitz, and Ann C. Bonham. NMDA receptors contribute to primary visceral afferent transmission in the nucleus of the solitary tract. J. Neurophysiol. 77: 2539–2548, 1997. The nucleus of the solitary tract (NTS) is the first central site where the reflex control of autonomic function is coordinated. The nucleus receives visceral afferent information from sensory endings in the large blood vessels, heart, lungs, and gastrointestinal organs; then, through primary reflex circuits within the brain stem or through more elaborate interconnections with higher brain regions, the sensory information is conditioned and ultimately transformed to regulate autonomic output. Primary afferent fibers from the target organs course in the solitary tract of the nucleus and then exit to synapse onto second-order neurons within the nucleus. The fibers terminate, to a large extent viscerotopically; cardiovascular, respiratory, and gastrointestinal afferent fibers terminate mainly in the intermediate and caudal NTS, whereas gustatory afferent fibers terminate in the rostral NTS (Loewy 1990).

Glutamate is the primary excitatory neurotransmitter released by the visceral afferent fibers in the intermediate and caudal NTS (Talman et al. 1980). It is well established that the non-N-methyl-D-aspartate [non-NMDA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate] receptors, which mediate the fast component of glutamate signaling, are activated by visceral afferent transmission to NTS neurons (Andresen and Yang 1990; Brooks and Spyer 1993; Brooks et al. 1992; Glaucom and Miller 1993, 1995). Whether the NMDA receptors, which mediate a slower-developing, longer-lasting component of glutamate signaling, are also activated is still in question. If only the non-NMDA receptors transmit primary sensory afferent signals to second-order neurons in the NTS, then the second-order neurons most likely serve to simply relay information from visceral sensory endings to higher-order neurons, as has been classically described (Spyer 1981). If, on the other hand, NMDA receptors are also activated by the primary visceral afferent input, then the second-order neurons can sustain a longer-lasting depolarization that allows for added signal conditioning capabilities such as the temporal integration of multiple inputs, rhythmic firing, and synaptic plasticity (Cotman and Iversen 1987). Unlike the non-NMDA receptors, the activation of NMDA receptors requires not only the presence of glutamate but also depolarization of the cell because of the Mg2+ block of the NMDA receptor channel at resting membrane potentials (Nowak et al. 1984). Consequently, NMDA-receptor-mediated synaptic responses are difficult to detect experimentally at resting membrane potentials, which may contribute to the uncertainty regarding the participation of NMDA receptors to primary visceral afferent transmission in the NTS.

Various experimental strategies have been used to investigate the role of non-NMDA and NMDA receptors in visceral afferent transmission in the NTS. In whole animals with
intact autonomic reflexes, microinjections of non-NMDA and NMDA receptor agonists and antagonists in the NTS have been utilized to mimic or block, respectively, the reflex responses evoked by stimulation of the visceral afferent fibers. Such studies have variably implicated the participation of either or both the non-NMDA and NMDA receptors on NTS neurons in several autonomic reflex pathways: the baroreceptor (Gordon and Leone 1991; Kubo and Kihara 1988; Ohta and Talman 1994), cardiopulmonary C fiber receptor (Vardhan et al. 1993), Breuer-Hering (Bonham et al. 1993), and superior laryngeal nerve inspiratory shortening reflex pathways (Karius et al. 1994). These variable findings regarding the role of non-NMDA and NMDA receptors in synaptic transmission in the NTS may be due to true differences in the glutamate receptor subtypes activated in these different autonomic reflex pathways or to limitations of the microinjection technique, which typically relies on relatively large volumes (10–100 nl) of highly concentrated agents that very likely affect many NTS neurons. Thus although microinjection studies provide crucial data regarding the functional significance of non-NMDA and NMDA receptors on NTS neurons for full expression of various autonomic reflex responses, they cannot determine with certainty the extent to which the receptors are located on second-order, higher-order, or other neurons not involved inafferent transmission. This limits the assessment of the relative contribution of the NMDA versus non-NMDA receptors to primary visceral afferent transmission in the NTS.

Extracellular recordings of NTS unit activity in the whole animal have more directly examined the role of non-NMDA and NMDA receptors in synaptic transmission between functionally identified visceral afferent fibers and NTS neurons. In a recent report, Zhang and Mifflin (1996), using iontophoretic application of non-NMDA and NMDA receptor antagonists, concluded that non-NMDA receptors transmit primary input from baroreceptor afferent fibers to second-order neurons, but that NMDA receptors transmit the information to higher-order NTS neurons. We similarly concluded that non-NMDA receptors largely mediate activation of NTS neurons by cardiopulmonary C fiber afferent input (Wilson et al. 1996). Together the data suggest that baroreceptor and cardiopulmonary C fiber afferent input is transmitted to second-order NTS neurons in the intermediate and caudal NTS largely by non-NMDA receptors. However, because the studies were performed on neurons at their resting membrane potentials and because extracellular Mg$^{2+}$ could not be depleted to remove the voltage-dependent block of the NMDA channel, the contribution of NMDA receptors to the synaptic transmission may not have been detected.

In in vitro studies in the medullary slice, Andresen and Yang (1990) reported that maximal selective blockade of non-NMDA receptors decreased the amplitude of short-latency excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the solitary tract by $>\text{85\%}$, whereas NMDA receptor blockade had relatively little effect, decreasing the amplitude by $<\text{20\%}$. Brooks et al. (1992) similarly demonstrated that non-NMDA receptor antagonism abolished solitary-tract-evoked EPSPs, although whether the recordings were made from second- or higher-order neurons was not determined. These in vitro findings considered along with the in vivo data have led to the proposal that NMDA receptors do not contribute to primary visceral afferent transmission to second-order neurons in the NTS (Andresen and Yang 1990). However, because the recordings were made in neurons at their resting membrane potentials, the Mg$^{2+}$ block of the NMDA receptor channel, maximal under these conditions, may have obscured NMDA-receptor-mediated responses. Indeed, NMDA receptor contribution to synaptic transmission may be missed altogether unless the NMDA currents are measured at membrane potentials positive to $-45\text{ mV}$. In the present study, to directly determine whether NMDA receptors contribute to primary visceral afferent transmission in the intermediate and caudal NTS, we used voltage-clamp recording to examine excitatory postsynaptic currents (EPSCs) monosynaptically evoked by solitary tract stimulation at a range of membrane potentials from $-90$ to $+60\text{ mV}$ in the medullary slice.

METHO DS

Experimental protocols followed in this work were reviewed and 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.

SLICE PREPARATION. Male Sprague-Dawley rats 3–4 wk old (60–120 g) were anesthetized with a combination of ketamine (35 mg/kg) and xylazine (2 mg/kg) given intramuscularly and were decapitated with a guillotine. The brain was rapidly exposed and submerged in ice-cold ($<-4^\circ\text{C}$) high-sucrose artificial cerebrospinal fluid that contained (in mM) 3 KCl, 2 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, 10 glucose, 220 sucrose, and 2 CaCl$_2$, pH 7.4, osmolality 300 mosM, constantly bubbled with 95% O$_2$-5% CO$_2$ (Parkis et al. 1995). After the brain stem was dissected, its caudal surface was cemented to the stage of a Vibratome 1000 (Technical Products International, St. Louis, MO) with cyanoacrylate glue, and its ventral surface was glued to an agar block. Coronal slices (300 μm thick) were obtained between 300 μm rostral and 1.200 μm caudal to the obex. Four slices were typically obtained from each brain stem; one slice caudal to the area postrema, two slices containing the area postrema, and one slice rostral to the area postrema. We used longitudinal slices (250–300 μm) in a few experiments to demonstrate that the findings in the coronal slices could be confirmed in this preparation, which has been used in other laboratories (Andresen and Yang 1990). The slices were incubated for 45 min at 37°C in a holding chamber filled with the high-sucrose artificial cerebrospinal fluid constantly bubbled with 95% O$_2$–5% CO$_2$. The slices were then transferred to a chamber filled with artificial cerebrospinal fluid (normal perfusate) containing (in mM) 125 NaCl, 2.5 KCl, 1 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 25 NaHCO$_3$, 10 glucose, and 2 CaCl$_2$, pH 7.4, osmolality 300 mosM, continuously bubbled with 95% O$_2$–5% CO$_2$ and held at room temperature (22°C). Slices were incubated in the normal perfusate for 1 h before whole cell recordings were begun, at which time a slice was transferred to the recording chamber, held in place with a nylon mesh, and continually perfused with the normal perfusate at a rate of 3 ml/min at room temperature. All the experiments were performed at 22°C.

WHOLE CELL PATCH-CLAMP RECORDING. Borosilicate glass electrodes were filled with a CsF solution containing (in mM) 145 CsF, 5 NaCl, 1 MgCl$_2$, 3 K-ATP, 0.2 sodium guanosine 5’- triphosphate (Na-GTP), 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.4, 300 mosM, for voltage-clamp experiments, or with a KCl solution containing (in mM) 140 KCl, 5 NaCl, 1 MgCl$_2$, 3 K-ATP, 0.2 Na-GTP, 10 EGTA, and 10 HEPES, pH 7.4, 300 mosM, for current-
clamp experiments. Electrodes had a resistance of 3–5 MΩ. Whole cell recordings in NTS cells were made with the Axoclamp 1D patch-clamp amplifier (Axon Instruments, Foster City, CA) in voltage-clamp or current-clamp mode. Whole cell currents and voltages were filtered at 2 kHz with the use of the Axoclamp amplifier four-pole Bessel filter, digitized at 10 kHz with the use of a DigiData 1200 Interface (Axon Instruments), and stored in a 386 DX computer. Data were analyzed off-line with the use of pClamp 6 software (Axon Instruments). EPSCs evoked by solitary tract stimulation were pharmacologically isolated by constant perfusion with the competitive γ-aminobutyric acid-A (GABA_A) receptor antagonist picrotoxin (100 μM) or the noncompetitive GABA_A receptor antagonist bicuculline (10 μM) or both. The synaptic currents were no larger than 800 pA and the measured series resistance was <30 MΩ.

SOLITARY TRACT STIMULATION. Either a custom-made bipolar electrode in which two 35-μm Pt/Ir wires were twisted and juxtaposed, or a commercially available bipolar tungsten stimulating electrode with two 25-μm tips (Frederick Haer), was placed in the solitary tract. Single stimuli (1–25 V, 100-μs pulses) were delivered to the solitary tract ipsilateral to the recording site at 0.2 or 0.5 Hz. To determine that responses to solitary tract stimulation were synaptically activated, we compared the solitary-tract-evoked EPSCs before and in the presence of the calcium channel blocker CdCl_2 (100 μM).

PROTOCOLS. The NTS and solitary tract were visualized in the slice with a ×10 objective. A bipolar stimulating electrode was placed in the solitary tract, and whole cell recordings (Hamill et al. 1981) were obtained with the use of the blind-patch technique. The recording sites were drawn on a representative slice at the time of recording and subsequently reconstructed on one of three representative slices drawn from 40-μm histological sections with the aid of a camera lucida drawing tube.

For each solitary-tract-evoked EPSC, we measured the amplitude of the current at the peak of the response, where the fast component of the EPSC is predominant, and at 20 ms after the peak, where the slow component is predominant (Hestrin et al. 1990). Solitary-tract-evoked EPSCs were determined to have both a fast and a slow component if the ratio of the amplitude of the current measured at 20 ms after the peak to the amplitude of the current measured at the peak of the EPSC was ≥0.6 when measured at voltages positive to +40 mV.

Cells were classified as second-order neurons if the onset latencies were short (<4.5 ms) and varied by <0.5 ms. To independently verify that these short, invariant onset latencies were consistent with monosynaptic activation, we assessed the ability of five cells with latencies ≤4.5 ms to discharge action potentials to each of two solitary tract stimuli delivered with an interpulse interval of 5 ms. On the basis of previously established criteria (Miles 1986), each of the two stimuli were required to evoke an action potential for the cell to be considered to receive monosynaptic input.

For antagonist studies, solitary-tract-evoked EPSCs were obtained in the normal perfusate and then 2–6 min after application of either the NMDA receptor antagonist DL-2-amino-5-monophospho- valeric acid (APV) or the non-NMDA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoline (NBQX). The responses recovered after 2–5 min in the presence of normal perfusate. For the nominally Mg^{2+}-free studies, EPSCs were first obtained in the nominally Mg^{2+}-free perfusate and then measured 4–6 min after the change to the normal perfusate (which contained 1 mM Mg^{2+}).

DATA ANALYSES. Currents shown in the traces and current-voltage plots are averages of two to four traces. Each voltage was corrected by subtracting the voltage error (the product of the series resistance and steady-state current) from the command voltage. Currents obtained at voltages negative to −70 mV in nominally Mg^{2+}-free perfusate were fitted with two exponentials, with time constants and amplitudes determined with the use of the pClamp6 simplex method. We used these same time constants to fit the curves obtained in the normal-Mg^{2+} perfusate and then determined the amplitude of the two exponentials. Data are expressed as means ± SE unless otherwise indicated. The EPSCs in the control conditions and after interventions were compared by the use of the paired t-test. Differences were considered significant at P < 0.05.

CHEMICALS. NaCl, KCl, MgCl_2, sucrose, and CdCl_2 were purchased from Fisher; NaHCO_3, K-ATP, Na-GTP, EGTA, HEPES, bicuculline, picrotoxin, and APV from Sigma; NaH_2PO_4 and CaCl_2 from Mallinkrodt; glucose from Baker; CsF from Aldrich; and NBQX from RBI.

RESULTS

NTS sites of whole cell patch-clamp recordings

Whole cell patch-clamp recordings were obtained from 177 NTS neurons, 126 of which received excitatory input from the solitary tract. In all of the four cells tested, the EPSCs evoked by solitary tract stimulation were blocked by the calcium channel blocker CdCl_2 (100 μM), indicating that the currents evoked by solitary tract stimulation were synaptically mediated. A photomicrograph of a medullary slice in the recording chamber is shown in Fig. 1A, bottom. The positions of the stimulating electrode in the solitary tract and the recording electrode just medial to the tract are indicated in Fig. 1A, inset. The locations of the recording sites are shown in three schematic representations of coronal slices in Fig. 1B. All recordings were made just medial to the solitary tract and between 300 μm rostral and 1,200 μm caudal to the obex. The majority of recordings was made in the intermediate NTS at the level of the area postrema, whereas fewer were made rostral or caudal to the area postrema.

Onset latencies and voltage dependence of solitary-tract-evoked EPSCs

EPSCs evoked by single stimuli delivered to the solitary tract were recorded in 32 neurons at a series of voltage steps from −90 to +60 mV. In 28 of the 32 neurons, the solitary-tract-evoked EPSCs had two components, a fast and a slow component; in the remaining four neurons the EPSCs had only a fast component. The fast component was maximal at 4.19 ± 1.80 (SD) ms after the onset of the EPSC and was negligible at 20 ms after the peak; the slow component developed more slowly, was clearly evident 20 ms after the peak, and had a duration of 100 ms. For the cells with both components (Fig. 2A), the currents had a fast onset but decayed slowly when the cell was depolarized to voltages positive to −45 mV. For the cells with only a fast component, the currents had a fast onset and decayed rapidly and similarly at all voltages (Fig. 2B). For all 28 cells with both components, the ratio of the amplitude of the current measured at 20 ms after the peak (where the contribution of the fast component was negligible) to the amplitude of the current measured at the peak averaged 0.78 ± 0.04 at voltages positive to +40 mV. For the remaining four cells, in which the EPSCs had only a fast component, the ratio was 0.26 ±
FIG. 1. Position of recording electrode, stimulating electrode, and recording sites. A: photomicrograph showing a medullary slice in the recording chamber. A inset: showing stimulating electrode in the solitary tract and recording electrode located medial to the tract. B: location of recording sites (hatched areas) superimposed on 3 schematic coronal sections. All recordings were made medial to the solitary tract; most were made in the intermediate nucleus of the solitary tract (NTS) at the level of the area postrema (AP; −100 to −800 μm caudal to the obex). ts, solitary tract; c, central canal.

The onset latencies of the 28 neurons with both the fast and slow components averaged 3.42 ± 1.03 (SD) ms and ranged from 2.2 to 7.7 ms. The onset latencies for the remaining four neurons in which the EPSCs had only the fast component were not significantly different (P = 0.33; unpaired t-test), averaging 4.00 ± 1.79 (SD) ms and ranging from 2.0 to 5.7 ms. The EPSCs with short (≤4.5 ms) and invariant (varied by <0.5 ms) onset latencies were considered to be evoked by monosynaptic activation. Independent evidence that these onset latencies were the result of monosynaptic activation was obtained in five cells that had similar onset latencies (3.02 ± 0.94 ms) and consistently discharged action potentials to each of two stimuli delivered at 5-ms intervals (Miles 1986), thus confirming that the solitary-tract-evoked EPSCs were measured in second-order neurons.

We also measured solitary-tract evoked EPSCs in NTS cells in longitudinal slices in which the stimulating electrode was placed in the solitary tract ~1 mm away from the recording site. The current-voltage relationships for the peak current and for the current at 20 ms after the peak were the same as for those obtained in coronal slices. Three of six neurons with onset latencies of 4.5 ± 0.6 (SD) ms had both the fast and slow components. The remaining three neurons had only the fast component and had onset latencies of 4.1 ± 0.8 (SD) ms.

Although the fast and slow components overlap in time (Figs. 2 and 3), the fast component of the EPSC is predominant at the peak of the response, with only a minor contribution from the slow component, and the slow component is predominant at 20 ms after the peak, with a small contribution from the fast component. Thus the fast component of the EPSC is represented in the peak current-voltage relationship, whereas the slow component is represented in the current-voltage relationship for the current measured at 20 ms after the peak. The current-voltage relationships were plotted for the solitary-tract-evoked EPSCs that met the criteria for monosynaptic activation given above and had both a fast and slow component. An example is shown in Fig. 3. At hyperpolarized potentials, the EPSCs had a fast onset and fast decay, corresponding to the predominance of the fast component. At depolarized potentials, the EPSCs had a fast onset and a much slower decay, reflecting the presence of both components (Fig. 3A). The current-voltage relationships for the peak current and the current at 20 ms after the peak for this neuron are shown in Fig. 3B. The amplitude of the fast component was linearly related to
Effect of absence of Mg$^{2+}$ on solitary-tract-evoked EPSCs

NMDA receptor currents are relatively small at membrane potentials negative to $-45 \text{ mV}$ because of Mg$^{2+}$ block of the channel (Nowak et al. 1984). To further demonstrate that the slow component of the solitary-tract-evoked EPSCs is mediated by NMDA receptors, we examined the EPSCs in the absence of Mg$^{2+}$ (nominally Mg$^{2+}$-free perfusate) and then in the presence of Mg$^{2+}$ (normal perfusate) ($n = 5$). An example is shown in Fig. 5A. At $-82 \text{ mV}$, the amplitude of the slow component measured in the absence of Mg$^{2+}$ was greater than in the presence of Mg$^{2+}$. At $+1$ and $+8 \text{ mV}$, where the Mg$^{2+}$ block of the NMDA channel is negligible, the amplitude of the slow component was similar in the absence or presence of Mg$^{2+}$. The current-voltage relationship of the slow component for this same neuron in the absence of Mg$^{2+}$ shows a linear behavior of the current over a wider voltage range than in the presence of Mg$^{2+}$, reflecting the voltage-dependent Mg$^{2+}$ block of the NMDA receptor channel (Fig. 5B).

For all five neurons tested, the NMDA current at $-82 \text{ mV}$ was significantly greater in the absence ($-177 \pm 56 \text{ pA}$) than in the presence ($-52 \pm 21 \text{ pA}$) of Mg$^{2+}$ $(P = 0.03)$; paired $t$-test). Moreover, when the decay of the currents was fitted with two exponentials at $-90 \text{ mV}$, the ratio of the amplitude of the slow to the fast exponential was significantly greater in the absence (2.37 ± 0.49) than in the presence (0.613 ± 0.37) of Mg$^{2+}$ $(P = 0.007)$. The amplitude of the slow component at voltages positive to $+45 \text{ mV}$ was not different in the absence (127 ± 16 pA) or in the presence (139 ± 24 pA) of Mg$^{2+}$ $(P = 0.707)$.

Effect of NBQX on solitary-tract-evoked EPSCs

We compared the EPSCs of nine neurons before and after application of the non-NMDA receptor antagonist NBQX (3 $\mu$M; $n = 9$). Six of the nine neurons tested with NBQX exhibited both fast and slow components; the remaining three neurons tested with NBQX had only the fast component. An example of the effect of NBQX on the fast and slow components of solitary-tract-evoked EPSCs at three voltages before and in the presence of NBQX is shown in Fig. 6. At all three voltages NBQX nearly abolished the fast component of the response and had no effect on the slow component.

For the six neurons exhibiting both fast and slow compo-
FIG. 3. Voltage dependence of solitary-tract-evoked EPSCs. A: EPSCs were recorded at −88, −73, −59, −43, −29, −14, 0, +13, +25, +38, and +50 mV. B: current-voltage plot for same cell at peak current shows a linear fast component (○). Current-voltage plot for current at 20 ms after peak shows a nonlinear slow component (●).

nents of solitary-tract-evoked EPSCs, NBQX significantly decreased the amplitude of the fast component, measured at voltages positive to +40 mV, from 102 ± 25 pA to 35 ± 12 pA (P = 0.014; paired t-test) while having no effect on the slow component, which was 85 ± 18 pA before and 61 ± 18 pA in the presence of NBQX (P = 0.134; paired t-test). The responses recovered after 2–5 min in the presence of normal perfusate.

DISCUSSION

This is the first study to demonstrate that dual non-NMDA and NMDA receptor mechanisms operate in synaptic transmission between primary visceral (sensory) afferent fibers and second-order neurons in the intermediate and caudal NTS. Two issues critical to the conclusions drawn from this study are whether the solitary-tract-evoked EPSCs were obtained in second-order neurons and whether the solitary-tract-evoked EPSCs obtained in these second-order neurons had an NMDA-receptor-mediated component.

Two related observations indicate that the recordings in the present study were obtained from second-order neurons that received monosynaptic input from the solitary tract: 1) the onset latencies of the synaptically evoked EPSCs were short and invariant (Figs. 2–5), and 2) the neurons reliably discharged an action potential after each of two pulses separated by 5 ms was delivered to the solitary tract (Miles 1986). The onset latencies in our study are the same as those observed by others for solitary-tract-evoked EPSCs (Glaum and Miller 1993) and EPSPs (Andresen and Yang 1990; Champagnat et al. 1986) in second-order neurons in this region of the NTS.

Previous patch clamping and intracellular studies in vitro have provided persuasive evidence that non-NMDA receptors mediate primary visceral afferent inputs from the solitary tract to second-order neurons within the intermediate and caudal NTS (Andresen and Yang 1990; Brooks and Snyder 1993; Brooks et al. 1992; Drewe et al. 1990). Our finding that NBQX blocked the fast component of solitary-tract-evoked EPSCs is consistent with these previous observations, and confirms that non-NMDA receptors mediate the fast component of glutamate signaling in primary visceral afferent transmission.

It is well established that functional NMDA receptors are present on NTS neurons, as evidenced by in vivo and in vitro findings that direct application of NMDA evokes excitatory responses in NTS cells receiving visceral afferent input (Brooks et al. 1992; Drewe et al. 1990; Wilson et al. 1996). However, whether NMDA receptors contribute to visceral afferent synaptic transmission to NTS cells has been more controversial. Recording in a medullary slice, Brooks et al. (1992) determined that non-NMDA receptor blockade abolished solitary-tract-evoked EPSPs. Although it was not determined whether the EPSPs were recorded from second- or higher-order neurons, the data suggest that NMDA receptors play no significant role in visceral afferent transmission in the NTS. Andresen and Yang (1990) similarly found a predominant contribution of non-NMDA receptors to short-latency, solitary-tract-evoked EPSPs; this has led to the suggestion that, in contrast to the non-NMDA receptors, the NMDA receptors probably do not mediate synaptic transmission between primary visceral afferent fibers and second-order neurons, but rather may mediate transmission to higher-order NTS neurons, presumably via polysynaptic pathways (Andresen and Kunze 1994). This proposal that NMDA receptors mediate visceral afferent transmission to
higher-order neurons is supported by findings by Brooks and Spyer (1993) that, in the presence of non-NMDA receptor blockade, stimulation in the area of the solitary tract occasionally evoked small NMDA-receptor-mediated EPSPs, suggesting that NMDA receptors may contribute to synaptic responses in higher-order neurons evoked by excitation of neurons outside the solitary tract. However, the previous observations were made in experiments in which EPSPs were recorded in NTS neurons at their resting membrane potentials, conditions in which the voltage-dependent block of NMDA channels may have obscured the contribution of NMDA-receptor-mediated currents. In the present study, in which we examined excitatory currents in NTS neurons over a range of membrane potentials, we were able to observe, in addition to the fast non-NMDA-receptor-mediated component, a slow component of solitary-tract-evoked EPSCs in second-order neurons. The following lines of evidence suggest that the slow component was mediated by NMDA receptors: 1) the current-voltage relationship for the current measured at 20 ms after the peak of the EPSCs was characteristic of NMDA receptor currents (Figs. 2A and 3), exhibiting appreciable outward currents at positive potentials but only small inward currents at negative potentials (Hestrin et al. 1990); 2) the slow component was significantly attenuated by NMDA receptor antagonism with APV (Fig. 4), but not by non-NMDA receptor blockade with NBQX; and 3) in the absence of Mg$^{2+}$ from the perfusate, the slow component was significantly enhanced at hyperpolarized membrane potentials (Fig. 5), at which Mg$^{2+}$ block of the NMDA receptor channel prevents conductance (Nowak et al. 1984). This enhanced amplitude of the slow component in the absence of Mg$^{2+}$ was corroborated by an increased contribution of the exponential with a slow time constant. Taken together, the findings indicate that NMDA receptors participate in synaptic transmission between visceral afferent fibers and second-order neurons in the NTS.

On the other hand, certain limitations of this study must be considered. First, we demonstrated that NMDA receptors contributed to visceral afferent transmission in the NTS in 3- to 4-wk-old rats. Although NMDA receptor characteristics have not been specifically studied during postnatal development in the rat NTS, data from other CNS regions suggest that NMDA receptor density is maximal in 2- to 3-wk-old rats and slowly declines up to the age of 1 yr (Erdo and Wolff 1990), and that NMDA receptors are less sensitive to Mg$^{2+}$ blockade in 3- to 4-wk-old rats compared with adult rats 10–12 wk old (Morrisett et al. 1990). These observa-
FIG. 5. Effect of Mg$^{2+}$ on solitary-tract-evoked EPSCs. A: EPSCs recorded initially in nominally Mg$^{2+}$-free medium ($-\text{Mg}^{2+}$) and in the presence of 1 mM Mg$^{2+}$ (normal perfusate). B: current-voltage plots measured at 20 ms after the peak of the EPSC in the absence of Mg$^{2+}$ (▲) and in the presence of 1 mM Mg$^{2+}$ (●).

The majority (88%) of the cells had NMDA receptor currents in addition to non-NMDA currents, indicating that in the intermediate and caudal NTS, NMDA receptors contribute to primary visceral afferent transmission to a high percentage of second-order neurons in the NTS. Although the modalities of the sensory endings and thus the function of the individual NTS cells cannot be determined in the slice, the majority of the cells had both NMDA and non-NMDA receptor currents. Thus it seems likely that the dual receptors are a common mechanism for primary afferent transmission in the NTS, where neurons receive primary afferent input largely from cardiovascular and respiratory sensory endings (Jordan and Spyer 1986; Loewy 1990; Spyer 1981). This dual participation of NMDA and non-NMDA receptors in synaptic transmission has also been observed in the hippocampus (Hestrin et al. 1990) and visual cortex (Artola and Singer 1987).

The significance of NMDA receptors in primary visceral afferent transmission in the NTS may reside in three characteristics of the receptors that allow for added signal processing capacity beyond that provided by non-NMDA receptors. First, NMDA receptor channel conductance is maximal both when glutamate is present and the cell is depolarized. This suggests that NMDA receptor contribution may be en-
enhanced during high-frequency sensory input from visceral afferent fibers or under conditions in which excitatory inputs from other sources are integrated. In that regard, bursting patterns of second-order neurons in the NTS have been observed following high-frequency stimulation of the solitary tract (20–50 Hz for 100–600 ms) (Tell and Jean 1991) and from direct application of NMDA (Tell and Jean 1993). It may be that these second-order neurons having NMDA receptors can transduce sensory input, which has no apparent pattern, into these bursting patterns for signal transmission at later synapses in the reflex circuitry. Second, the prolonged depolarization due to NMDA receptor currents lengthens the time during which otherwise ineffective inputs can be integrated with the primary visceral afferent input. Third, calcium entry through the channel activates second-messenger cascades, which are important in modification of synaptic efficacy such as occurs in long-term potentiation (Artola and Singer 1987; Collingridge et al. 1983) and in sustained increases in discharge (windup) of dorsal horn neurons (Mendell 1966; Woolf and Swett 1984).

In conclusion, this study offers the first direct evidence for the contribution of NMDA receptors to synaptic transmission between primary visceral afferent fibers and second-order neurons in the NTS. The second-order neurons may play a more complex role, beyond that of a simple relay neuron, in the coordination of reflex control of autonomic function.

The authors acknowledge Dr. Pedro Maldonado for the stimulating electrode, J. Stewart for technical assistance; Dr. Pam Pappone, Dr. Jesse Joad, and A. Gupta for reviewing the manuscript; and E. Walker for clerical support. This work was supported by National Institutes of Health Grants HL-52165, HL-48584, and DK-32907.

Address for reprint requests: A. C. Bonham, University of California, Davis, Cardiovascular Medicine, TB 172, Biederly Way, Davis, CA 95616.

Received 10 September 1996; accepted in final form 9 January 1997.

REFERENCES


LOEWY, A. D. Central autonomic pathways. In: Central Regulation of Auto-


