Vagal afferent transmission in the NTS mediating reflex responses of the rat esophagus

WEI YANG LU AND DETLEF BIEGER
Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Canada A1B 3V6

Lu, Wei Yang, and Detlef Bieger. Vagal afferent transmission in the NTS mediating reflex responses of the rat esophagus. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1436–R1445, 1998.—In urethan-anesthetized rats, esophageal distension evoked volume-dependent reflex contractions with phase-locked multiunit discharges in the central subnucleus of the solitary tract complex (NTS C) and the nucleus ambiguous. During blockade of solitarial, but not peripheral, muscarinic cholinoreceptors, the volume-response relationship of reflex contractions was shifted rightward with a depression in pressure wave amplitude. Concurrently, premotor NTS C responses were attenuated and nucleus ambiguous activity was abolished during esophagomotor inhibition. Both NTS C discharges and reflex responses were eliminated, or strongly inhibited, during blockade of excitatory amino acid receptors (EAARs) with 6-cyano-7-nitroquinoxaline-2,3-dione, γ-glutamylglycinor 2-amino-7-phosphonohexanate. In brain stem slice preparations, whole cell recordings in the NTS C region revealed fast excitatory postsynaptic potentials (EPSPs) with spikes in response to electrical stimulation of the solitary tract. Although spiking was facilitated by muscarine, EPSPs were resistant to cholinoreceptor antagonists but sensitive to EAAR blockers. We conclude that esophageal vagal afferents excite ipsilateral NTS C interneurons via activation of glutamate receptors of the δ- or K, α-amin-3-hydroxy-5-methylisoxazole-propionic acid and N-methyl-o-aspartate subtypes. Cholinergic input to the NTS C probably derives from propriobulbar sources and serves to modulate the responsiveness of reflex interneurons.

central subnucleus; nucleus ambiguous; acetylcholine; glutamate; nucleus of the solitary tract

The synaptic transmitter released by esophageal vagal afferents at interneurons of the central subnucleus of the solitary tract complex (NTS C) is yet to be identified. Both muscarinic cholinoreceptors (mACHRs) (26) and N-methyl-o-aspartate (NMDA) subtype glutamate receptors (7) are present in the NTS C and have been shown to mediate discrete esophagomotor responses (14). The intriguing possibility that vagal afferents are cholinergic merits consideration because 1) choline acetyl transferase (ChAT)-immunoreactivity has been demonstrated in nodose ganglionic perikarya of the rat (22) and rabbit (25), 2) the NTS C contains a dense field of ChAT-immunoreactive terminals (24), and 3) activation of mACHRs in the rat NTS C produces patterned esophageal peristalsis (4, 5).

However, the pharmacological evidence available at present is equally compatible with a glutamatergic mechanism, first, because focal stimulation of glutamate receptors in the NTS C produces an esophagomotor response (4) that is blocked by NMDA receptor antagonists (14) and, second, because fictive primary (swallow induced) esophageal peristalsis can be inhibited by the NMDA antagonist dizocilpine (13).

The purpose of the present study was to investigate the involvement of ACh and excitatory amino acids (EAsAs) in esophageal primary afferent transmission at the level of the central subnucleus. In particular, an attempt was made to determine the role of both transmitter substances in the generation of excitatory postsynaptic potentials (EPSPs) evoked by electrical stimulation of solitary tract afferents in a brain stem slice preparation. Parts of the present investigation have been reported in preliminary form (21).

METHODS

General Procedures

In vivo experiments were performed in 41 male Sprague-Dawley rats anesthetized with urethan (1.2 g/kg i.p.). General surgical procedures, intraluminal pressure recording, and techniques used to evoke reflex contractions by stationary intraluminal balloon catheters were identical to those described by Lu and Bieger (20). Briefly, after anesthesia was administered, a tracheal tube was inserted and spontaneous respiratory activity derived from intratracheal tidal pressure fluctuations was continuously recorded. The right jugular vein was cannulated for intravenous infusion of saline and drugs. Rectal temperature was maintained between 37.5 and 38°C.

Esophageal manometry and distension were performed by means of miniature balloon-tipped catheters that were filled with water and connected to pressure transducers. Pressure signals for each transducer were displayed on a polygraph. When positioned in the esophagus, the balloon-tipped catheters were equilibrated with atmospheric pressure for 5–10 s. The balloon for distension was connected in parallel to a 500-μl syringe. Esophageal distension was performed manually or by a variable-speed infusion pump. For observation of responses occurring proximally and distally to the distended segment, the distension balloon was placed in the thoracic segment (9 cm from the upper incisors) with two additional recording balloons being placed 7–8 and 10–11 cm, respectively, from the upper incisors. For investigating proximal responses to distension of the distal esophagus, the distension balloon was positioned distally (11 cm from upper incisors). In most experiments, a recording balloon was placed in the pharynx to monitor pharyngeal responses.

For extracellular recordings of brain stem esophageal neuronal activity, the animals were mounted in a stereotaxic frame after intrasophageal manometric catheters were secured in their appropriate positions. The caudal roof of the fourth ventricle and surrounding structures of dorsal medulla were surgically exposed under a dissection microscope. Cerebrospinal fluid was drained continuously with a wick. Extracellular microelectrodes consisted of a glass micropipette containing a fine carbon fiber and filled with 4 M NaCl. Based on previous work (1, 4, 13, 14), the electrode was stereotaxically inserted into either the medial portion of the NTS or the...
rostral portion of the nucleus ambiguus (AMB) in the medulla oblongata. The electrode was advanced in 50-µm dorsoventral steps with esophageal distension applied between each step. Unit discharges were monitored on an oscilloscope and discriminated by means of a spike-trigger (Neurolog NL200; Digitimer). Discharge frequency was displayed on a Grass-polygraph through a ratemeter along with intraesophageal pressure signals.

Pharmacological Procedures

Drugs were given systemically by bolus injection via the right jugular vein. For local chemostimulation of medullary neurons, aqueous drug solutions were applied by means of a microliter syringe to the extraventricular surface of the NTSC region, including an area 0–100 µm rostral to the cranial edge of the area postrema (AP) and between 500 and 700 µm lateral to the midline. For control, dissolved drugs were applied at adjacent sites on the dorsal surface of the medulla oblongata as follows: 1) the midline at the cranial edge of the AP, 2) 1,150–1,250 µm lateral to the midline at the cranial margin of the AP, 3) 500 µm rostral to the cranial margin of the AP and 800–900 µm lateral to the midline. The volume applied was kept within 0.05 µl to limit spread beyond the targeted region.

In another series of experiments, drugs were pressure ejected in the NTSC or the compact formation of the AMB (AMBc) by means of a three-barrel glass micropipette containing sodium glutamate (0.2 M), 2-amino-5-phosphonoheptanoate (50 mM), or methscopolamine (10 mM). A nitrogen-pressured Picospripter pump (General Valve) allowed for precise delivery of ejection over a range of 20–100 pl. The diameter of the ejected droplet was measured under a microscope at an accuracy of ±10 µm to obtain an estimate of the amount of drug delivered per pulse. As described previously (4, 14, 28), this technique permitted accurate localization of deglutitive neurons in both the intermediate NTSC and rostral AMB region.

Brain Stem Slice Preparation

Sprague-Dawley rats weighing 120–250 g were anesthetized with urethane (0.8–1.0 g ip). The brain was rapidly removed and placed in cooled (1–4°C) oxygenated (95% O2–5% CO2) modified artificial cerebrospinal fluid (aCSF). The lower brain stem was isolated and glued to a mounting block. Horizontal slices of 350–400 µm were cut on a vibratome and continuously bathed in oxygenated modified aCSF at room temperature. The procedure was completed within 10 min. After 40–60 min of recovery at room temperature in modified aCSF, slices were transferred to a submerged type recording chamber and perfused with normal aCSF at a flow rate of 2.0 ml/min and a temperature of 32–34°C. Normal aCSF consisted of (in mM) 126 NaCl, 3 KCl, 2 CaCl2, 2 MgCl2, 1.2 KH2PO4, 26 NaHCO3, and 10 glucose and was continuously bathed in oxygenated modified aCSF at room temperature. The procedure was completed within 10 min. After 40–60 min of recovery at room temperature in modified aCSF, slices were transferred to a submerged type recording chamber and perfused with normal aCSF at a flow rate of 2.0 ml/min and a temperature of 32–34°C. Normal aCSF consisted of (in mM) 126 NaCl, 3 KCl, 2 CaCl2, 2 MgCl2, 1.2 KH2PO4, 26 NaHCO3, and 10 glucose and was continuously bubbled with 95% O2–5% CO2 to maintain a pH of 7.3–7.4. In modified aCSF, NaCl was replaced by isoosmolar sucrose (29).

Recording of EPSPs

The NTSC region was visually identified at ×40 magnification in 32 horizontal slice preparations with reference to previous anatomic studies (1). Whole cell recordings were made in the NTSC region by means of patch pipettes that had resistances measured in aCSF ranging between 8 and 12 MΩ. Patch pipettes contained (in mM) 145 K-gluconate, 2 MgCl2, 5 HEPES, 1,1,1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, 0.1 CaCl2, and 5 K3ATP. A bipolar microelectrode made of sharpened tungsten wires was placed in the solitary tract of the slice. Single monophasic square-wave pulses (0.1 ms, 0.5–30 V) and/or pulse trains (1–5 V, 1–10 Hz, 1–5 s) were delivered to the solitary tract fibers to elicit postsynaptic potentials in NTSC neurons. After stable NTSC EPSPs were obtained, effects of antagonists were examined. Drugs were either pressure ejected in pulses of 50–200 pl from a multibarreled pipette positioned within 100 µm from the recorded cell or applied by bath perfusion. The membrane potential of the neurons was recorded with an Axoclamp II amplifier, displayed and captured on a Nicolet 310 digital oscilloscope, and saved on a computer for offline analysis. Data were averaged and analyzed using a suite of software routines written in Asyst (Asyst Laboratory Technology, Rochester, NY).

Drugs

(±)-Muscarnine chloride, (-)-scopolamine hydrobromide, methscopolamine or (-)-scopolamine methylbromide, gluta-

mate, γ-glutamyl-glycine (γ-DGG), kynurenic acid, (±)-2-

amino-5-phosphonoheptanoic acid (AP-5), and (±)-2-amino-7-

phosphonoheptanoic acid (AP-7) were obtained from Sigma Chemical; 6-cyano-7-nitroquininaline-2,3-dione (CNQX) from Research Biochemical International; and ketamine hydrochlo-

dride from Rogar/STB (London, ON, Canada). The neuronal-
type nicotinic cholinceptor blocker, dihydro-β-erythroidine hydrobromide (D-β-E, Merck Sharp & Dohme Research Laboratory) was generously donated by the manufacturers.

Statistics

Data are presented as means ± SD. Paired t-tests were done with a statistical software (SigmaStat, Jandel Scientific). P < 0.05 was considered to be significant. Unless noted otherwise, all drug effects were replicated at least three times in separate experiments.

RESULTS

Reflex Responses to Esophageal Distension

Effects of ACh receptor blockade. Vagovagal reflex responses evoked by esophageal distension included the following three types described in detail by Lu and Bieger (20): 1) a nonrhythmic monophasic pressure wave (type I) in the lower cervical and thoracic esophagus (Fig. 1), 2) a rhythmic propulsive pressure wave activity in the intercrural (diaphragmatic) portion (Fig. 2), and 3) an "off" or deflation response in the thoracic and the intercrural portion (Fig. 1). Intravenous administration of 0.2 µmol/kg methscopolamine, a blood-

brain barrier (BBB)-impermeant mAChR antagonist, was without effect on type I, type II, or deflation responses (Figs. 1 and 2). However, an equimolar intravenous dose of the BBB-permeant mAChR antagonist scopolamine produced a prolonged inhibition of all responses. The amplitude of the type I activity was reduced from 4.2 ± 0.9 to 0.6 ± 0.3 kPa (n = 12 trials from 4 animals). The off response was abolished in all cases (n = 4). Type II responses were either depressed (n = 9; Fig. 2A3) or completely inhibited even at inflation volumes as high as 160–180 µl (n = 5), equivalent to stimuli four to five times the threshold volume (V1). Responses obtained after scopolamine were evident only at inflation volumes >100 µl and showed a blunted volume dependence (Fig. 2B).

Muscarine 20–35 nmol/kg iv evoked a transient, slow pressure rise (0.2–0.4 kPa) in the cervical, thoracic,
and distal esophagus (n = 3; not shown) that was abolished by methscopolamine 0.2 µmol/kg iv.

Application of either methscopolamine or scopolamine (50–100 pmol in 0.01–0.02 µl) to the extraventricular NTS surface strongly inhibited the type I, type II, and deflation responses. In 2 of 12 methscopolamine-treated animals, type II responses were absent even at high inflation volumes (160–180 µl). In the other 10 animals, an elevation in reflex threshold and reduction in mean amplitude were evident (Fig. 3, A and B). A partial recovery was seen 2–3 h after topical application of methscopolamine. In control tests (n = 3 of each), methscopolamine (100 pmol in 0.02 µl) applied topically to the dorsal surface of the medulla oblongata surrounding the NTS (n = 4) or pressure ejected into the AMBc (n = 3) was without effect. Furthermore, the central nervous system-type nicotinic cholinoceptor antagonist, dihydroβ-erythroidine (0.1 nmol), applied to the extraventricular surface of the NTS (n = 3) was ineffective.

Effects of EAAR blockade. When applied bilaterally to the NTS surface, the competitive kainate/DL-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) subtype glutamate receptor antagonist CNQX (0.2 to 2.0 nmol) reversibly depressed both type I and type II responses (n = 6; not shown).

At doses of 1–2 µmol/kg iv, the noncompetitive NMDA antagonist ketamine induced a complete inhibition of type II responses (n = 7; Fig. 4A1) within 5 min. The response partially recovered in 15–20 min and was fully restored in 50–60 min. After systemic ketamine, occasional buccopharyngeal swallows were observed; however, these lacked an esophageal component (not shown). Application of 100 nmol ketamine to the NTS surface eliminated the type II activity for 30–50 min (n = 4; Fig. 4A2).

Topical application of the competitive NMDA receptor antagonist AP-5 (10–50 nmol) to the NTS surface eliminated both the type I (n = 4) and type II (n = 9; Fig. 4A) activity and the off response (n = 4; Fig. 4B). Inhibition of type II responses reached a maximum within 2–5 min, when contractions failed to be evoked by high volume (>120 µl) inflation. Partial recovery

![Fig. 1. Inhibition of distension-evoked esophageal reflex response by blockade of central muscarinic cholinoceptors (mAChRs). Three balloons are positioned in the cervical (CE), the thoracic (TE), and distal esophagus (DE). During sustained inflation (> at the thoracic level, a high-amplitude pressure wave (type I response) is seen in the inflated segment and low-amplitude pressure waves at CE and DE levels. Deflation (<) triggers a pressure wave (off response) in the distal but not the cervical segment. Reflex responses are not affected by intravenous methscopolamine (MSCP, 0.2 µmol/kg), but inhibited by an equimolar intravenous dose of scopolamine (Scop).](http://ajpregu.physiology.org/Downloadedfrom)
occurred after 15–30 min, full recovery in 30–60 min (Fig. 4, A and B). When 50–100 pmol of AP-5 was unilaterally injected into the region of the NTS C, the threshold of the type II reflex response increased with a concomitant decrease in amplitude of the pressure waves (Fig. 4A3).

Brain Stem Neural Responses Evoked by Esophageal Distension

Effects of ACh receptor blockade in NTS. During inhibition of reflex peristalsis after topical application of methscopolamine (0.1–1.0 nmol) to the ipsilateral NTS surface, distension-evoked burst discharges in both the NTS C and the AMB C were significantly altered. In the NTS C region, both type I (n = 4; Fig. 5A) and type II activity (n = 7; Fig. 5B) persisted, although the peak frequency (F_peak) of burst responses was gradually reduced to 77.27 ± 8.53% of the control (Fig. 5D). The rhythmicity of type II discharges became indistinct (Fig. 5B) as rhythmic esophageal pressure waves disappeared. By contrast, in the AMB C region, evoked unit discharges ceased completely when the esophagomotor response had become undetectable (n = 4; Fig. 5C). Application to the ipsilateral NTS surface of D-β-E (10–20 nmol) did not affect the unit discharges in the NTS C region (n = 3; not shown).

Effects of EAAR blockade in NTS. Both type I (Fig. 6A) and type II (Fig. 6B) discharges in the NTS C were reversibly depressed by the application of CNQX (0.2–2.0 nmol) to the ipsilateral NTS surface. The effect reached a maximum within 5 min, NTS C discharges being completely abolished in three of eight recordings, and in five other cases the F_peak of the discharges was reduced to 5.6 ± 6.58% of the control level. Within 30–40 min, the F_peak of the unit discharges recovered to 93 ± 12% of the control (Fig. 6D1). Applied by the same
route, γ-DGG (20 nmol, n = 2), a nonselective competitive glutamate receptor antagonist, qualitatively produced the same effect as CNQX (not shown), whereas the selective NMDA receptor antagonist AP-7 (5.0–10 nmol) decreased the $F_{\text{Peak}}$ of the evoked NTS C discharges to 15.5 ± 2.5% of the control (n = 4). Recovery to 88 ± 5.5% of the control occurred during the following 30 min (Fig. 6D2). Furthermore, NTS surface application of either CNQX (n = 6; Fig. 6C) or AP-7 (n = 3; not shown) reversibly abolished the ipsilateral AMB$_C$ unit discharges together with reflex esophagomotor output.

**In Vitro Responses in Subnucleus Centralis Region Evoked by Solitary Tract Stimulation**

EPSPs and evoked miniature spikes. Successful recordings were obtained from 87 cells in the NTSC$_C$ region of the horizontal brain stem slice preparation (Fig. 7A). Cells were identified as neurons based on the presence of spikes (spontaneous or evoked by suprathreshold depolarizing current steps). The membrane potential of the neurons, after rupturing the membrane to achieve the whole cell configuration, varied from $-42$ to $-67$ mV ($-52 \pm 8.0$ mV). Most of the neurons with membrane potentials lower than $-50$ mV fired spontaneously with a prominent spike afterhyperpolarization. Thus the membrane potential of the cells was current clamped to $-60$ mV in the bridge mode. Fast EPSPs were evoked in 56 of 87 recorded neurons by stimulation of the solitary tract with a single electrical pulse (0.1 ms). The peak amplitude and duration of the EPSPs varied with the intensity of the stimulation. At intensities of 6–8 V, the neurons produced EPSPs...
(12–25 mV, mean 17.2 ± 3.6 mV, n = 56), some of which had superimposed spikes (Fig. 7B). On stimulation of the solitary tract with pulse trains (2–5 V, 2–10 Hz, 1–5 s), no slow or late EPSPs were observed (n = 17). However, when current clamped at potentials of −45 to −50 mV, two of six neurons produced a group of miniature spikes immediately after the stimulus train.

Effects of muscarinic receptor stimulation or blockade. In 56 neurons that generated a synaptic response to solitary tract stimulation (6–8 V at a holding potential of −60 mV), pulses of muscarine (100 pmol) produced a low-amplitude (3–5 mV), long-lasting (3–10 min) membrane depolarization (28 of 56) or hyperpolarization (4 of 56). Concomitantly, the peak amplitude of the EPSPs increased by 23 ± 11% of the control (n = 32). Furthermore, the number of spikes riding on the EPSP was increased in 11 neurons (Fig. 8A). However, the peak amplitude of the EPSPs in all neurons tested (n = 56) was unaffected by bath perfusion of the slices with methscopolamine (1–20 µM; Fig. 8B).

Effect of EAA antagonists. Bath application of the nonselective EAA receptor antagonist kynurenate (1 mM) eliminated the fast component of the EPSP (n = 9; Fig. 9A2), and the peak amplitude of the EPSP was reduced to 28 ± 12% of the control (Fig. 9B). The effects on EPSPs of bath-applied CNQX (10 µM, n = 5) and γ-DGG (50 µM, n = 2) were qualitatively similar to that of kynurenate. When combined with kynurenate (1 mM), AP-7 (50 µM) further depressed the slow component of the EPSP (Fig. 9A3). The peak amplitude of the EPSPs was reduced to 7.5 ± 2.8% of the control (n = 7; Fig. 9C). The EPSPs recovered after a 15–30 min washout of the antagonist.

DISCUSSION

Sensory input required for the reflex regulation of esophageal motility in all mammals studied to date is thought to be conveyed by vagal afferents (3). In the rat, vagal esophageal afferents terminate massively in the NTSC (1), the hypothesized neural correlate of the medullary pattern generator for esophageal peristalsis (5). The present investigation provides suggestive evidence concerning the nature of excitatory transmission at synapses of vagal esophageal afferents in the subnucleus centralis. Although the pharmacological data presented do not directly identify the vagal afferent transmitter in question, they clearly favor the involvement of glutamate (or a related EAA), rather than ACh.
ous work in this laboratory (14) has shown that the EAAergic input to esophageal loci in the NTS\textsubscript{C} is mediated by different EAA receptor subtypes including AMPA and NMDA.

As expected, NMDA antagonists were effective in blocking esophageal reflex responses. Specifically, our data corroborate the NTS as a major site of action of AP-7 and ketamine. Interestingly, the blocking effect of ketamine was also seen with systemic administration at fairly low dosage. However, the apparent antimuscarinic action of this drug (9, 12) may be a contributing factor, as well as blockade of esophageal motoneuronal NMDA receptors (19, 29). It should be noted that ketamine has been widely employed as a general anesthetic agent for experimental work, including studies of deglutitive function in the rat (17). Because NMDA receptors appear to be involved at different levels of esophageomotor control, ketamine-anesthetized rats would be expected to show impaired peristaltic activity in response to both swallow and balloon distension of the esophagus. Whereas similar caveats may apply to urethan, previous work in the rat from this laboratory has consistently shown that urethan anesthesia enables a propulsive esophageomotor component to be demonstrated during different forms of experimentally induced swallowing (4, 5, 13, 14, 28).

Consistent with previous work (14), AMPA receptors also play a part in generating esophageal premotor activity. The observed blocking effects of CNQX (15) on all types of distension-evoked reflex activity and NTS\textsubscript{C} firing are consistent with an involvement of AMPA receptors in the excitation of esophageal second-order

**Fig. 6.** Effect of glutamate receptor blockade in the NTS on esophageal inflation-evoked neural activity in the NTS\textsubscript{C} and AMB\textsubscript{C}. Topical application of 6-cyano-7-nitroquinolin-2,3-dione (CNQX; 0.2–1.0 nmol) to the NTS surface reduces type I (A) and type II (B) burst discharges in the NTS\textsubscript{C} and completely eliminates the latter in the AMB\textsubscript{C} (C) together with esophageomotor output. Application of (+)-2-amino-7-phosphonohexanoic acid (AP-7; 5.0–10 nmol) to the NTS surface exerts similar effects (D). After CNQX (D1) or AP-7 (D2), the \( F_{\text{peak}} \) of NTS\textsubscript{C} discharges is significantly and reversibly reduced to 5.6\( \pm \) 6.5\% and 12.5\( \pm \) 2.5\% of the control value of 125\( \pm \) 17 Hz (*\( P < 0.05 \)), respectively.
sensory neurons by vagal afferents from the esophagus. As in the case of NMDA blockers, this interpretation hinges on the assumption that NTSC interneurons all project to esophagomotor neurons and do not activate the latter via other oligosynaptic links within the NTS region.

Our in vitro data provide further evidence for an EAAergic vagal input to neurons of the NTSC region. Neurons responsive to solitary tract stimulation invariably showed fast EPSPs. Although these were not tested for sensitivity to CNQX, the synergistic blockade by kynurenic acid and AP-7 (30) supports the hypothesis that both AMPA and NMDA receptors mediate vagal afferent transmission in the NTSC.

Central cholinergic mechanisms. The present study demonstrates that cholinergic transmission in the NTS plays an important part in reflex esophageal peristalsis. As demonstrated previously, intravenous administration of the muscarinic antagonist scopolamine eliminates the esophageal stage of fictive swallowing (4, 28). Conversely, focal stimulation of mAChRs in the NTSC produces rhythmic esophageal peristaltic contractions (4), implicating cholinergic synapses in the NTSC in the generation of esophageal activity. As shown here, all types of reflex peristalsis in the rat esophagus, including the "inflation" and "deflation" response, were sensitive to muscarinic antagonists, provided that the drugs gained access to central cholinergic synapses. Thus extracerebral muscarinic cholinergic receptors, including those in esophageal smooth muscle and intramural ganglia, are not likely to contribute to the esophageal reflexes studied. The central muscarinic receptors appear to operate at the level of NTSC interneurons, rather than AMBc motoneurons or ventral medullary interneurons, because methscopolamine produced its inhibitory effects only on application to the NTS, but not the AMBc. However, the cholinergic synapses in the NTSC do not appear to form an intrinsic link of the esophagomotor reflex arc but instead may modulate the responsiveness of NTSC premotor neurons to afferent inputs from the esophagus.

In humans (23), cats (6), and opossums (18), centrally mediated smooth muscle responses evoked by esophageal distension are atropine sensitive. This effect generally has been attributed to a peripheral mechanism. Arguably, this action of atropine could also involve central muscarinic cholinceptors, because atropine readily crosses the BBB. Although only sketchy evidence is available with regard to striated muscle esophaga-

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**Fig. 7.** Excitatory postsynaptic potentials (EPSPs) evoked by solitary tract (ST) stimulation in the subnucleus centralis region. A: schematic sketch showing horizontal brain stem slice preparation. A bipolar electrode is placed in the ST for electrical stimulation (ES), and whole cell recordings are made in the NTSC region (shaded area). IV, fourth ventricle. B: EPSPs in the same NTSC neuron evoked by single pulse (0.1 ms) stimulation. Weak stimulation evokes a fast EPSP only, whereas more intense stimulation elicits EPSPs with superimposed spikes.

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**Fig. 8.** Muscarinic facilitation of synaptic responses in the NTSC. After a subthreshold pulse (+, ~20 pmol) of muscarine applied to NTSC region, the number and amplitude of spikes superimposed on the EPSP are increased (A). Bath perfusion of MSCP (10 µM) does not affect the EPSP of another cell in the NTSC (B). Intrinsic membrane potential of these two cells is ~52 and ~56 mV. EPSP of both neurons is elicited at a holding potential of ~60 mV (dashed line).
geal peristalsis in the cat (6), the role of central cholinergic mechanisms in esophageal peristalsis needs to be confirmed in other mammals, including humans.

If mAChRs in the NTS region (26) were innervated by vagal afferent fibers, slow long-lasting EPSPs might have occurred in NTSC neurons on solitary tract stimulation. However, in our in vitro studies, most of the recorded neurons showed fast synaptic responses to stimulation of the solitary tract and slow EPSPs were not observed in any of the neurons tested. The fast synaptic responses were methscopolamine resistant. Because nicotinic cholinoceptor blockade was ineffective in vivo, our in vitro results argue against the idea that vagal afferent transmission in the NTSC is mediated by cholinergic fibers, notwithstanding the reported presence of ChAT in nodose ganglionic cells (22, 25). Rather, our data support the alternative explanation that the cholinergic innervation of the NTSC comes from propriobulbar sources, i.e., interneurons of the lower brain stem.

The results of the present experiments further implicate NTSC mAChRs in the control of esophageal peristalsis. Although blockade of mAChRs in vitro did not disrupt synaptic transmission from vagal afferents to NTSC neurons, activation of mAChRs facilitated the production of spikes in these neurons. Furthermore, blockade of NTS cholinergic transmission in vivo resulted in an attenuation of evoked NTSC burst discharges and loss of AMB C motoneuronal output. Thus muscarinic cholinergic input to the NTSC appears necessary for maintaining the excitability of these esophageal premotoneurons at a level appropriate for activating their motoneuronal targets. Blockade of mAChRs moreover resulted in a loss of rhythmicity of NTSC unit discharges when the neuronal activity in the AMB C and the esophagomotor output in response to the esophageal distension had disappeared. A possible explanation for this phenomenon is that loss of motor output resulted in a loss of reafferent feedback, as in the case of neuromuscular paralysis (21), and hence an impaired synchronization of premotor activity.

Other transmitters could act as mediators of vagal esophageal afferents. However, the NTSC region is largely devoid of axon terminals immunoreactive for neuropeptides such as substance P and calcitonin gene-related peptide (unpublished data from this laboratory). As well, the role of nitric oxide in esophageal afferent transmission may merit attention, although our preliminary data suggest that inhibition of nitric oxide synthase in the NTS region does not acutely block rhythmic (type II) esophageal peristalsis.

In summary, 1) the brain stem neuron network controlling esophageal reflex peristalsis depends on cholinergic and glutamatergic neurotransmission in the NTSC region, presumably involving synapses located on esophageal interneurons in the NTSC; 2) vagal afferents from the esophagus employ an EAA rather than ACh to convey excitatory input to the ipsilateral NTSC; and 3) the cholinergic innervation of the NTSC probably originates from propriobulbar sources.

Perspectives

Although Dale (10) dismissed the notion of a cholinergic function in vagal sensory fibers, the issue as such
prompted him to formulate what subsequently became known as his “one neuron–one transmitter” principle. More than one-half a century later, this problem has resurfaced, because cholinergic properties have been ascribed to vagal afferents on the basis of immunohistochemical and nerve cross-suturing studies in rats and rabbits (22, 25). The functional data presented here fail to support a role for cholinergic transmission in vagal afferents from the rat esophagus. Instead, they strongly favor transmission mediated by glutamate or an EAAs. By implication then, cholinergic input to esophageal reflex interneurons may originate from a central source. If borne out by further experiments, the existence of a central cholinergic link forming part of esophageal premotor circuits would strengthen the idea that primary (swallow induced) differs from secondary (bolus induced) peristalsis in terms of being, at least in part, preprogrammed.

Our findings call for further investigations into 1) the identity of cholinergic neurons projecting to reflex interneurons of the rat medulla oblongata, 2) connections between the former and vagal afferent fibers, and 3) the characteristics of EAARs present on these cholinergic premotor neurons.

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Address reprint requests to D. Bieger.

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