Non-NMDA and NMDA receptors in the synaptic pathway between area postrema and nucleus tractus solitarius

MARIA LUZ AYLWIN,1 JOHN M. HOROWITZ,2 AND ANN C. BONHAM1

1Division of Cardiovascular Medicine, Department of Pharmacology, and 2Department of Neurobiology, Physiology, and Behavior, University of California, Davis, California 95616

Non-NMDA and NMDA receptors in the synaptic pathway between area postrema and nucleus tractus solitarius. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1236–H1246, 1998.—Area postrema (AP) modulates cardiovascular function through excitatory projections to neurons in nucleus tractus solitarius (NTS), which also process primary sensory (including cardiovascular-related) input via the solitary tract (TS). The neurotransmitter(s) and their receptors in the AP-NTS pathway have not been fully characterized. We used whole cell recordings in voltage- and current-clamp in the rat brain stem slice to examine the role of ionotropic glutamatergic receptors and α2-adrenergic receptors in the pathway from AP to NTS neurons receiving visceral afferent information via the TS. In neurons voltage clamped at potentials from −100 to +80 mV, AP stimulation (0.2 Hz) evoked excitatory postsynaptic currents having a fast component blocked by the non-N-methyl-D-aspartate (NMDA) receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzofuroxan (NBQX; 3 µM, n = 7) and a slow component blocked by the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV; 50 µM, n = 8). Although NBQX (3 µM, n = 14) abolished AP-evoked action potentials, APV (50 µM, n = 9 or 500 µM, n = 6) or yohimbine, (200 nM, n = 5 or 2 µM, n = 10) did not. Thus, although AP stimulation activates both non-NMDA and NMDA receptors on NTS neurons receiving TS input, only non-NMDA receptors are required for synaptic transmission.

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.

REFLEX CONTROL OF arterial blood pressure is finely tuned in the central nervous system (CNS) not only by neuronal influences but also by humoral influences by virtue of the circumventricular organs, the most caudal of which is the area postrema (AP). The AP is located on the dorsal surface of the medulla above the fourth ventricle and is well suited to regulate cardiovascular function. It lacks a complete blood-brain barrier, making it accessible to circulating substances with cardiovascular-related actions, including angiotensin II, vasopressin, and endothelin (10, 11, 20, 31), and it sends prominent projections to other CNS regions important in cardiovascular regulation (4, 25, 27, 29). Substantial evidence for AP modulation of cardiovascular function has been obtained by examining the consequences of either stimulation or lesions in the AP on cardiopulmonary receptor and baroreceptor reflex control of sympathetic nerve activity. AP stimulation has been shown to augment baroreceptor reflex-mediated inhibition of sympathetic nerve activity (14). AP lesions have been shown to decrease the ability of circulating vasopressin to augment either cardiopulmonary receptor or baroreceptor reflex-mediated inhibition of sympathetic nerve activity (7, 15, 31).

There is considerable evidence that the nucleus tractus solitarius (NTS) is the site in the primary baroreflex arc where AP neurons exert their actions on baroreceptor reflex function. The NTS, located in immediate contact with the AP, is the first central site where afferent fibers carrying baroreceptor and cardiopulmonary receptor-related information synapse (9, 19, 21). Neuroanatomic studies have shown that the AP sends prominent projections, specifically, to the dorsomedial NTS, where cardiovascular afferent fiber terminals are concentrated (25, 29, 32). Electrophysiological studies performed in the intact animal and in a medullary slice containing the AP and NTS have demonstrated that activation of AP neurons evokes mostly excitatory responses in NTS neurons. Importantly, the onset latencies for the AP-evoked action potential responses of the NTS neurons are similar in the slice and in the intact animal, findings consistent with a direct connection from the AP to the NTS without intervening synapses outside the AP-NTS axis (5, 16, 17). In further support of AP modulation of autonomic reflex function at the level of the NTS, we have previously shown that AP and aortic baroreceptor or vagal afferent fibers converge onto NTS neurons and that when the inputs are combined, they sum in a facilitative manner in half of the neurons tested (5). Similar results have been obtained in the medullary slice where AP stimulation has been shown to facilitate the number of tractus solitarius (TS)-evoked action potentials in NTS neurons (17). Recently, the same group, using intracellular recording in the slice, has shown that AP- or TS-evoked postsynaptic potentials evoked in NTS neurons are preponderantly excitatory (6). Interestingly, they also found that depending on the relative strengths of the inputs, AP and TS inputs could sum either in a facilitative or an occlusive manner. Together, these findings suggest that AP neurons send excitatory projections to NTS neurons that also receive visceral (including baroreceptor and cardiopulmonary) afferent inputs and that these inputs can interact in a facilitative fashion.

Although convergent inputs onto NTS neurons from the AP and baroreceptor or vagal afferent fibers have been documented in vivo or from the AP and sensory afferent fibers in the TS in vitro, the neurotransmitters and neuromodulators in the AP-NTS synaptic pathway have not been fully determined. Immunohistochemical studies have localized glutamate, norepinephrine, sub-
stance P, serotonin, and aspartate in the cell bodies of AP neurons (1, 22, 33). A more specific neurotransmitter role for glutamate in the AP-NTS pathway is suggested by its localization in the nerve terminals of AP neurons (33) and because of the ubiquitous nature of glutamatergic synapses in the CNS. Whether either or both non-N-methyl-D-aspartate (NMDA) and NMDA glutamatergic receptors mediate the AP-evoked excitatory responses in NTS neurons has not been determined.

Norepinephrine may also contribute to AP-NTS signal transmission because it has been shown to be released by potassium-induced depolarization of AP neurons (30). In addition, norepinephrine activation of $\alpha_2$-adrenergic receptors in the AP-NTS synaptic pathway has been suggested by studies in the medullary slice and intact animal. Bath application of the $\alpha_2$-adrenergic receptor antagonist yohimbine in the slice has been shown to inhibit AP-induced action potentials in NTS neurons in over half of the neurons tested by up to 74% (16). Moreover, in the intact animal, injection of yohimbine in the NTS has been shown to block AP-mediated augmentation of baroreflex function (13).

In the present study, we used whole cell patch-clamp recordings in both voltage- and current-clamp modes in the rat coronal brain stem slice to examine the role of ionotropic glutamatergic receptors and $\alpha_2$-adrenergic receptors in the synaptic pathway from the AP to NTS neurons that process visceral afferent information including cardiopulmonary and baroreceptor information via the TS. The primary aims were 1) to examine whether AP stimulation evokes both non-NMDA and NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) in NTS neurons and 2) to determine the extent to which both non-NMDA and NMDA receptors are required for synaptic transmission in the AP to NTS pathway, that is, for AP stimulation to evoke action potentials in NTS neurons. A secondary aim was to also determine the extent to which $\alpha_2$-adrenergic receptors are required for AP stimulation to evoke action potentials in NTS neurons.

METHODS

All experimental protocols 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 the National Institutes of Health Guide for the Care and Use of Laboratory Animals [Department of Health and Human Services Publication No. (NIH) 85–23, Revised 1985].

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), and after decapitation, the brain was rapidly exposed and submerged in ice-cold (<4°C) artificial cerebrospinal fluid (aCSF) that contained (in mM) 125 NaCl, 2.5 KCl, 1 MgCl$_2$, 1.25 Na$_2$HPO$_4$, 25 NaHCO$_3$, 10 glucose, and 2 CaCl$_2$, pH 7.4 when continuously bubbled with 95% O$_2$-5% CO$_2$ (300 mosM). Brain stem coronal slices (250 µm thick) were cut with the Vibratome 1000 (Technical Products International, St. Louis, MO). Two slices that included the AP, NTS, and TS were typically obtained from each rat. After incubation for 1 h at 37°C, the slices were placed in room temperature aCSF and continuously gassed with 95% O$_2$-5% CO$_2$. During the experiments, a single slice was transferred to the recording chamber, held in place with a nylon mesh, and continuously perfused with oxygenated aCSF at a rate of ~3 ml/min. All experiments were performed at room temperature.

Whole-Cell Patch-Clamp Recording

Borosilicate glass electrodes were filled with a cesium solution containing (in mM) 145 CsF, 5 NaCl, 1 MgCl$_2$, 3 K-ATP, 0.2 Na-GTP, 0.2 EGTA, and 10 HEPES, pH 7.4 (300 mosM), for voltage-clamp experiments or with a potassium solution containing (in mM) 145 potassium gluconate, 2 MgCl$_2$, 3 K-ATP, 0.2 Na-GTP, 1.1 EGTA, and 5 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). Whole cell voltages and currents were filtered at 2 kHz with a four-pole Bessel filter, digitized at 10 kHz with the DigiData 1200 Interface (Axon Instruments), and stored in a 386 DX computer. Data were analyzed off-line using the pClamp6 software (Axon Instruments). Somatic currents were no larger than 600 pA, and the measured series resistance was no larger than 30 MΩ. Steady-state currents were small at all potentials and did not introduce a significant voltage error through the series resistance.

AP and TS Stimulation

Bipolar tungsten electrodes with 25-µm tips or a concentric bipolar electrode with a 25-µm tip (Frederick Haer) were placed in the AP and TS. The AP-stimulating electrode was placed in the center of the AP. Single stimuli (1–20 V, 100 µs) were delivered to the AP and the TS ipsilateral to the recording site at a frequency of 0.2 Hz. To ensure that the responses to AP and TS stimulation were synaptically activated, we compared the action potentials evoked in aCSF with the action potentials evoked after bath application of the calcium channel blocker CdCl$_2$ (200 µM). In all nine cells tested, CdCl$_2$ abolished the action potentials in NTS neurons evoked by AP stimulation.

Protocols

The AP, NTS, and TS were located in the slice using a ×2 objective. The stimulating electrodes were placed in the AP and TS, and whole cell recordings (12) were obtained in visualized NTS cells using standard infrared videomicroscopy (8).

To determine whether non-NMDA and NMDA receptors were synaptically activated by AP stimulation, AP-evoked EPSCs in NTS neurons were measured at a series of potentials ranging from −100 to +80 mV in 20-mV steps. Using established criteria for isolating non-NMDA and NMDA receptor-mediated components of EPSCs (3, 18), for each AP-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 of the response where the slower NMDA receptor-mediated component is predominant. AP-evoked EPSCs were considered to have both a fast and a slow component if the ratio of the

Downloaded from http://ajpheart.physiology.org/ by 10.20.33.3 on October 14, 2017
amplitude of the current measured 20 ms after the peak response to the current amplitude measured at the peak response of the EPSC was > 0.5 at a holding potential of +60 mV (18). The fast and slow EPSC components were pharmacologically verified as being mediated by non-NMDA and NMDA receptors, respectively, by the application of specific non-NMDA and NMDA receptor antagonists. For the antagonists studies, AP-evoked EPSCs were obtained in normal perfusate and 2-6 min after bath application of either the specific NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV) or 1-3 min after bath application of the specific non-NMDA receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzoquinoxaline-7-sulfonamide (NBQX). EPSCs evoked by AP stimulation were pharmacologically isolated by constant perfusion with the specific GABA_A receptor antagonist bicuculline (10 µM).

To determine the extent to which non-NMDA and NMDA receptors were required for synaptic transmission between AP and NTS, specifically, for AP stimulation to evoke action potentials in NTS neurons, we recorded the voltage changes in NTS neurons in response to low-frequency AP stimulation (0.2 Hz). We calculated the percent response as the number of times an action potential was evoked in the NTS cell by AP stimulation divided by the number of AP stimuli delivered and multiplied the result by 100. We compared the percent response in the presence of ACSF during bath application of the appropriate antagonist and after reperfusion with ACSF. We used 3 µM NBQX, a concentration sufficient to block over 90% of the non-NMDA receptor-mediated component of the EPSC in voltage-clamp experiments. For the NMDA antagonist APV, we used two different concentrations: 50 µM, a concentration shown to selectively block the NMDA receptor-mediated EPSC component in voltage-clamp experiments by ~65%, and 500 µM, a 10 times higher concentration. For the α2-adrenergic receptor antagonist yohimbine, we used two different concentrations: 200 nM, shown to have an inhibitory response (n = 5) or an inhibitory response (n = 3) to AP stimulation. In general, larger stimulating voltages delivered to the AP were required to evoke NTS responses compared with TS stimulating voltages.

Data Analyses

Data are expressed as means ± SE unless otherwise indicated. The synaptic currents in response to AP stimulation shown in traces and current-voltage (I-V) plots are averages of two to four traces. The I-V plots were fitted with a second-order regression. The EPSCs were compared in the control condition and in the presence of antagonist using the paired t-test. The excitatory postsynaptic potentials (EPSPs) and action potentials shown are individual traces. Changes in the percent response of NTS neurons to AP stimulation were compared in the control condition, during the appropriate antagonist, and after reperfusion with ACSF using one-way repeated measures ANOVA, followed by Scheffe’s post hoc test when appropriate. Differences were considered significant at P < 0.05. Latencies to the synaptic responses were measured from the stimulus artifact to the beginning of the synaptic currents or the EPSP that preceded the action potential.

Chemicals

NaCl, KCl, MgCl_2, and CdCl_2 were purchased from Fisher. Potassium gluconate NaHCO_3, K-ATP, Na-GTP, EGTA, HEPES, APV, bicuculline, and yohimbine were from Sigma. NaH₂PO₄ and CaCl₂ were from Mallinckrodt. CsF was from Aldrich, and NBQX was from RBI.

RESULTS

AP and TS Inputs to NTS Neurons

Whole cell patch-clamp recordings were obtained from 350 dorsomedial NTS neurons; 125 of these neurons were studied in voltage-clamp mode to examine EPSCs and 225 NTS neurons in current-clamp mode to examine AP-evoked EPSP and action potentials. The number of neurons receiving excitatory input from AP alone, AP and TS, or TS alone are shown in Table 1. In an additional eight neurons studied in current-clamp mode, we observed a mixed excitatory-inhibitory response (n = 5) or an inhibitory response (n = 3) to AP stimulation. In general, larger stimulating voltages delivered to the AP were required to evoke NTS responses compared with TS stimulating voltages.

To focus on the role of the AP in modulating the integration of sensory afferent signals in the NTS, we examined the effect of AP stimulation on dorsomedial NTS neurons receiving visceral sensory information via the TS. This region of the NTS is the primary site where afferent fibers for cardiovascular-related receptors, including the baroreceptors, terminate (21). Thus the results described below are for those NTS neurons that received both AP and TS input.

AP-Evoked EPSCs in Neurons Receiving TS Input

Time course and voltage dependence of AP-evoked EPSC. In 13 neurons responsive to both AP and TS stimulation, AP-evoked EPSCs were recorded at a series of voltages from −100 mV to +80 mV in 20-mV steps. In 10 of these neurons, the AP-evoked EPSC had two components (Fig. 1A), a fast component that is predominant at the peak of the response and a slow component that is predominant at 20 ms after the peak of the response (3, 18). In the remaining three cells, the AP-evoked EPSCs had only the fast component according to the criteria described in METHODS. For the cells with both the fast and slow components, the ratio of the current amplitude measured at 20 ms after the peak of the response to the current amplitude measured at the peak of the response averaged 0.87 ± 0.09 at a holding potential of +60 mV. For the cells with only the fast component, this ratio was 0.29 ± 0.07, significantly less than the ratio for the cells with both components (P < 0.006, unpaired t-test). The average onset latency of AP-evoked EPSCs that had both components was 4.7 ±

| Table 1. Number of NTS neurons receiving excitatory input from AP, AP + TS, or TS |
|-------------------------------|-------------------|-------------------|
| Voltage-Clamp Mode EPSC       | Current-Clamp Mode EPSP or Action Potentials |
| Total                        | 125               | 225               |
| AP + TS                      | 57                | 96                |
| AP only                       | 1                 | 6                 |
| TS only                       | 48                | 53                |
| Neither                      | 19                | 70                |

AP, area postrema; TS, solitary tract; NTS, nucleus tractus solitarius; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential.
Effect of APV on AP-evoked EPSCs. To verify that the slow component of the AP-evoked EPSCs was mediated by NMDA receptors, we used the selective NMDA receptor antagonist APV. AP-evoked EPSCs having both the fast and slow components were measured before, during, and after the slice was superfused with APV (50 µM, n = 8). An example of the effect of APV (50 µM) on AP-evoked EPSCs is shown in Fig. 2A. At −100 mV, where NMDA receptor-mediated currents are negligible because of the voltage-dependent Mg2+ block of the channel (26), APV had no effect on the slow component (20 ms after the peak of the response). At −40 mV, where there is less voltage-dependent Mg2+ blockade of the channel and NMDA receptor-mediated currents become detectable, APV modestly inhibited the slow EPSC component. At +60 mV, where there is no appreciable Mg2+ blockade of the channel, APV markedly decreased the slow component and had no observable effect on the fast component. The response recovered 2–5 min after reperfusion with aCSF (data not shown). The I-V relationships for the same neuron shown in Fig. 2A were plotted at the peak of the response and at 20 ms after the peak of the response (Fig. 2B). The upper I-V plot shows that APV had no effect on the fast component of the EPSC. In contrast, the lower I-V plot shows that APV decreased the slow component of the EPSC at positive voltages (+40 and +60 mV) and had no effect at voltages negative to −50 mV.

For all eight cells, APV significantly decreased the amplitude of the slow component measured at +60 mV from 66 ± 7 to 23 ± 8 pA (P = 0.0005; paired t-test), while having no significant effect on the amplitude of the fast component which was 75 ± 12 pA in the control condition and 122 ± 36 pA in the presence of APV (P = 0.17; paired t-test).

Effect of NBQX on AP-evoked EPSC. To pharmacologically demonstrate that the fast component of the AP-evoked EPSCs was mediated by non-NMDA receptors, we used the selective non-NMDA receptor antagonist NBQX. AP-evoked EPSCs having both the fast and slow components were measured before, during, and after the slice was superfused with NBQX (3 µM, n = 7). Five of the seven cells had both the fast and slow components, and the remaining two cells had only the fast component. An example of the effect of NBQX on the AP-evoked EPSCs with both the fast and slow components is shown in Fig. 3A. At −100 mV, where only non-NMDA receptor-mediated currents are present, NBQX abolished the EPSC. At −40 mV, where NMDA receptor-mediated currents become detectable, NBQX abolished only the fast component of the EPSC. Moreover, at +60 mV, where both non-NMDA and full-fledged NMDA receptor-mediated currents are present, NBQX inhibited the fast component but did not affect the slow component. The synaptic currents recovered after 10 min of reperfusion with aCSF (data not shown). The I-V relationships for the same neuron shown in Fig. 3A were plotted at the peak of the response and at 20 ms after the peak of the response (Fig. 3B). The upper I-V plot shows that NBQX reduced the amplitude of the fast component (measured at the slow component predominates at 20 ms after peak response (labeled peak), and slow component overlapping fast and slow components. Fast component predominates at peak of response (dotted line labeled peak), and slow component predominates at 20 ms after peak response (dotted line labeled 20 ms after peak).
peak of the response) at all voltages. The lower I-V plot shows that NBQX had only a small effect on the slow component (measured at 20 ms after the peak of the response) at any voltage.

For all seven cells, NBQX significantly decreased the amplitude of the fast component measured at $-100 \text{ mV}$ from $-264 \pm 26$ to $-37 \pm 7 \text{ pA}$ ($P = 0.00005$, paired t-test), data consistent with NBQX blockade of a non-NMDA current. For all five cells that had both components, NBQX also had a small yet statistically significant effect on the slow component ($-18 \pm 3 \text{ pA}$ before and $-7 \pm 3 \text{ pA}$ during NBQX, $P = 0.009$, paired t-test). In contrast, at $+60 \text{ mV}$, where NMDA receptor-mediated currents are maximal, NBQX had no significant effect on the slow component, which was $45 \pm 13 \text{ pA}$ before and $83 \pm 28 \text{ pA}$ during NBQX ($P = 0.07$, paired t-test).

Synaptic Transmission Between AP and NTS

AP-evoked action potentials and/or EPSPs were recorded in 25 neurons that were responsive to both AP and TS stimulation. The average onset latency of the AP-evoked action potentials or EPSCs was $6.6 \pm 0.6 \text{ ms}$ and was longer than the average latency ($4.5 \pm 0.3 \text{ ms}$) of the AP-evoked EPSCs ($P = 0.02$; unpaired t-test).

Fig. 2. Effect of N-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV) on AP-evoked EPSC in an NTS neuron. A: EPSC shown before (thick line) and in presence (thin line) of APV (50 µM) at 3 different voltages. B: current-voltage plots for same cell as in A. Top panel shows EPSC at peak of response before (●) and in presence (○) of APV. Bottom panel shows EPSC measured at 20 ms after peak response before (▲) and in presence (△) of APV.

Fig. 3. Effect of non-NMDA receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzoquinazoline-7-sulfonamide (NBQX) on AP-evoked EPSC in an NTS neuron. A: time course of EPSC shown before (thick line) and in presence (thin line) of NBQX (3 µM) at 3 different voltages. B, top panel: current-voltage plot of currents at peak response before (●) and in presence (○) of NBQX. Bottom panel: current-voltage plot of currents measured at 20 ms after peak response before (▲) and in presence (△) of NBQX.
Effect of NBQX on synaptic transmission between AP and NTS. To determine the role of non-NMDA receptors in mediating low-frequency synaptic transmission between AP and NTS, specifically, in mediating AP-evoked action potentials in NTS neurons, we compared the percent response of the NTS neurons to AP stimulation in the presence of aCSF (control), during NBQX (3 µM), and after reperfusion with aCSF (recovery). Figure 4 shows a typical response of an NTS neuron to five AP stimuli before (A), during (B), and after recovery (C) from NBQX. NBQX reversibly blocked both AP-evoked action potentials and EPSPs.

The results obtained with NBQX are summarized in Fig. 5. In all 14 cells tested, NBQX abolished AP-evoked action potentials; the percent response decreased from 93 ± 4% in the control condition to 0 ± 0% during NBQX and partially recovered to 63 ± 9% after reperfusion with aCSF (ANOVA, \( P = 0.0001 \); Scheffe's post hoc, \( P < 0.05 \)).

Effect of APV on synaptic transmission between AP and NTS. To determine the role of NMDA receptors in low-frequency synaptic transmission between the AP and NTS, we compared the percent response ratio of the NTS neurons to AP stimulation in the presence of

![Fig. 4. Effect of NBQX on AP-evoked action potentials and excitatory postsynaptic potential (EPSP) in an NTS neuron. A representative example of 5 consecutive synaptic responses is shown before (A; control), in presence of 3 µM NBQX (B), and after 10 min reperfusion with artificial cerebrospinal fluid (C; recovery).](image)

![Fig. 6. Effect of APV on AP-evoked action potentials and EPSP in an NTS neuron. A representative example of 5 consecutive synaptic responses is shown before (A; control) and in presence of 50 µM APV (B).](image)
failed to trigger an action potential but still evoked EPSP.

In three of nine cells tested, APV (50 µM) decreased the percent response to AP stimulation by at least 20% (1 of 5 AP stimuli did not evoke an action potential in an NTS cell). A summary of the results obtained with 50 µM APV (n = 9) and 500 µM APV (n = 6) are shown in Fig. 7. As shown in Fig. 7A for 50 µM APV, the percent response was 98 ± 2% in the control condition, 82 ± 10% during APV, and 87 ± 7% after reperfusion with aCSF; these values were not statistically significantly different (ANOVA, P = 0.2). NBQX (3 µM) was subsequently perfused over eight of the nine cells. In contrast to APV, NBQX abolished the AP percent response ratio of NTS neurons to AP stimulation-evoked action potentials; the percent response decreased from 87 ± 6% in aCSF to 0 ± 0% in the presence of NBQX and partially recovered to 35 ± 12% after reperfusion with aCSF (ANOVA, P = 0.0001; Scheffé's post hoc, P < 0.05). In voltage-clamp mode, we observed that 50 µM APV decreased the slow component of the EPSC by −65%.

To ensure that all NMDA receptors were blocked, we increased the APV concentration to 500 µM and still found no statistically significant effect on the six cells tested (Fig. 7B); the percent response was 97 ± 3% in control condition, 100 ± 0% in the presence of APV, and 100 ± 0% in the recovery period (during reperfusion with aCSF) (ANOVA, P = 0.4). NBQX (3 µM) was subsequently perfused over the same six cells and abolished the AP-evoked action potentials; the percent response decreased from 100 ± 0 to 0 ± 0% and recovered to 83 ± 8% (ANOVA, P = 0.0001, Scheffé's post hoc, P < 0.05).

Effect of yohimbine on synaptic transmission between AP and NTS. To examine the role of α2-adrenergic receptors in low-frequency synaptic transmission between the AP and NTS, we compared the percent response of NTS neurons to AP stimulation before, during perfusion with the selective α2-adrenergic receptor antagonist yohimbine at two concentrations (200 nM and 2 µM), and after reperfusion with aCSF. An example of the effect of 2 µM yohimbine on the response of an NTS neuron to five AP stimuli is shown in Fig. 8. Under control conditions (Fig. 8A), each AP stimulus evoked an action potential. In the presence of yohimbine (Fig. 8B), the third AP stimulus failed to evoke an action potential but still evoked an EPSP. The response recovered after reperfusion with aCSF (Fig. 8C).

Yohimbine at a concentration of 200 nM decreased the AP-evoked percent response in only one of the five NTS neurons tested. A summary of the results obtained with 200 nM yohimbine (n = 5) and 2 µM yohimbine (n = 10) is shown in Fig. 9. As shown in Fig. 9A, the percent response was 100 ± 0% in the control condition, 96 ± 4% during yohimbine (200 nM), and 96 ± 4% after reperfusion with aCSF. These values were not statistically significantly different (ANOVA, P = 0.6). NBQX (3 µM) was subsequently perfused over four of these
cells and abolished the AP-evoked action potentials; the percent response decreased from 95 ± 5% in the control condition to 0 ± 0% during NBQX and recovered to 75 ± 10% (ANOVA, \( P = 0.0001 \); Scheffé’s post hoc, \( P < 0.05 \)). Yohimbine at a concentration of 2 µM decreased the AP-evoked percent response by at least 20% in 4 of the 10 NTS neurons tested. As shown in the summary data (Fig. 9B), the percent response was 98 ± 2% in the control, 84 ± 8% in the presence of yohimbine, and 98 ± 2% after reperfusion of aCSF. Although there appeared to be a trend for the response to decrease in the presence of yohimbine, the values were not statistically significantly different (ANOVA, \( P = 0.08 \); Fig. 9). NBQX was subsequently perfused over 6 of the 10 NTS cells and abolished the AP-evoked action potentials; the percent response decreased from 97 ± 3% in the control to 0 ± 0% in the presence of NBQX and partially recovered to 37 ± 17% (ANOVA, \( P = 0.0001 \); Scheffé’s post hoc, \( P < 0.05 \)).

**DISCUSSION**

The principal finding was that AP activation of non-NMDA receptors is essential for low-frequency synaptic transmission in the AP-NTS synaptic pathway, despite the coexistence and synaptic activation of both non-NMDA and NMDA receptor-mediated currents. A secondary finding was that α2-adrenergic receptors are also not required for low-frequency AP synaptic transmission to NTS neurons. These observations are the first to suggest that glutamate is necessary for transmission of signals from the AP to the NTS. The roles played by non-NMDA, NMDA, and α2-adrenergic receptors in this pathway are discussed below.

**NTS Neuronal Responses to AP and TS Stimulation**

In general, the percentages of NTS neurons that were excited by both AP and TS stimulation in voltage-clamp (46%) and in current-clamp mode (41%) are consistent with previous experiments using extracellular recordings in rabbit brain stem slices (16, 17) and with our previous findings with extracellular recordings in vivo in which 51% of the NTS cells were excited by both AP and vagus nerve stimulation (5). Not surprisingly, in the same study, we found a smaller percentage (24%) of the NTS cells responsive to combined stimulation of the AP and the aortic depressor nerve (5). Moreover, the mean onset latency for the AP-evoked EPSPs and action potentials in this study (~7 ms) are similar to previous findings in the rabbit medullary slice (~9–10 ms) (16, 17), although, as expected, somewhat shorter than the average onset latency of ~12 ms observed in vivo (5). Viewed collectively, the data obtained in vitro and in vivo demonstrate that stimulation of AP neurons evokes excitatory responses in almost half of NTS neurons that process visceral afferent sensory information and support the proposal that AP neurons can directly modulate sensory processing at the level of the NTS in autonomic reflex pathways.

The circuitry linking the AP and NTS is complex, and inhibitory responses, although less prominent, are also present in NTS neurons after AP stimulation. Although the frequency with which we observed inhibitory responses was lower (2% mixed excitatory and inhibitory, 1% inhibitory) than the 10% observed by Cai et al. (6) in intracellular recordings in rabbit brain stem slices, our observations confirm their report of inhibitory responses in NTS neurons.

**Non-NMDA and NMDA Receptors Exist in the AP-NTS Synaptic Pathway**

AP-evoked EPSCs in the majority of NTS neurons that responded to both AP and TS stimulation had two components with distinct temporal patterns and I-V relationships (Fig. 1A) as well as sensitivity to glutamatergic antagonists. The fast component peaked on average at 4.2 ms after the EPSC onset, then rapidly decayed to a low amplitude at 20 ms after the peak of the response; this component displayed a linear I-V relationship for voltages from −100 to +20 mV and, most importantly, was abolished by the non-NMDA receptor antagonist NBQX (Fig. 3), findings consistent with activation of a non-NMDA receptor-mediated current (3, 18).

The slow component of the AP-evoked EPSCs developed more slowly, being most clearly present at 20 ms after the peak of the response and having a duration >60 ms. The I-V relationship showed that the slow component had appreciable outward currents only at positive voltages and only negligible inward currents at voltages negative to −40 mV (Fig. 1B). Finally, the amplitude of the slow component (Fig. 2) was attenuated (65%) by the selective NMDA receptor antagonist
APV (50 µM), findings consistent with activation of an NMDA receptor-mediated current (3, 18).

These observations provide the first evidence that non-NMDA and NMDA receptors are present in the AP-NTS synaptic pathway and can be activated by AP stimulation. Non-NMDA and NMDA glutamatergic receptors also coexist on cells and respond to afferent stimulation in the hippocampus (18) and visual cortex (2), and on NTS neurons that are activated by TS stimulation (3).

Non-NMDA and NMDA Receptors in Synaptic Transmission From AP to NTS

Although both non-NMDA and NMDA receptors on NTS neurons were activated by low-frequency AP stimulation as described above, current-clamp experiments showed that only non-NMDA receptor-mediated currents evoked by AP stimulation were sufficient to depolarize NTS neurons to the threshold range and trigger action potentials. NBQX abolished AP-evoked synaptic responses in NTS cells including action potentials and EPSPs (Figs. 4 and 5). In contrast, APV (50 µM), at a concentration that significantly attenuated AP-evoked NMDA receptor-mediated EPSCs, marginally reduced the number of action potentials in NTS neurons evoked by AP stimulation in only three of the nine neurons tested and in no case had a detectable effect on AP-evoked EPSPs (Figs. 6 and 7). Thus, even though the grouped data for all nine cells suggested a trend for a decrease (16%) in the percent response ratio in the presence of APV (Fig. 7A), the difference was not statistically significant. Moreover, a 10-fold higher concentration of APV (500 µM) had no statistically significant effect on the percent response ratio (Fig. 7B).

Taken together, these results indicate that functional non-NMDA receptors are sufficient and essential for low-frequency synaptic transmission between AP and NTS. More specifically, these receptors are necessary for low-frequency AP stimulation to evoke action potentials in NTS neurons. Although NMDA receptors are not essential for synaptic transmission during low-frequency AP stimulation, these receptors may become important during high-frequency input from the AP, or when excitatory inputs from other sources depolarize the NTS cell and relieve the Mg²⁺ blockade of the NMDA receptors (26).

Of related interest to the nature of the AP synaptic input to the NTS neurons was the consistent onset latencies of the synaptic responses (<1 ms variability). Such invariant onset latencies suggest that the AP input to the NTS neurons may be monosynaptic. However, another characteristic of many monosynaptic pathways was not met as the NTS cells failed to reliably discharge an action potential in response to each of two AP stimuli separated by 5 ms (data not shown) (23). The inability of the NTS neurons to follow both stimuli 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 that have been described as small, multipolar, and having either diffuse short dendrites or a few long branching dendrites (24).

Although further experiments are required to unequivocally determine whether the AP-NTS pathway is primarily monosynaptic or polysynaptic, our results provide information on the number and location of glutamatergic synapses in the pathway. Current-clamp recordings showed that AP stimulation evoked no detectable synaptic responses in NTS cells when non-NMDA receptors were blocked with 3 µM NBQX (Fig. 4), indicating that NMDA receptors cannot, by themselves, support low-frequency transmission across the AP-NTS synaptic pathway. However, voltage-clamp recordings in cells clamped at +60 mV and perfused with the same concentration of NBQX used in the current-clamp recordings showed that only the non-NMDA receptor-mediated component was still present. If there were multiple glutamatergic synapses in this pathway, NBQX would block signal transmission at the first synapse, and no action potentials would reach the following synapses; hence, no AP-evoked NMDA receptor-mediated component would be detected in the NTS neuron. Taken together, these results (Figs. 3 and 4) suggest that the AP-NTS synaptic pathway is either 1) monosynaptic and glutamatergic or 2) polysynaptic with only one glutamatergic synapse at the NTS cell. Further experiments are necessary to determine which of these alternatives describes the AP-NTS synaptic pathway. Nonetheless, the observation that AP stimulation activates glutamatergic neurons that synapse onto NTS cells also receiving afferent input from the TS provides a first step in linking specific neurotransmitters to AP neurons that modulate sensory afferent signal processing in the NTS.

α₂-Adrenergic Receptors in Synaptic Transmission From AP to NTS

Evidence that norepinephrine acting at α₂-adrenergic receptors is involved in the AP-NTS synaptic pathway has been obtained from anatomic as well as physiological studies. Immunocytochemical studies have indicated that α₂-adrenergic receptors are regionally distributed in the brain stem with a predominance in the dorsomedial NTS (28), the site where afferent fibers carrying baroreceptor information first synapse. Moreover, norepinephrine can be released by potassium-induced depolarizations of AP neurons (30). Hay and Bishop (16) found that 200 nM yohimbine decreased the number of action potentials in NTS neurons evoked by AP stimulation in 9 of 14 cells tested; the percent response decreased by 74% from a control response of 66% to 17% in the presence of yohimbine (16). These experiments suggested that AP-evoked NTS responses require activation of α₂-adrenergic synapses (perhaps via a polysynaptic pathway). To test this proposal, we applied yohimbine at two different concentrations and determined whether AP-NTS signal transmission was...
blocked. At a concentration of 200 nM, yohimbine failed to block transmission and only slightly decreased the number of AP-evoked action potentials in one of five cells tested; of note were the findings that EPSP were still evoked in that cell (Fig. 8). Yohimbine at a 10-fold higher concentration (2 µM) decreased the number of AP-evoked action potentials in 4 of 10 cells, but the overall decrease (15%) was still not statistically significant (Fig. 9). In the majority of these cells, NBQX perfused after recovery from yohimbine washout abolished AP-evoked action potentials and EPSP. Thus our findings suggest that norepinephrine acting at a2-adrenergic receptors is not required for the induction of action potentials in NTS cells evoked by low-frequency AP activation in the rat medullary slice.

An alternative role for a2-adrenergic receptors, consistent with our results described above and those of Hay and Bishop (16, 17), is that, although not required for AP-evoked generation of action potentials in NTS neurons, norepinephrine acting at a2-adrenergic receptors can effectively modulate synaptic transmission. Hay and Bishop (16), using extracellular recording, stimulated the AP with currents sufficient to evoke an average action potential response rate of 66% during the control conditions. In our study, we stimulated the AP with voltages slightly above the threshold but sufficient to evoke close to a 100% average action potential response rate. The lower response rate in the Hay and Bishop (16) study suggests that their cells were operating in the steep region of the sigmoid curve that relates stimulation strength and synaptic response, the part of the curve where neurons are more susceptible to modulation. Thus it seems reasonable to assume that a2-adrenergic receptors play some role in modulating synaptic transmission in the AP-NTS synaptic pathway. Further experiments are needed to fully delineate the conditions of activation of the a2-adrenergic receptors that suppress action potentials in NTS cells evoked by AP stimulation.

In summary, our data provide further support for the hypothesis that autonomic reflex pathways, including the baroreceptor pathway, contain NTS neurons strategically placed to also receive synaptic signals from the AP. The voltage-clamp recordings confirm that AP stimulation evokes short-latency excitatory responses and document that almost half the NTS neurons that generate EPSCs in response to AP stimulation also generate EPSCs in response to TS stimulation. The data further demonstrate that within the AP-NTS synaptic pathway both non-NMDA and NMDA receptors exist and can be synaptically activated, but that, at least in vitro, only non-NMDA receptor activation is required for low-frequency AP stimulation to evoke action potentials in NTS neurons. It should be acknowledged, however, that the slice preparation eliminates inputs from CNS regions outside the AP-NTS axis, some of which may provide sligh depolarizing influences sufficient to minimize the Mg2+ block to increase the contribution of NMDA receptors to synaptic transmission in the AP-NTS pathway in the intact organism.

We gratefully acknowledge Judy Stewart for technical assistance and Drs. Pedro Maldonado and Pam Pappone for reviewing the manuscript.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-52165 (to A. C. Bonham). M. L. Aylwin was supported, in part, by a postdoctoral fellowship from the American Heart Association, California Affiliate.

Present address of M. L. Aylwin: Departamento de Farmacologia Molecular y Clinica y Departamento de Patologia, Instituto de Ciencias Biomedicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Address for reprint requests: A. C. Bonham, Div. of Cardiovascular Medicine, Univ. of California, Davis, TB 172 One Shields Ave., Davis, CA 95616.

Received 29 April 1998; accepted in final form 10 June 1998.

REFERENCES


