Cholinergic systems in the nucleus of the solitary tract of rats

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Cholinergic systems in the nucleus of the solitary tract of rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1141–R1148, 1999.—The pharmacological and physiological properties of excitatory amino acid and ACh systems in the nucleus of the solitary tract (NTS) were studied in slices of rat brain stem by extracellular and intracellular recordings from neurons activated by solitary tract (ST) stimulation. These neurons were characterized as having several long dendrites with multiple varicosities. Synaptic activation of the medial NTS (mNTS) neurons by ST stimulation was mediated by non-N-methyl-D-aspartate (NMDA) glutamate (Glu) receptors, because the excitation was blocked by 6-cyano-7-nitroquinolinic acid, but not by NMDA, nicotinic, or muscarinic antagonists. Identified mNTS neurons were excited by iontophoresis of both Glu and ACh. The most sensitive region of the cell was on the dendrites ~100 µm from the cell body for both putative neurotransmitters. Nicotinic and/or muscarinic excitatory ACh responses were detected on the mNTS neurons. Our observations suggest that both types of ACh receptors may contribute to the attenuation of the baroreceptor reflex, but the functional correlation of this receptor profile remains to be determined.

glutamate; brain stem

THE NUCLEUS OF THE SOLITARY tract (NTS) is the first projection site of afferent fibers from arterial baroreceptors, chemoreceptors, cardiopulmonary mechanoreceptors, and other visceral receptors (14–16, 19, 21, 30, 38). As such the NTS plays an important role in the integration of autonomic and visceral functions relevant to the cardiovascular system (15, 16, 22, 30, 38).

ACh is the neurotransmitter most often assumed to be central in regulation of cardiovascular function. ACh has a widespread distribution throughout the central nervous system and contributes to central autonomic regulation, including control of arterial pressure. A cholinergic system in the medial NTS (mNTS) was identified by the presence of choline acetyltransferase, ACh esterase, and ACh in this region (15, 26, 27) and labeling by [3H]ACh (28). Glycine injected into the NTS was shown to induce the release of ACh from a portion of locally synthesized neurotransmitter stores (31). Furthermore, microinjection of ACh and nicotine into the NTS elicited hypotension and bradycardia responses similar to those induced by stimulation of arterial baroreceptors (5, 13), and these effects of ACh could be inhibited by pretreatment with a muscarinic receptor antagonist, atropine, but not by a nicotinic receptor antagonist, hexamethonium (5). Autoradiographic and histocytochemical studies demonstrated, moreover, that this region is rich in muscarinic receptors (10, 12). Electrophysiological experiments on acutely dissociated neurons from the NTS found, however, that the NTS neurons had nicotinic, but no muscarinic, ACh receptors (36), and microinjection of nicotine into the NTS elicited decreases in arterial pressure and heart rate that were inhibited by pretreatment with hexamethonium (13). Thus, although it is generally accepted that ACh plays an important role for the central regulation of arterial pressure in the mNTS, there is no agreement concerning which type of ACh receptor works in the mNTS. In addition, little is known about the electrophysiological and pharmacological mechanisms of cholinergic systems on the neurons in the mNTS that respond to solitary tract (ST) stimulation.

Despite all of the evidence for a role of cholinergic systems in regulation of cardiovascular function, strong evidence has been demonstrated that glutamatergic systems play a central role (25, 32, 34). It was demonstrated that injection of L-glutamate into the NTS produces a dose-dependent hypotension and bradycardia (33) and that electrical stimulation of vagal C fibers causes release of [3H]Glu into the NTS (35). Thus both cholinergic and glutamatergic systems may be important in the circuitry of the mNTS.

In the present study, we used a brain stem slice maintained in vitro that allows identification of neurons excited by ST stimulation, and we characterized both the pharmacological sensitivities of the endogenous neurotransmitter and responses to iontophoretic application of ACh and Glu.

METHODS

Preparation of slices. Wistar-Kyoto rats (100–150 g) were used in all experiments. Under ether anesthesia, a rat was euthanized by cervical dislocation and the brain stem and cerebellum were rapidly removed to cold Krebs-Ringer solution containing (in mM) 126 NaCl, 5 KCl, 2.4 CaCl2, 1.3 MgSO4, 1.26 KH2PO4, 26 NaHCO3, and 10 d-glucose, saturated with 95% O2 and 5% CO2. The cerebellum was then removed, and the brain stem was trimmed to a length of ~7 mm centered on the obex rostrally and caudally. With the use of cyanoacrylate glue, the ventral surface was fixed on a stage that had an angle of ~10° (9, 20, 29) in a chamber of a vibratome. A single 400-µm slice from the semihorizontal...
section was obtained, containing the ST, the NTS, and the area postrema. In this slice, the ST provides major afferent inputs to the NTS neurons and was long enough to allow synaptic activation of the NTS neurons on ST stimulation. Before the electrophysiological recording, the slices were incubated for at least 2 h in Krebs-Ringer solution bubbled with 95% O₂ and 5% CO₂ at 34°C (11, 29).

Recording systems. Slices were submerged on a Plexiglas mesh in the recording chamber and covered with a nylon mesh on which a silver wire coil was placed to prevent movement of the tissue. This chamber was perfused with oxygenated Krebs-Ringer solution at 34°C at ~3 ml/min. A concentric stimulating electrode was placed on the ST 1.0–2.0 mm from the recording electrode. Square wave pulses (50 µs in duration, 15 V at 0.2 Hz, supramaximal strength of a threshold) were delivered through a stimulator for monosynaptic activation of the neurons in the mNTS. In the 400-µm-thick semis horizontal section, the ST was visible under a dissecting microscope (SV-6, Zeiss, Germany) as a white fiber bundle on the cut surface, and stimulating and recording electrodes were manipulated under direct observation. There was sufficient distance between the stimulating and recording electrodes so as not to stimulate the mNTS neuron directly.

Single-unit discharges were recorded with a glass micropipette filled with Krebs-Ringer solution (3–5 MΩ) or Lucifer yellow CH (Sigma, 10% in distilled water, ~60 MΩ) to mark the neuron being recorded. The electrode was driven into the mNTS until an action potential evoked by ST stimulation was encountered. Responses were amplified with both an analog-current (AC) amplifier and a digital-current (DC) amplifier. Output was led into a pulse counter modified to function as a ratemeter. The spikes and firing rate were simultaneously displayed on an oscilloscope and recorded on a pen recorder.

Intracellular recordings without Lucifer yellow injection were performed on the mNTS neurons with glass microelectrodes (80–120 MΩ) pulled with a Brown-Fleming model P-80 puller and filled with 3 M potassium acetate. The recording electrode was connected to a DC amplifier with a bridge circuit (model IR 183, Neuro Data Instrument).

Histological studies. In some experiments, after extracellular recordings, intracellular recordings were performed using Lucifer yellow CH, an attempt was made to penetrate the neuron and inject the label to identify the location and morphology of the cell. After penetration, the Lucifer yellow was injected into the recorded cell by application of 2 nA negative current pulse of 250 ms duration at 2 Hz for 1.5–2 min. After injection, the slices were fixed in 4% paraformaldehyde in phosphate-buffered saline and frozen sections were cut at 100 µm thickness and stained with neutral red with standard histological methods. Lucifer yellow-containing neurons were observed with a fluorescent microscope using mounting fluid (FA Mounting fluid, Difco Lab).

Drug applications. After neurons were identified as being mNTS cells on the basis of receiving a monosynaptic excitation on stimulation of the ST, agonists and NaCl, as a current control, were iontophoretically applied to the dendritic trees in an automated sequence through a three-barreled iontophoretic electrode, controlled independent of the recording electrode. Agonists were prepared at 0.5 M in distilled water, pH 3.5 for ACh and 7.5 for Glu. For positioning of the iontophoretic electrode on the dendritic tree of a neuron being recorded, the tip of iontophoretic and recording electrodes were dipped in water-proof ink (Magic Ink) to allow visualization of both electrode tips under the dissecting microscope. The iontophoretic current used was usually 1-s pulses of ~10 to ~30 nA for Glu, 20–50 nA for ACh, and ±20–50 nA for NaCl, using a Neurophore model BH-2 control unit. Backing current was not applied routinely. These agonists were usually given in fixed sequence at 1-min intervals to eliminate the possibility of desensitization. Antagonists were added to Krebs-Ringer solution and perfused over the slices for 5 min. Antagonists used for ACh were atropine for muscarinic receptors and curare for nicotinic receptors; antagonists for the excitatory amino acids were 6-cyano-7-nitroquinolinol-2,3-dione (CNQX) for D1-α-amino-3-hydroxy-5-methylisoxazole-propanionic acid (non-N-methyl-d-aspartate [NMDA] receptor), and DL-2-amino-5-phosphonovaleric acid for NMDA receptors. In some experiments, a modified Ringer solution containing low Ca²⁺ (0.1 mM) and high Mg²⁺ (4.3 mM) was perfused to block neurotransmitter release. The sequence of steps of experiments was first to find a spontaneously active neuron with extracellular recording in the mNTS, then to check monosynaptic excitation on stimulation of the ST. Then the iontophoretic electrode filled with Glu, ACh, and NaCl was positioned at various points in the dendritic tree so as to find the most active site, and the responses to Glu and ACh and their antagonists were determined. Then the recording electrode was inserted into the cell to be injected with Lucifer yellow, and it was injected as described above.

Results

Thirty-seven neurons located in the mNTS and receiving monosynaptic excitation on stimulation of the ST were studied. Figure 1 shows extracellular recordings of a single-unit discharge evoked by ST stimulation and the effects of various antagonists. In this record, the cell body of the neuron being recorded was ~1.5 mm from the stimulation electrode. The delay of action potentials evoked by ST stimulation was between 5 and 10 ms. The variation in this delay is probably due to the spontaneous activity of the cell. Immediately after a spontaneous discharge, ST stimulation could not evoke an action potential (21). Another possibility is that inhibitory mechanisms may be involved (22). All of the neurons examined showed spontaneous discharge (0.4–6.1 spikes/s). Among the four antagonists studied, only CNQX (5 × 10⁻⁵ M, n = 5) blocked the synaptic transmission. Apparently the neurotransmitter released from the ST terminals to mNTS neurons is Glu and the primary postsynaptic receptor is a non-NMDA type of Glu receptors, as previously shown in vitro (2).

This conclusion is also consistent with reports from in vivo studies showing that the neurotransmitter from primary arterial baroreceptor afferents to the mNTS region is Glu, on the basis of microinjection studies (32, 34) and L-[³H]Glu uptake experiments (24).

Figure 2A shows a photomicrograph of an identified neuron that was injected with Lucifer yellow. Figure 2B shows the response of this neuron to iontophoretically applied Glu and ACh. The responses to ACh and Glu were determined in a total of 21 identified neurons, and five of these were intracellularly injected with Lucifer yellow as in the neuron illustrated; the identified cells
had two or more long (150 µm or more) dendrites, plus numerous smaller ones, all with many varicosities (not clear in this figure). The position of the iontophoretic electrode that gave the largest response from the 21 neurons investigated was on average ~100 µm from the tip of the recording electrode, which we presumed to be close to the synaptic sites. Responses were amplified with an AC amplifier. This suggests that both types of ACh receptors are maximally concentrated on the dendritic trees at this distance from the cell body. When the tip of the iontophoretic electrode was moved, the response rapidly declined and usually was no longer discernible when the tip had been moved by ~25 µm. This suggests that under the circumstances of our experiments iontophoresis spreads putative transmitters over an area whose diameter is ~50 µm.

To identify the location of the tip of recording and iontophoretic electrodes, the recording electrode was filled with Lucifer yellow (10% in distilled water) and one of the double-barreled iontophoretic electrodes was filled with Lucifer yellow and the other with 0.5 M Glu.

First, the recording electrode was driven until an action potential evoked by ST stimulation was encountered (Fig. 3B); second, the iontophoretic electrode was driven until responses evoked by Glu could be detected (Fig. 3C). Finally, a 5-nA negative current was passed through the iontophoretic electrode filled with Lucifer yellow at 0.5 Hz for 5 min, and the recording electrode filled with Lucifer yellow was advanced to the cell body and 2-nA negative current at 2 Hz for 1.5 min was applied (Fig. 3A).

When putative neurotransmitters are applied by iontophoresis, it is possible that they activate receptors on the neurons being recorded directly or, alternatively, that they activate neighboring neurons that make synaptic contact on the recorded neuron. To ascertain whether the observed excitation with Glu and ACh was direct or indirect, the brain slice was perfused with a modified Ringer solution (0.1 mM Ca²⁺ and 4.3 mM Mg²⁺) that blocks all synaptic input by preventing neurotransmitter release from the ST (n = 5). Figure 4A shows the reversible blockade of excitation of a neuron on ST stimulation, whereas Fig. 4B shows the responses of this neuron to ACh in the low-Ca²⁺ and high-Mg²⁺ medium. Iontophoretically applied ACh activated the mNTS neuron in a dose-dependent manner (Fig. 4B). Unlike the neurons shown in Fig. 2, this response did not show the second inhibitory phase, suggesting that this phase is synaptically mediated. Twenty of twenty-one mNTS neurons recorded were activated by ACh but one neuron showed only inhibition (not illustrated).

Fig. 1. Pharmacological identification of neurotransmitter released from solitary tract (ST). Unit discharges evoked by ST stimulation were recorded from medial nucleus of the ST (mNTS) neurons. ACh and amino acid antagonists was perfused with Ringer solution at 5 × 10⁻³ M for 5 min, and washing time was >20 min. 5-AP, DL-2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitro-quinoxaline-2,3-dione.
To determine whether the responses to ACh applied iontophoretically were mediated by nicotinic or muscarinic receptors, the nicotinic receptor antagonist curare or the muscarinic receptor antagonist atropine were perfused at concentrations of 10⁻² to 5 × 10⁻⁵ M. In the 20 neurons excited by ACh, the responses were attenuated by curare in 12 (Fig. 5A) and by atropine in 8 (Fig. 5B). The spontaneous firing of these neurons was reduced by curare in 12 (from 2.1 ± 0.3 to 1.8 ± 0.3 spikes/s, P < 0.05) and by atropine in 5 (from 2.0 ± 0.3 to 1.6 ± 0.2 spikes/s, P < 0.01) and by both curare and atropine in three neurons (Table 1).

Fig. 3. Identification of sites of recording and iontophoretic electrodes. A: confocal image of Lucifer yellow-positive sites. Double-barreled iontophoretic electrodes (ion elec) were filled with Lucifer yellow CH (Lucifer) and Glu, and a recording electrode (rec elec) was filled with Lucifer yellow CH. B: a unit discharge evoked by ST stimulation (s). C: responses to iontophoretic application of Glu (~25 nA).

Fig. 4. Excitatory response to iontophoretically applied ACh on mNTS neuron. A: unit discharges evoked by ST stimulation in normal and low-Ca²⁺ and high-Mg²⁺ Ringer solution. B: dose-dependent responses (unit discharges) to iontophoretically applied ACh on the mNTS neuron under the condition of synaptic blockade by low-Ca²⁺ and high-Mg²⁺ Ringer solution. Top trace shows raw neurogram, and bottom trace shows firing rates of spikes for 1 s.

Fig. 5. Effect of nicotinic and muscarinic antagonists on ACh responses. A: muscarinic neuron. B: nicotinic neuron. Control, Krebs-Ringer; curare, at 5 min after perfusion with 5 × 10⁻³ M curare; atropine, at 5 min after perfusion with 5 × 10⁻³ M atropine; Wash, after >20 min washing with Krebs-Ringer solution. Ejection current of ACh, 18 nA. Top traces show raw neurograms, and bottom traces show firing rates.
In six identified mNTS neurons, intracellular recordings were performed to characterize the voltage dependency of the ACh response. Nicotinic responses on central neurons are expected to result from conductance increases to monovalent cations and therefore should decrease with depolarization and increase with hyperpolarization. In contrast, muscarinic responses are known to result from closure of the M-type potassium channel (1) and consequently are increased with depolarization as one goes farther from the potassium equilibrium potential and decreased with hyperpolarization. Figure 6 shows intracellular recordings from neurons whose responses to ACh were pharmacologically characterized. Figure 6A shows a response to ACh that is blocked by curare but unaffected by atropine. The depolarization caused by ACh iontophoresis was clearly increased by hyperpolarization (Fig. 6B). Similar results were obtained in a total of three neurons. In contrast, Fig. 6C shows recordings from a neuron where the response to ACh was blocked by atropine but unaffected by curare. This response was reduced by hyperpolarization (Fig. 6D), consistent with the conclusion that the receptor is a muscarinic receptor coupled to an M channel. This result was obtained in a total of three neurons showing atropine-sensitive ACh responses.

DISCUSSION

Our results suggest two distinct conclusions: first, that the primary neurotransmitter released on stimulation of the ST is Glu acting through non-NMDA receptors and, second, that both nicotinic and muscarinic ACh receptors occur on subpopulations of cells in the mNTS. The strong glutamatergic responses on stimulation of the ST are consistent with previous in vitro and in vivo experiments that have also documented a role for Glu receptors in the mNTS (2, 32, 34). We stimulated the ST at low frequency (0.1 Hz) but we cannot rule out synaptic transmission involving metabotropic receptors in in vivo systems (8). Our results are surprising, however, in the lack of evidence for ACh action after stimulation of the ST, because numerous microinjection experiments of ACh (5) or its antagonists (36) into the NTS suggested a strong role for ACh.

A major goal of this investigation was to resolve the controversy over whether ACh receptors in the mNTS neurons were nicotinic (13, 37) or muscarinic (5, 12, 36). This brain stem slice preparation is ideal for investigation of the question because it allows clear visualization of the region of the NTS being recorded, absolute certainty that neurons are directly excited via the ST, and the added possibility of applying putative transmitters and pharmacologically characterizing their responses by perfusion of various antagonists. We need,

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<td>ACh responses (iontophoresis)</td>
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<td>Spontaneous discharge</td>
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Values are no. of recorded neurons. Effects of ACh antagonists on responses to iontophoretically applied ACh and effects of ACh antagonists on spontaneous discharges of ACh-sensitive neurons are shown.

Fig. 6. Effects of membrane potential on a nicotinic and muscarinic receptor activation. A: antagonistic effect of curare on intracellular responses to Ach (20 nA). B: voltage dependency for Ach response (10 nA) in same neuron as in A. C: antagonistic effect of atropine on intracellular responses to Ach (15 nA). D: voltage dependency for Ach response (15 nA) in same neuron as in C.
However, more detailed anatomic studies because the ST is not consistent with homogeneous fibers. Our observations clearly show that both nicotinic and muscarinic receptors are found on the NTS neurons. We found 12 of 20 neurons to have nicotinic receptors, and 8 of 20 to have muscarinic receptors, on the basis of sensitivity of the iontophoretic responses to the nicotinic and muscarinic antagonists curare and atropine, respectively. There were three neurons whose frequency of spontaneous discharge was somewhat reduced by both curare and atropine, but this may reflect synaptic inputs rather than the nature of the postsynaptic receptors on that cell. These results suggest that the nicotinic and muscarinic receptors are located on different populations of cells. However, spontaneous discharge from nine neurons that responded to ACh were not affected by both curare and atropine. Concentrations of these antagonists might not be enough to penetrate to ACh receptor sites of recorded neurons during perfusion.

The intracellular experiments indicate that the cholinergic responses are similar to those described elsewhere within the central nervous system. The atropine-sensitive responses show the characteristics of M channels, a voltage-dependent potassium channel known to be closed by activation of muscarinic receptors (1). The responses are reduced by hyperpolarization and increased by depolarization, reflecting proximity to the potassium equilibrium potential. In contrast, the nicotinic response displays a similar voltage dependence to responses at the neuromuscular junction due to an increase in both sodium and potassium conductance. As at the neuromuscular junction, the nicotinic responses increased with hyperpolarization and decreased with depolarization, reflecting an equilibrium potential in the depolarizing direction.

In dissociated NTS neuron, Ueno et al. (37) found only nicotinic receptors. They were found in 30% of NTS neurons and evoked inward currents. But they comment that the dissociated neurons have a cell soma and short dendrites, and therefore muscarinic receptors might be absent as a result of the absence of longer dendrites. On the other hand, as shown in the vagus nerve by Zarbin et al. (41), the muscarinic receptor may undergo axonal transport associated with regulatory proteins during their life cycle. However, we also found nicotinic receptors on only a portion of the mNTS neurons and have no reason to believe that the nicotinic receptors are more localized to the cell body as contrasted to the dendrites. Our observations are more consistent with the conclusion that there are two subgroups of neurons in the mNTS, one with nicotinic receptors and one with muscarinic receptors. With regard to the location of the nicotinic receptor subtypes in the NTS, a recent study suggested that $^{125}$I-labeled α-bungarotoxin binding sites are found almost extensively in the caudal part of the NTS whereas $[^{3}H]$nicotine binding sites are restricted to the more rostral regions of the NTS (18).

It is not clear from these investigations where the cholinergic inputs, whether nicotinic or muscarinic, come from. ACh is widely distributed in the region of the NTS (6, 12), microinjection of ACh into the NTS is known to elicit hypotension and bradycardia (5), and there is considerable indirect evidence for the presence of ACh, choline acetyltransferase, and ACh esterase in the NTS (26, 27). The NTS may be innervated by the axons of the cholinergic interneurons in the NTS (12) or of the dorsal motor vagal nucleus and nucleus ambiguus (7). It is possible that the primary afferent fibers in the ST use glutamate as a neurotransmitter but activate interneurons that use ACh. If this is the case, we would not have studied the cells because the major criterion for inclusion was a monosynaptic excitation on stimulation of the ST. It is also possible that because the cells were found on the basis of their spontaneous discharge, we might select a subpopulation for investigation.

Iontophoretic application of both Glu and ACh caused a strong excitation of the mNTS neurons that was not due to a current artifact. Furthermore, both agents are still effective under circumstances where synaptic transmission is blocked in a low-Ca$^{2+}$ and high-Mg$^{2+}$ medium, indicating that the excitation seen is a direct result of excitatory receptors on the mNTS neurons. These studies were possible because of development of a 400-µm brain stem slice cut semihorizontally and containing the NTS and the area postrema and sufficient length of the ST so as to allow ST activation without direct current spread to the mNTS neurons. The delay between stimulation and neuronal discharge that we have observed was similar to that reported from in vivo systems (5–10 ms) (21). There was significant variation in the synaptic delay even at constant stimulation of the ST, which is undoubtedly due to the fact that all recorded neurons showed spontaneous discharge at a frequency of 1–15 Hz (23). Because this slice is a totally isolated system, this spontaneous discharge must reflect a combination of endogenous pacemaker discharge from somewhere within the brain slice and resulting synaptic interactions. It was previously demonstrated that some brain stem neurons are endogenous pacemakers (4), although the mNTS neurons were not specifically investigated. All of the neurons recorded exhibited spontaneous discharge of a somewhat irregular nature. This discharge must reflect some endogenous pacemaker activity within the brain stem slice, as is known to occur in other brain stem areas (4). The fact that the spontaneous discharge in the mNTS neurons was somewhat irregular is an indication that the endogenous activity is modulated by synaptic input from other neurons. These observations, however, do not necessarily indicate that the pacemaker activity is from the mNTS neurons, because they could be driven synaptically by other neurons in the slice. The observation that curare alone, atropine alone, or curare plus atropine caused a reduction in the rate of spontaneous activity in a portion of the cells indicates that cholinergic synaptic activity contributes to the spontaneous discharge, at least in some cells.
The morphology of neurons monosynaptically activated by ST stimulation and then filled with Lucifer yellow is identical to that of neurons described by Whitehead (40) and Barnes et al. (3). The neurons have two or more long dendrites of more than 150 μm length in parallel with the fibers of the ST and several short dendrites, all with multiple varicosities. We found the dendrite to be considerably more sensitive to application of both ACh and Glu than the cell body. This observation is consistent with the location of the dendrites in close proximity to the ST fibers.

Although these studies did not involve direct activation of the baroreceptor reflex, our observations are relevant to the various points of view regarding the roles of Glu and ACh in the NTS regulation of cardiovascular function. If indeed the NTS is the major first-order synapse of cardiovascular afferents and if these afferents are activated by stimulation of the ST, our evidence supports the conclusions of Talman et al. (34) that the neurotransmitter is Glu, not ACh. Talman et al. (32) presented considerable evidence that excitatory amino acid antagonists, but not cholinergic antagonists, block the baroreceptor reflex. This is consistent with our demonstration that the neurotransmitter released from the ST terminals is Glu. However, we cannot rule out the possibility that some small fraction of afferent input is cholinergic.

In conclusion, by use of a semihorizontal slice of rat brain stem containing the mNTS and the ST we showed that the neurotransmitter released from the ST terminals is Glu acting via non-NMDA receptors. Iontophoretic application of Glu and ACh showed that both are excitatory on almost all identified neurons receiving monosynaptic activation after ST stimulation. The responses to ACh are, however, pharmacologically distinct. Individual neurons exhibited either nicotinic or muscarinic excitatory receptors, but no neuron appeared to have both types. Although there is a large body of evidence indicating a role for ACh in the regulation of the baroreceptor reflex, our observations are consistent with other evidence that Glu serves as the primary neurotransmitter in this pathway, while ACh modulates responses to Glu through both nicotinic and muscarinic receptors (17).

Perspectives

What then is the role of ACh? ACh is known to have a modulating role at many sites in the central nervous system, operating particularly through muscarinic receptors and M channels (1). Muscarinic receptors are concentrated in the NTS (6, 13), but there are also nicotinic receptors widely distributed in the brain stem (6). In some brain stem areas such as the nucleus ambiguus it has been demonstrated that both nicotinic receptor activation causes a depolarization and that it potentiates the action of Glu (39). Thus ACh receptors, both muscarinic and nicotinic, may play a role in the modulation of excitability and of Glu responses even if ACh is not the primary transmitter mediating the baroreceptor reflex. Our iontophoretic results suggest the presence of ACh receptors in the mNTS. These receptors may represent the ST projection that has a response that is masked by a stronger glutamatergic response in our experiments or may represent an alternate pathway that provides cholinergic control of baroreceptor and other reflexes. Such alternate pathways might be extrinsic or intrinsic to the mNTS. Further investigation of these possibilities is warranted.

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