Endogenous Inhibition of the Trigeminally Evoked Neurotransmission to Cardiac Vagal Neurons by Muscarinic Acetylcholine Receptors

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Submitted 17 May 2010; accepted in final form 13 August 2010

INTRODUCTION

Activation of the trigeminocardiac reflex by mechanical or electrical stimulation of the trigeminal nerve evokes a pronounced decrease in heart rate, significant fall in blood pressure, and apnea and is among the most powerful autonomic responses. These responses slow the heart rate and reduce myocardial oxygen consumption. Although normally cardio-protective, exaggeration of this reflex can be detrimental and has been implicated in cardiorespiratory diseases, including sudden infant death syndrome (SIDS). An essential component of the diving response and trigeminocardiac reflex is activation of the parasympathetic cardiac vagal neurons (CVNs) in the nucleus ambiguus that control heart rate. This study examined the involvement of cholinergic receptors in trigeminally evoked excitatory postsynaptic currents in CVNs in an in vitro preparation from rats. CVNs were identified using a retrograde tracer injected into the fat pads at the base of the heart. Application of the acetylcholinesterase inhibitor neostigmine significantly decreased the amplitude of glutamatergic neurotransmission to CVNs on stimulation of trigeminal fibers. Whereas nicotine did not have any effect on the glutamatergic responses, the muscarinic acetylcholine receptor (mACHr) agonist bethanechol significantly decreased the excitatory neurotransmission. Atropine, an mACHr antagonist, facilitated these responses indicating this trigeminally evoked brain stem pathway in vitro is endogenously inhibited by mACHRs. Tropicamide, an m4 mACHr antagonist, prevented the inhibitory action of the muscarinic agonist bethanechol. These results indicate that the glutamatergic synaptic neurotransmission in the trigeminally evoked pathway to CVNs is endogenously inhibited in vitro by m4 mACHRs.

Address for reprint requests and other correspondence: D. Mendelowitz, George Washington University, Department of Pharmacology and Physiology, 2300 Eye St NW, Washington, DC 20037 (E-mail: dmendel@gwu.edu).

Gorini C, Philbin K, Bateman R, Mendelowitz D. Endogenous inhibition of the trigeminally evoked neurotransmission to cardiac vagal neurons by muscarinic acetylcholine receptors. J Neurophysiol 104: 1841–1848, 2010. First published August 18, 2010; doi:10.1152/jn.00442.2010. Stimulation of the nasal mucosa by airborne irritants or water evokes a pronounced bradycardia accompanied by peripheral vasconstriction and apnea. The dive response, which includes the trigeminocardiac reflex, is among the most powerful autonomic responses. These responses slow the heart rate and reduce myocardial oxygen consumption. Although normally cardio-protective, exaggeration of this reflex can be detrimental and has been implicated in cardiorespiratory diseases, including sudden infant death syndrome (SIDS). An essential component of the diving response and trigeminocardiac reflex is activation of the parasympathetic cardiac vagal neurons (CVNs) in the nucleus ambiguus that control heart rate. This study examined the involvement of cholinergic receptors in trigeminally evoked excitatory postsynaptic currents in CVNs in an in vitro preparation from rats. CVNs were identified using a retrograde tracer injected into the fat pads at the base of the heart. Application of the acetylcholinesterase inhibitor neostigmine significantly decreased the amplitude of glutamatergic neurotransmission to CVNs on stimulation of trigeminal fibers. Whereas nicotine did not have any effect on the glutamatergic responses, the muscarinic acetylcholine receptor (mACHr) agonist bethanechol significantly decreased the excitatory neurotransmission. Atropine, an mACHr antagonist, facilitated these responses indicating this trigeminally evoked brain stem pathway in vitro is endogenously inhibited by mACHRs. Tropicamide, an m4 mACHr antagonist, prevented the inhibitory action of the muscarinic agonist bethanechol. These results indicate that the glutamatergic synaptic neurotransmission in the trigeminally evoked pathway to CVNs is endogenously inhibited in vitro by m4 mACHRs.

Previous work has shown stimulation of trigeminal sensory fibers elicits a polysynaptic excitatory glutamatergic synaptic neurotransmission to cardiac vagal neurons (CVNs) in the nucleus ambiguus (NA) (Gorini et al. 2009). Furthermore, serotonin (5-HT1A and 5-HT2A/C) receptors are endogenously active in vitro and differentially modulate this pathway in the brain stem (Gorini et al. 2009). However, little is known about the cholinergic modulation of the trigeminally evoked responses in CVNs. The goals of this study were to characterize the role of nicotinic and mACHRs

Goksoy et al. 2002. Activation of the dive response, through stimulation of nasotrigeminal sensory fibers, evokes an increase in parasympathetic cardiac activity and pronounced bradycardia (Elsner et al. 1971; Gandevia et al. 1978).

Although the trigeminocardiac reflex and diving responses are typically protective, exaggeration of the resulting bradycardia could prove detrimental. Sudden infant death syndrome (SIDS) is the leading cause of death in infants ages 1 mo to 1 yr, occurring in 0.3 per 1,000 live births in the United States (Hunt and Hauck 2006). The most prevalent and predictive event in infants monitored for SIDS is bradycardia (Cote et al. 1998). This bradycardia precedes or is accompanied by centrally mediated life-threatening apnea (Fewell et al. 2001; Meny et al. 1994; Nachmanoff et al. 1998; Poets et al. 1999). Although the cause(s) for SIDS remains unknown, studies suggest an exaggeration of parasympathetic control of cardiac function may be involved (Divon et al. 1986; Harper and Bandler 1998; Meny et al. 1994; Schechtman et al. 1992; Spyer and Gilbey 1988). SIDS has also been linked to an abnormal or exacerbated response to trigeminal sensory nerve stimulation (Lobban 1991, 1995; Morpurgo et al. 2004). A recent case study describes an infant who succumbed to SIDS hours after having been given a bath. It was determined SIDS was triggered by temporarily plunging the infant’s head underwater during bathing (Maturri et al. 2005). SIDS has also been associated with both abnormal brain stem serotonergic function and cholinergic dysfunction (Frank et al. 2001; Hunt 2005; Hunt and Hauck 2006; Kinney 2009; Kinney et al. 1995; Panigrahy et al. 2000; Paterson et al. 2006). Interestingly, deficits in cholinergic receptor density, in addition to binding dysfunction of cholinergic receptors, have been implicated as potential risk factors for SIDS (Frank et al. 2001; Kinney et al. 1995; Panigrahy et al. 2000). Infants who succumb to SIDS exhibit a decrease in choline acetyltransferase (ChAT) activity, in addition to a reduction in muscarinic acetylcholine receptor (mACHr) binding capacity (Kubo et al. 1998; Slotkin et al. 1999) and ChAT immunoreactivity (Kalaria et al. 1993; Kinney et al. 1995; Mallard et al. 1999; Sparks and Hunsaker 1991).

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in the trigeminally evoked excitatory neurotransmission to parasympathetic CVNs in the NA.

METHODS

In an initial surgery, 2- to 5-day-old Sprague-Dawley rats were anesthetized with hypothermia, which also decreased heart rate and aided in recovery. A right thoracotomy was performed and 50 μL of 1–5% of the retrograde fluorescent tracer X-rhodamine-5-(and-6)-isothiocyanate (Molecular Probes, Eugene, OR) was injected into the pericardial sac and onto the fat pads at the base of the heart. After 24–48 h of recovery, animals were anesthetized with isoflurane and killed by cervical dislocation, after which the brain tissue was placed in a 4°C physiologic saline solution [containing (in mM) 140 NaCl, 5 KCl, 2 CaCl₂, 5 glucose, and 10 HEPES] bubbled with 100% O₂ (pH 7.4). All animal procedures were performed with the approval of the Animal Care and Use Committee of The George Washington University in accordance with the recommendations of the panel on euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication, Guide for the Care and Use of Laboratory Animals.

The medulla was removed with care to preserve the trigeminal cranial nerve rootlet, mounted on a cutting block, and placed into a vibrating blade microtome (Leica, Nussloch, Germany). A modified horizontal slice (800–900 μm) was obtained to stimulate the trigeminal nerve rootlet and record synaptic responses in CVNs. The tissue was submerged in a recording chamber that allowed perfusion (4 ml/min) above and below the slice with artificial cerebrospinal fluid [ACSF; containing (in mM) 125 NaCl, 3 KCl, 2 CaCl₂, 26 NaHCO₃, 5 glucose, and 10 HEPES], equilibrated with 95% O₂/5% CO₂ (pH 7.4).

CVNs in the NA were identified by the presence of the fluorescent tracer as described previously (Mendelowitz and Kunze 1991). Briefly, slices were viewed with infrared illumination and differential interference optics (Zeiss, Oberkochen, Germany) and under fluorescent illumination with an infrared-sensitive cooled charged-coupled device camera (Photometrics, Tucson, AZ). Neurons that contained the fluorescent tracer were identified by superimposing the fluorescent and infrared images on a video monitor (Sony, Tokyo). Our labeled preparation did not include esophageal motoneurons nor did neurons in the nucleus ambiguus pars compacta contain any significant fluorescent labeling. Patch pipettes (2.5–3.5 MOhm) were visually guided to the surface of individual CVNs using differential interference optics and infrared illumination (Zeiss). CVNs were voltage-clamped at a holding potential of −80 mV. The patch pipettes were filled with a solution that consisted of (in mM) 135 K-glucic acid, 10 HEPES, 10 EGTA, 1 CaCl₂, 1 MgCl₂, and 0.005 QX-314 at a pH of 7.3.

Afferent fibers in the trigeminal nerve were stimulated, 1–2 ms duration, 2-Hz frequency, with a bipolar concentric stimulating electrode (WPI, Sarasota, FL) using a stimulator isolator (AMPI, Jerusalem, Israel). Stimulus intensity was increased in each experiment, ranging from 0.1 to 1 mA until a consistent synaptic pathway was evoked in each CVN during control conditions and then the stimulus parameters were maintained at that intensity and duration throughout the experiment. A series of 10 consecutive stimulations in each neuron was averaged and this mean value from each neuron in the population was then averaged to create a summary of results for each condition. Excitatory events were measured using pClamp 8 software (Molecular Devices, Sunnyvale, CA), which analyzes excitatory postsynaptic current (EPSC) amplitudes as peak currents subtracted from baseline currents. Baseline measurements were taken as an average over 400 ms before the stimulation and the maximum peak amplitude was detected within 20 ms, beginning from stimulation. Results are presented as mean ± SE and statistically compared with a paired Student’s t-test or ANOVA with repeated measures, as appropriate (for significance of difference, P < 0.05). Only one experiment was performed per preparation. Stimulations were withheld for a period of about 5 min between stimulations 10 and 11.

The following pharmacological agents were applied by inclusion in the perfusate: the acetylcholinesterase inhibitor neostigmine (10 μM), the nicotinic acetylcholine receptor agonist nicotine (100 μM), the nicotinic acetylcholine receptor antagonist dihydro-β-erythroidine (DhbE, 100 μM), the mAChR agonist carbamyl-β-methylcholine chloride (bethanechol, 100 μM), and the mAChR antagonist atropine (10 μM). The following subtype-specific mAChR antagonists were applied: m1, pirenzepine dihydrochloride (pirenzepine, 10 μM); m2, 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one (AF-DX, 0.1 μM); m3, p-fluorohexahydro-sila-difenidol hydrochloride (pFHHSiD, 1 μM); and m4, tropicamide (1 μM). All drugs were purchased from Sigma-Aldrich (St. Louis, MO), with the exception of AF-DX and pirenzepine, which were purchased from Ascent Scientific (Princeton, NJ). Subtype-specific mAChR antagonist concentrations were chosen based on the literature. Specifically, in the case of m1 mACHRs, Sun et al. (2009) previously showed that 10 μM is the lowest concentration for which pirenzepine is significantly selective for m1 mAChRs in rat brain stem slices.

RESULTS

To elucidate the role of endogenous acetylcholine synaptic release in trigeminally evoked EPSCs in CVNs the acetylcholinesterase inhibitor neostigmine (10 μM) was included in the perfusate. Peak amplitudes of EPSCs, represented by a ▲, evoked in CVNs on stimulation of trigeminal afferents, were significantly reduced on application of neostigmine by 58.5 ± 3.7% (from −38.1 ± 3.9 to −15.8 ± 2.3 pA, n = 8, P < 0.05) as shown in a typical experiment (Fig. 1A). The summary data from eight experiments are represented in the bar graph (Fig. 1B) and the percentage maximum EPSC amplitude from eight experiments is illustrated in the scatterplot (Fig. 1C), indicating endogenous acetylcholine release inhibits trigeminally evoked responses in CVNs.

To identify whether nicotinic acetylcholine receptors (nAChRs) and/or muscarinic acetylcholine receptors (mAChRs) are involved in the cholinergic modulation of this trigeminally evoked response pathway, the effects of different nAChR and mAChR agonists and antagonists were tested. The mAChR agonist nicotine (100 μM) did not have any significant effect on the excitatory synaptic response in CVNs on trigeminal afferent stimulation (n = 8, P > 0.05). Summary data from eight experiments are shown in the bar graph in Fig. 2B, with a typical experiment illustrated in the traces (Fig. 2A), with trigeminally evoked glutamatergic neurotransmission indicated by a ▲. A scatterplot representing the percentage maximum EPSC amplitude from eight experiments is shown in Fig. 2C. Additionally, application of the nAChR antagonist DhbE (100 μM) also did not have any significant effect on trigeminal-induced EPSCs in CVNs (data not shown; n = 7, P > 0.05).

The broad mAChR agonist bethanechol (100 μM) significantly inhibited the excitatory neurotransmission to CVNs, decreasing peak amplitude from −34.1 ± 9.2 to −15.9 ± 3.7 pA (n = 8, P < 0.05). A typical experiment is shown in the top traces in Fig. 3A, with a ▲ designating the evoked synaptic response and summary data from eight experiments in Fig. 3, B and C. Conversely, the mAChR antagonist atropine (10 μM) was shown to significantly facilitate the EPSC evoked in CVNs on trigeminal afferent stimulation by 33.8 ± 20.9% (from −63.1 ± 27.4 to −84.4 ± 33.1 pA, n = 7, P < 0.05),
providing additional evidence that this pathway is endogenously inhibited in vitro by acetylcholine and, in particular, mAChR activation. A ▲ indicates the peak synaptic event as seen in a typical experiment, illustrated in Fig. 4A, with summary data from seven experiments shown in the bar graph (Fig. 4B) and percentage maximum EPSC amplitude (Fig. 4C).

To identify the specific subtypes of mAChRs involved in the endogenous inhibition, the broad agonist bethanechol (100 μM) was applied in conjunction with subtype-specific mAChR antagonists. Tropicamide (1 μM), an m4 mAChR antagonist, prevented the inhibitory action of bethanechol (100 μM), as shown in a typical experiment (Fig. 5A), with the summary data from eight cells in the bar graph along with the percentage maximum EPSC amplitude from eight cells. The ▲ symbols are representative of trigeminally evoked glutamatergic synaptic neurotransmission to CVNs.

The m1, m2, and m3 mAChR receptor antagonists, pirenzepine (10 μM, n = 8), AF-DX (0.1 μM, n = 7), and pFFHSiD (1 μM, n = 8), respectively, did not significantly alter the synaptic excitatory response in CVNs (P > 0.05). Additionally, the m1, m2, and m3 antagonists did not significantly alter the inhibition of the excitatory glutamatergic response in CVNs evoked by stimulation of trigeminal afferents on the addition of the mAChR agonist bethanechol (100 μM). Figure 5 shows the summary data illustrating the maintained significant decrease in peak amplitude on application of bethanechol with pirenzepine, AF-DX, and pFFHSiD (Fig. 5B). These data indicate that m4 receptors are the most likely mAChRs endogenously modulating the excitatory glutamatergic neurotransmission to CVNs on trigeminal afferent stimulation.

**DISCUSSION**

This study was carried out to elucidate the role of brain stem cholinergic receptors in the trigeminally evoked excitation of CVNs, providing three major conclusions: 1) neostigmine, an acetylcholinesterase inhibitor, significantly inhibited the excitatory glutamatergic trigeminally evoked responses in CVNs; 2) nicotinic acetylcholine receptors do not significantly alter this neurotransmission; and 3) this evoked response in CVNs is endogenously inhibited in vitro by m4 mAChRs.
Cholinergic modulation of brain stem cardiorespiratory network function is complex. Previous work has shown that nAChRs play a major role in brain stem cardiorespiratory function (Dwyer et al. 2008; Huang et al. 2007; Kamendi et al. 2006; Wang et al. 2003). Activation of nAChRs, specifically beta2 nAChRs, at presynaptic terminals by endogenous acetylcholine release facilitates GABAergic inhibitory inputs to CVNs and likely generates respiratory sinus arrhythmia (Evans et al. 2005; Wang et al. 2003). CVNs in the NA receive cholinergic inputs from multiple areas in the brain stem (Bieger 1993; Milner et al. 1989). Release of acetylcholine increases the frequency of glutamatergic synaptic inputs thought to originate from synaptic terminals of nucleus tractus solitarius neurons via activation of presynaptic alpha7-containing nicotinic receptors (Wang et al. 2001). Other sources of cholinergic inputs to CVNs could include the pedunculopontine tegmental nucleus as well as the laterodorsal tegmental nucleus (Jones and Beaudeut 1987; Shiromani et al. 1988), both of which send projections to the cranial nerve nuclei, including the NA. It is also possible that cholinergic inputs to CVNs originate from surrounding cholinergic cells in the NA (Bouairi et al. 2006), lateral tegmentum area (Winzer-Serhan and Leslie 1997), and passing fibers from pontine structures (Jones 1990).

Interestingly, however, our data suggest that nicotinic receptors do not play a significant role in modulation of the trigeminally evoked responses in CVNs because the glutamatergic neurons via activation of presynaptic alpha7-containing nicotinic receptors (Wang et al. 2001). Other sources of cholinergic inputs to CVNs could include the pedunculopontine tegmental nucleus as well as the laterodorsal tegmental nucleus (Jones and Beaudeut 1987; Shiromani et al. 1988), both of which send projections to the cranial nerve nuclei, including the NA. It is also possible that cholinergic inputs to CVNs originate from surrounding cholinergic cells in the NA (Bouairi et al. 2006), lateral tegmentum area (Winzer-Serhan and Leslie 1997), and passing fibers from pontine structures (Jones 1990).

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neurotransmission evoked on trigeminal nerve stimulation did not exhibit any significant change on application of nicotine or the nicotinic receptor antagonist DhβE. Surprisingly, however, the responses evoked by trigeminal afferent stimulation are endogenously modulated by mAChRs. Neostigmine decreased the trigeminally evoked activation of CVNs and, demonstrating a role of muscarinic receptors, the muscarinic receptor antagonist atropine increased the neurotransmission to CVNs and bethanechol, a muscarinic receptor agonist, decreased this response pathway. This modulation is mediated by m4 mAChRs.

Prenatal nicotine exposure is a known cause of change in both nAChR and mAChR function and density and is thought to be one likely cause of SIDS (Campos et al. 2009; Coddou et al. 2009; Fewell et al. 2001; Matturri et al. 2004; Thiriez et al. 2009). Prenatal exposure to nicotine has been shown to exaggerate and alter the GABAergic pathways to CVNs as well as the synaptic pathways to CVNs in response to hypoxia (Huang et al. 2007; Kamendi et al. 2006). In addition to changes in nicotinic receptor function, fetal exposure to nicotine also decreases brain stem mAChR expression and binding in rat neonates through suppression of mRNA expression (Zhu et al. 1998). Reduced cholinergic binding has been suggested to be responsible for the perturbed ventilatory response in sudden infant death (Kinney et al. 1995; Kubo et al. 1998; Matturri et al. 2004). However, this cholinergic receptor irregularity may also be linked to a decrease in choline acetyltransferase (ChAT) activity (Oda 1999). Victims of SIDS have exhibited a reduction in ChAT-immunoreactive neurons in both the hypoglossal and dorsal motor nucleus (Mallard et al. 1999; Oda 1999). A decrease in cholinergic activity in the brain stem would likely lead to a reduction in endogenously active in vitro m4 mAChR activity, causing a disinhibition and an exaggerated trigeminocardiac reflex. Future experiments are necessary.

FIG. 5. Identification of subtype-specific mAChRs involved in this reflex pathway was accomplished through application of specific receptor antagonists along with the broad mAChR agonist bethanechol (100 μM). m4 AChR antagonist, tropicamide (1 μM) significantly inhibited the glutamatergic neurotransmission to CVNs by 26.7 ± 3.1%, reducing peak amplitude from 36.7 ± 4.7 to 26.9 ± 3.7 pA (n = 8, P < 0.05). Tropicamide also blocked the inhibitory effect of the mAChR agonist bethanechol (100 μM) on the evoked excitatory synaptic transmission to CVNs. A typical experiment is shown in top traces and summary data from 8 experiments in the bar graph and scatterplot (A). Application of the m1, m2, and m3 AChR antagonists, pirenzepine dihydrochloride (pirenzepine, 10 μM), 11-[2-(diethylamino)methyl]-1-piperidinyl[acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one (AF-DX, 0.1 μM), and p-fluorohexahydro-sila-difenidol hydrochloride (pFHHSiD, 1 μM), respectively, did not significantly inhibit the excitatory glutamatergic transmission to CVNs on trigeminal afferent stimulation. Additionally, each antagonist did not block the inhibitory effect of bethanechol (100 μM) on the evoked synaptic transmission. The summary results for pirenzepine (n = 8), AF-DX in B (n = 7), and pFHHSiD (n = 8) are shown in B. All evoked synaptic glutamatergic neurotransmission is indicated by a ▲.
to test whether fetal nicotine exposure alters the excitatory glutamatergic neurotransmission to CVNs on trigeminal afferent stimulation via changes in mAChR function and may provide a new mechanism responsible for the exaggeration of the trigeminocardiac reflex that occurs in some victims of SIDS.

In addition to regulatory modulation of CVNs, cholinergic receptors and, in particular mAChRs, also play a major role in sleep-dependent changes. Brain stem cholinergic neurotransmission is an integral component of rapid-eye-movement (REM) sleep generation (Vazquez and Baghdoyan 2004; Verret et al. 2005). Changes in cholinergic receptor activation and potentiation of ethmoidal nerve-evoked respiratory suppression (Dutschmann and Herbert 1999) have been associated with altered infant sleep–wake cycles, which is also associated with SIDS (Datta et al. 2009; Frank et al. 2001; Oda 1999). Activation of sensory trigeminal afferents during REM sleep has been shown to contribute to the REM sleep-associated respiratory failures in SIDS (Dutschmann and Herbert 1999; Gould et al. 1988).

It is likely that muscarinic receptors alter the sleep–wake cycles via alterations in protein kinase A (PKA) activity (Caulfield and Birdsall 1998; Gillin and Sitaram 1984; Wess et al. 2007). mAChRs are G-protein coupled receptors (GPCRs) (Caulfield and Birdsall 1998; Wess et al. 2007), with m4 mAChRs primarily coupling to G/G, type G proteins (Sawaguchi et al. 2002; Wess et al. 2007). Once activated, m4 mAChRs activate a series of second-messenger systems that inhibit PKA and, subsequently, down-regulate cyclic adenosine monophosphate (cAMP) (Caulfield and Birdsall 1998; Datta et al. 2009; Wess et al. 2007). PKA is critical for the generation of REM sleep and regulation of sleep and wakefulness (Datta et al. 2009; Frank et al. 2001). Abnormalities in REM sleep, specifically longer latencies to REM sleep and lower REM sleep amounts (Coons and Guilleminault 1985; Frank et al. 2001; Harper et al. 1983), evoke changes in cardiovascular system activity, including highly variable heart rate (del Bo et al. 1982; George and Kryger 1985; Koehler et al. 1988). Additionally, mAChRs mediate REM sleep as well as parasympathetic cardiovascular regulation via vasoactive neuromodulators (VN) and cAMP production, respectively (Staines 2005, 2006). Changes in cAMP levels have been shown to cause both irregular VN production and VN dysfunction (Staines 2005, 2006). It has also been suggested that VN abnormalities increase expression of N-methyl-D-aspartate receptor mRNA on postganglionic parasympathetic neurons (Robertson et al. 1998). The changes in cAMP levels by endogenous activation of m4 mAChRs by the trigeminocardiac reflex are a possible cellular target for alterations in sleep–wake states that may also increase the risk of SIDS.

In summary, results from this study demonstrate that the trigeminally evoked synaptic responses to CVNs are endogenously inhibited in vitro by mAChRs, specifically m4 AChRs, but are unaffected by nicotinic receptor activation. These results provide a cellular mechanism by which cholinergic dysfunction may play a role in victims of SIDS and describe a novel muscarinic modulation of trigeminally evoked responses in CVNs.

GRANTS
This work was supported by National Heart, Lung, and Blood Institute Grants HL-59895 and HL-72006 to D. Mendelowitz.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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J Neurophysiol Vol 104 October 2010 www.jn.org


