Pharmacology of Nicotinic Receptors in PreBöttinger Complex That Mediate Modulation of Respiratory Pattern

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Shao, Xuesi M., and Jack L. Feldman. Pharmacology of nicotinic receptors in preBöttinger Complex that mediate modulation of respiratory pattern. J Neurophysiol 88: 1851–1858, 2002; 10.1152/jn.00179.2002. Nicotine regulates respiratory pattern by modulating excitatory neurotransmission affecting inspiratory neurons within the preBöttinger Complex (preBötC). The nicotinic acetylcholine receptor (nAChR) subtypes mediating these effects are unknown. Using a medullary slice preparation from neonatal rats, we recorded spontaneous respiratory-related rhythm from the hypoglossal nerve (XIIN) and patch-clamped inspiratory neurons in the preBötC simultaneously. The α7 nAChR antagonists α-bungarotoxin or methyllycaconitine (MLA) had little effect on the actions of low concentrations of nicotine (0.5 μM), which included an increase in respiratory frequency; a decrease in amplitude of XIIn inspiratory bursts; an tonic inward current associated with an increase in membrane noise; an increase in the frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs), and; a decrease in the amplitude of inspiratory drive current in voltage-clamped preBötC inspiratory neurons. These nicotinic actions were completely reversed by dihydro-β-erythroidine (DH-β-E) or hexamethonium and reduced by D-tubocurarine. Comparable concentrations of RJR-2403 (0.5–1 μM), an agonist selective for α4β2 nAChRs, increased respiratory frequency to 186% and decreased the amplitude of XIIn inspiratory bursts to 83% of baseline. In voltage-clamped preBötC inspiratory (including pacemaker) neurons, RJR-2403 induced a tonic inward current of −15.2 pA associated with an increase in membrane noise, increased the frequency to 157% and amplitude to 106% of spontaneous EPSCs, and decreased the amplitude of inspiratory drive current to 80% of baseline. MLA had little effect on RJR-2403 actions, while DH-β-E completely reversed them. These results suggest that the predominant subtype of nAChRs in preBötC in neonatal rats that mediates the modulation of respiratory pattern by low concentrations of nicotine is an α4β2 combination and not an α7 subunit homomer. We do not exclude the possibility that co-assembly of α4β2 with other subunits or other nAChR subtypes are also expressed in preBötC neurons. The parallel changes in the cellular and systems level responses induced by different nicotinic agonists and antagonists support the idea that modulation of excitatory neurotransmission affecting preBötC inspiratory neurons is a mechanism underlyling the cholinergic regulation of respiratory pattern (Shao and Feldman 2001). This study provides a useful model system for evaluating potential therapeutic cholinergic agents for their respiratory effects and side effects.

INTRODUCTION

Nicotinic receptors are targets for the neurotransmitter acetylcholine (ACh) and exogenous cholinergic ligands such as nicotine from cigarette smoke. Nicotonic ACh receptors (nAChRs) are expressed in many brain stem areas involved in cardiorespiratory control (Dominguez del Toro et al. 1994; Wada et al. 1989), and ACh plays a role in cardiovascular and respiratory regulation (Burton et al. 1994; Shao and Feldman 2001; Wang et al. 2001; Weinstock et al. 1981). Nicotine is implicated in various cardiorespiratory disorders. Maternal smoking, a potent source of nicotine in fetal brain, is a major risk factor for sudden infant death syndrome (SIDS) (Klonoff-Cohen et al. 1995; Taylor and Sanderson 1995). Smoking is also a risk factor for sleep-disordered breathing, i.e., sleep apnea (Wetter et al. 1994). Prenatal nicotine exposure delays early postnatal changes in breathing pattern and increases the frequency of apnea in mice (Robinson et al. 2002). Low concentrations of nicotine affect respiratory pattern in vivo (Howell 1995; Stepens and Wilkerson 1998). We have shown that activation of nAChRs in the preBöttinger Complex (preBötC), the hypothesized site for respiratory rhythm generation (Gray et al. 1999; Smith et al. 1991), affects respiratory frequency and pattern in vitro. Activation of nAChRs modulates excitatory neurotransmission by potentiating tonic excitatory input to, and inhibiting excitatory coupling between, preBötC inspiratory neurons (Shao and Feldman 2001). The nicotinic receptor subtypes that mediate these effects are unknown.

nAChRs are ligand-gated ion channels formed as pentameric assemblies of subunits. Ten α (α1–10), four β (β1–4), and γ, δ, and ε subunits have been identified (Lukas et al. 1999; Lustig et al. 2001). Different combinations of these subunits can form functional nAChRs often with distinct pharmacological profiles (Chavez-Noriega et al. 1997; Luetje and Patrick 1991; McGehee and Role 1995). Different subtypes of nAChRs formed from a variety of nicotinic subunit combinations are found in the mammalian CNS (Jones et al. 1999; Klink et al. 2001; le Novere et al. 1999; Zoli et al. 1998). Are these diverse nAChR subtypes physiologically significant? Are different nAChRs linked to different functions? Resolving these questions is an ongoing challenge for the field of nicotinic pharmacology with obvious clinical implications for neurological diseases related to deficit of cholinergic functions (Clementi et al. 2000; Picciotto et al. 2000).

In the ventrolateral medulla (which includes the preBötC), the nAChR subunits α4, α7, and β2 are present (Dominguez del Toro et al. 1994; Wada et al. 1989). The purpose of this study was to identify the subtypes of nAChRs mediating the
effects of nicotine/ACh on central control of respiration. We examined the differential effects of subtype selective nicotinic agonists and antagonists. Pharmacological characterization of these nAChRs may provide a basis for treatment strategies for SIDS and sleep apnea (Gothe et al. 1985) as well as central respiratory failure during organophosphate poisoning due to pesticides or nerve gases, e.g., sarin (Lotti 1991; Rickett et al. 1986). Such characterization may also provide a basis for understanding and ultimately reducing the respiratory side effects of therapeutic use of cholinergic agents for Parkinson’s disease, Alzheimer’s disease, schizophrenia, and algnesia (Jones et al. 1999; Rezvani and Levin 2001; Rusted et al. 2000).

METHODS

Slice preparation

Experiments were performed on a medullary slice preparation that retains functional respiratory networks and generates respiratory rhythm in vitro (Smith et al. 1991). Briefly, Sprague-Dawley neonatal rats (0–3 days old) were anesthetized by hypothermia (incubated on ice for 3–4 min) and then promptly decerebrated. The cerebellum was removed and the brain stem-spinal cord was isolated. The brain stem-spinal cord was mounted in the specimen vise of a Vibratome (VT 100, Technical Products International) oriented vertically with the rostral boundary of preBo at the level of the obex. Coronal sections (0.25 mm thick) were cut. The slice was transferred to a recording chamber of 3-ml volume and stabilized with a threaded frame. The dissection and slicing were performed in an artificial cerebrospinal fluid (ACSF) bubbled with 95% O2–5% CO2 at room temperature. The ACSF contained (in mM) 128 NaCl, 3.0 KCl, 1.5 CaCl2, 1.0 MgSO4, 23.5 NaHCO3, 0.5 NaH2PO4, and 30 glucose. During electrophysiological recording, the slice was continuously superfused (2.5–3.5 ml/min) with ACSF with increased KCl (9 mM) that was recycled into a reservoir equilibrated with 95% O2–5%CO2. The ACSF in the recording chamber was maintained at 27 ± 1°C. All slices studied had rhythmic activities from XIIh that were similar in frequency and in temporal pattern to the respiratory activities recorded from en bloc brain-stem-spinal cord preparations (Smith et al. 1991).

Electrophysiological recording

Neurons within 100 μm of the slice surface were visualized with an infrared-differential interference contrast (IR-DIC) microscope (Axioskop, Zeiss). The respiratory neurons we recorded in this study fired in phase with the inspiratory bursts from XIn and were located ventral to the nucleus ambiguus. Patch electrodes were pulled from thick-wall (0.32 mm) borosilicate glass with tip size of 1–2 μm. The cut ends of XIIh roots were recorded with a suction electrode. The electrode-filling solution contained (in mM) 140 K-glucuronate, 5.0 NaCl, 0.1 CaCl2, 1.1 EGTA, 10 HEPES, and 2.0 ATP (Mg2+–salt), pH adjusted to 7.3 with KOH. Intracellular signals were amplified with a patch-clamp amplifier (AXOPATCH 200A, Axon Instruments, Foster City, CA). A −10-mV junction potential was determined experimentally; reported values of potential are corrected values.

Respiratory-related rhythmic motor activity was recorded from the cut ends of XIIh roots with a suction electrode, amplified 10,000–20,000 times and band-pass filtered (3–3,000 Hz) with an amplifier (GRASS Instruments). Both signals from intracellular recording and from XIIh roots were recorded on video cassettes via pulse code modulation (A. R. Vetter.). Selected segments of intracellular signals were low-pass filtered at 1 kHz (except otherwise stated) with a 8-pole Bessel filter (Frequency Devices) and XIIh nerve activity were rectified and integrated (Paynter filter, τ = 15 ms), then both were digitized at 4,000 Hz sampling frequency with DIGIDATA 1200 and software CLAMPEX 8 (AXON Instruments) on a Pentium-based computer. For measuring the phasic inward current of inspiratory neurons, membrane current signals were filtered at 20 Hz and digitized at a sampling frequency of 100 Hz.

Drug application

Nicotinic agonists or antagonists were applied to the perfusate. For agonists, the baseline was measured immediately prior to the application and the effects were measured 3–5 min after adding them. For antagonists, the effects were measured 4–6 min after adding them. (−)-Nicotine (hydrogen tartrate salt), RIR-2403 (hemigalactarate salt), α-bungarotoxin (α-BgTx), t-tubocurarine chloride (t-TC), Methyllycaconitine citrate, dihydrol-β-erythroidine hydrobromide, hexamethonium chloride (HMT), were obtained from SIGMA/RBI (Sigma-Alrich).

Data analysis

Respiratory periods were averaged from 10 consecutive periods in the baseline or drug application condition for each preparation and were used in statistical tests. Respiratory frequency was taken as reciprocal of period. The inspiratory amplitude of XIIh activity and the phasic inward current amplitude of inspiratory neurons were measured from averaged envelope of 5 consecutive respiratory periods triggered by the up-stroke of the integrated inspiratory XIIh bursts (CLAMPEX 8). Then they were averaged across neurons or preparations and presented as mean ± SD, n = number of cells (for whole cell recording) or preparations (for XIIh motor output recording) is indicated. Usually, we measured electrophysiological parameters before (predrug baseline), during application of agonist and during agonist + antagonist for one neuron or one slice. Repeated-measures ANOVA (Neter et al. 1990) was used to test the statistical significance of the responses. Post hoc multiple comparison analyses in different situations, when necessary, are described in the Figure Legends. The procedure MIXED in the data analysis software package SAS (V8.2, SAS Institute,) was used for these analyses. P < 0.05 was the criterion for statistical significance.

Spontaneous EPSC (sEPSC) data were analyzed with a program written in AXOBASIC (AXON Instruments). This program read the Axon Binary Files (ABF) containing two channels of digitized data: the whole cell patch-clamp signal and the integrated XIIh activity. The program detected sEPSCs during inspiratory periods by setting a threshold for the derivative of the neuronal signal and then measured the time as well as the peak amplitude of sEPSCs. The program ignored the neuronal activity and the time during inspiratory periods, when neurons receive substantial endogenous currents. Statistical significance for difference in rates, i.e., frequency, of sEPSCs was analyzed with a method detailed in Shao and Feldman (2001). Because the amplitude of sEPSCs is not normally distributed, statistical significance for difference in sEPSC amplitude was analyzed with Kolmogorov-Smirnov test (Mini Analysis Program V5, Synaptosoft, GA). Rates and amplitudes of sEPSCs were tested during application of cholinergic agents versus baseline conditions for each neuron.

RESULTS

We examined the effects of the nAChR antagonists on the responses of respiratory-related rhythmic XIIh activity pattern and inspiratory neurons induced by low concentrations of nicotine [0.5 μM, equivalent to the arterial blood nicotine concentration after a cigarette has been smoked (Henningfield et al. 1993)]. The various nicotinic antagonists α-BgTx (0.2 μM, Fig. 1A), MLA (1 μM, Fig. 1B), DH-β-E (0.2 μM, Fig.
were applied immediately after the maximum effects of nicotine were observed, which was usually at 3–5 min (Fig. 2A). To examine the possible confounding effects of nAChR desensitization, we analyzed the time course of the nicotine-induced increase in respiratory frequency. Under baseline conditions, the XIIn burst frequency was 6.64 ± 1.9/min. Within 3–5 min after bath application of nicotine, the frequency increased to 276 ± 56% (n = 18) of baseline; this effect slightly desensitized in the continuous presence of nicotine (Fig. 2A). At about 10 min after application, the frequency decreased to 254 ± 36% of baseline and did not return to the baseline level in the presence of nicotine for as long as 30 min (n = 3). α-BgTx or MLA, potent antagonists for α7 nAChR, had no significant effect on the frequency compared with control group (application of nicotine alone; frequency was measured at the time points equivalent to time points when the antagonist effects were measured following application of antagonists; Fig. 2B). In contrast, either DH-β-E or HMT reduced the frequency close to baseline levels, whereas d-TC induced a partial reduction. The decrease in frequency with DH-β-E, HMT, and d-TC were significant compared with the control desensitization while the decrease with α-BgTx or MLA was not (analyzed by 2-way repeated-measures ANOVA and the post hoc analysis based on Dunnett (SAS Institute, 1999). When we switched the order by adding α-BgTx before adding nicotine in the bath, we got the similar results (n = 2, data not shown).

Under baseline conditions, the amplitude of integrated XIIn inspiratory bursts was 128 ± 82 V. Nicotine (0.5 μM) decreased the amplitude to 78 ± 11% of baseline; DH-β-E, HMT, or d-TC reversed this effect while α-BgTx and MLA did not (Fig. 2C).

In preBötz inspiratory neurons voltage-clamped at −60 mV [n = 15 including 3 pacemaker neurons, a subset of inspiratory neurons which fire ectopic bursts of action potentials during the normally silent expiratory periods if depolarized to −45 to −55 mV, (Smith et al. 1991)], nicotine induced a tonic inward current of −17 ± 13.6 pA associated with an increase in membrane noise (random current fluctuations) and decreased the phasic inspiratory drive current to 64 ± 12% from a baseline level of −63 ± 40 pA. DH-β-E, HMT and d-TC reversed these effects while α-BgTx and MLA did not (Fig. 3,
Fig. 2. Systems level effects of Nic and Antag. A: Nic (0.5 μM) increased respiratory frequency; this effect slightly desensitized. Nic was bath applied at time 0 min and was continuously present for 30 min (n = 3). B: the effects of nicotinic antagonists α-BgTx (n = 4) or MLA (n = 6) on Nic-induced increase in respiratory frequency are not significant compared with control (Ctrl). DH-β-E (n = 4), HMT (n = 5) and n-TC (n = 6) reversed these effects. Control value was taken at about 10 min during bath application of nicotine in the group of control experiment as A without adding antagonist. Ten minutes was equivalent to the time the effects of antagonists were measured (4–6 min after Antag application and Antag was applied 3–5 min after Nic application) in the groups of antagonist experiments. Two-way repeated-measures ANOVA and the post hoc analysis based on Dunnett were used to analyze the change in frequency with antagonists compared with the control desensitization. C: the effects of α-BgTx or MLA on nicotine-induced decrease in amplitude of inspiratory bursts were not significant, while DH-β-E, HMT and n-TC reversed these nicotinic effects. One-way repeated-measures ANOVA for each group and post hoc analysis based on Tukey (SAS Institute 1999) were used. *, statistically significant. The frequency and amplitude data were normalized to baseline values prior to Nic application for each preparation. Raw data (nonnormalized) were used in statistical tests.

A and B). The nicotine-induced tonic inward current appeared to partially recover during MLA application, but this was not statistically significant (Figs. 1 and 3A).

Bath application of nicotine increased the frequency of sEPSC to 158 ± 76.5% from a baseline of 3.12 ± 1.6/s (n = 14) and increased the amplitude of sEPSC to 115 ± 31.6% from a baseline of −20.2 ± 5.4 pA in voltage-clamped inspiratory neurons during expiratory periods. The increase in frequency of sEPSCs was statistically significant in 9 of 14 neurons. Figure 3F summarizes the effects of α-BgTx, MLA, DH-β-E, or HMT on sEPSC frequency for these nine neurons. The sEPSC frequency of all four neurons in the group that antagonist DH-β-E or HMT was applied (filled symbols in Fig. 3F) was significantly decreased by the antagonist; while the frequency of four of the five neurons that antagonist α-BgTx or MLA was applied (open symbols in Fig. 3F) was not significantly decreased by the antagonist. Figure 3D shows a representative neuron in which 10 μM HMT reversed the nicotine-induced increase in spontaneous EPSC amplitude while α-BgTx did not (Fig. 3C). We analyzed the sEPSC amplitude with Kolmogorov-Smirnov test for each neuron. Most neurons (7 of 9) in which the sEPSC frequency was increased by nicotine also exhibited an increase in amplitude. Figure 3E summarizes the effects of α-BgTx, MLA, DH-β-E, or HMT on sEPSC amplitude of the seven neurons with which sEPSC amplitude was increased by nicotine. The sEPSC amplitude of three of four neurons was significantly decreased by the antagonist DH-β-E or MLA (filled symbols in Fig. 3E), while the amplitude of two of three neurons was not significantly decreased by α-BgTx or MLA (open symbols in Fig. 3E).

Bath application of RJR-2403 (0.5–1 μM, comparable to the concentrations of nicotine we used), an agonist selective to αβ2 nAChR (Bencherif et al. 1996), increased respiratory frequency to 186 ± 48% of baseline of 9.3 ± 3.2/min and decreased the amplitude of inspiratory bursts in XIn to 83 ± 13% of baseline level of 107 ± 36 μV (n = 7; Figs. 4, A and B, and 5, A and B). In preBötC inspiratory neurons voltage-clamped at −60 mV (n = 9 including 3 pacemaker neurons), RJR-2403 induced a tonic inward current of −15.2 ± 10.1 pA associated with an increase in membrane noise and decreased the amplitude of phasic inspiratory drive current to 80 ± 9% of baseline −65 ± 42 pA (Figs. 4, A and B, and 6, A and B). The frequency of sEPSCs during expiratory periods was 4.5 ± 4.5/s and the amplitude was −27 ± 12 pA in baseline conditions (n = 4). RJR-2403 increased the frequency of these sEPSCs to 157 ± 54% and the amplitude to 106 ± 25% of baseline (Figs. 4, A and B, and 6C). Statistical analyses were done for frequency and amplitude of sEPSCs (refer to METHODS) for each neuron. The increase in frequency was significant in five of eight neurons and in four of these five neurons; the amplitude of sEPSC was also increased by RJR-2403 (Fig. 6D). The effects of RJR-2403 at both systems and cellular levels were similar to the effects of nicotine, while the changes induced by RJR-2403 were smaller than those induced by nicotine. These effects were reversed by DH-β-E (0.2 μM) but only minimally affected by MLA (1 μM; Figs. 4, A and B, 5, A and B, and 6, A and B). Figure 6, C and D, summarizes the effects of MLA or DH-β-E on RJR-2403-induced changes in sEPSCs. In the five neurons with RJR-2403-induced increase in sEPSC frequency, the frequency was decreased significantly by DH-β-E in two of three neurons; the frequency was not decreased by MLA in one of two neurons. RJR-2403 induced increase in sEPSC amplitude in four neurons, the amplitude was decreased by MLA (n = 2) or by DH-β-E (n = 2).

We observed parallel changes in preBötC inspiratory neurons and in the respiratory-related motor output induced by various nicotinic agonists and antagonists. Whenever a nicotinic agonist induced a tonic inward current associated with an increase of membrane noise, decreased the phasic inspiratory drive current, and increased the frequency and amplitude of sEPSCs during expiratory periods, we concurrently observed an increase in frequency and a decrease in amplitude of respiratory-related rhythmic motor activity in the XIn. When the
Nicotinic agonist-induced responses at the cellular level were reduced by an antagonist, the increase in frequency and decrease in amplitude of the respiratory-related motor activity in the XIIn were concurrently reduced.

**Discussion**

We demonstrated that the pharmacological properties of the nAChR subtypes in the neonatal rat preBöC mediating the modulatory effects of low concentrations of nicotine on respiratory pattern were different from those of α7 subunit-containing receptors. The nicotinic receptor antagonists α-BgtTx or MLA had little effect on the nicotinic actions, which included increasing respiratory frequency, decreasing the amplitude of inspiratory bursts of respiratory-related motor activity from XIIn, inducing a tonic inward current associated with an increase in membrane noise, increasing the frequency and amplitude of sEPSCs during the expiratory period, and decreasing the amplitude of phasic inspiratory drive inward current in preBöC inspiratory neurons. These nicotinic effects were completely reversed by DH-β-E or HMT and reduced by d-TC.

The nicotinic agonist RJR-2403 had effects similar to those of nicotine at both the systems level, i.e., respiratory frequency and pattern of XIIn, and the cellular level. MLA had little effect on the actions of RJR-2403 while DH-β-E completely reversed these nicotine-induced responses. The parallel changes in cellular events in preBöC inspiratory neurons and in the respiratory-related motor output pattern induced by different nicotinic agonists and antagonists support the hypothesis that nicotine/ACh regulate respiratory frequency and pattern by modulating excitatory neurotransmission via an enhancement of the tonic excitatory input to, and an inhibition of the phasic excitatory coupling between, preBöC inspiratory neurons (Shao and Feldman 2001).

**FIG. 3.** Cellular level effects of Nic and Nic Antag in preBöC inspiratory neurons voltage-clamped at −50 mV. The effects of α-BgtTx (n = 4) or MLA (n = 4) on Nic-induced tonic inward current (A) and decrease in amplitude of phasic inspiratory drive current (B) were not significant; DH-β-E (n = 5), HMT (n = 4), and d-TC (n = 4) reversed these nicotine-induced responses. The amplitude of phasic inspiratory drive current was normalized by the values prior to application of nicotine (baseline: BasL). One-way repeated-measures ANOVA for each group and posthoc analysis based on Tukey were used for A and B. *p < 0.05, statistically significant. C: α-BgtTx did not reduce nicotine-induced increase in amplitude of spontaneous EPSCs (sEPSCs). Cumulative (Cumul) histogram from 1 representative neuron. D: HMT reversed the Nic-induced increase in amplitude of sEPSCs. Summaries of the effects of α-BgtTx, MLA, DH-β-E, and HMT on the Nic-induced changes in amplitude (E) and frequency (F) of sEPSCs. sEPSCs were recorded for 1–2 min, and 100–600 events were collected in each condition for each neurons. Each symbol indicates 1 neuron. Refer to RESULTS for statistical analyses for C–F.

**FIG. 4.** Nic agonist RJR-2403 (RJR, 0.5–1 μM) induced responses in inspiratory neurons and pattern of respiratory-related rhythmic activity from XIIn. Nic Antag MLA (1 μM; A) did not, but DH-β-E (0.2 μM; B) did, reverse the RJR-induced responses. Right: low-pass filtered (10 Hz) traces of average of 5 consecutive inspiratory drives of the neurons and integrated inspiratory bursts of XIIn at extended scales. Baseline, RJR and RJR plus Antag conditions and were superimposed. Averages were triggered by the onset of integrated inspiratory bursts from XIIn. The vertical scales represent 40 pA.
related and nonrespiratory neurons can be blocked by DH-
H9252
respiratory pattern is
blocked these responses. These data suggest the nAChR sub-
and partially blocked by D -TC. This antagonist pro-
duced responses at both the cellular and systems levels, but
forms of nicotine
Antagonist profile of nAChRs that mediate the modulatory
effects of nicotine

The predominant forms of functional nAChRs in the brain are
tetrameric heteromeric assemblies of α4 and β2 subunits
and homomorphic assemblies of α7 subunits. The α7 homomeric
nAChR is α-BgTx sensitive and is rapidly desensitized. The
α4β2 heteromer is insensitive to α-BgTx and MLA but sensi-
tive to DH-β-E (Jones et al. 1999). We showed that α-BgTx
and MLA had minimal blocking effects on the nicotine-ind-
duced responses at both the cellular and systems levels, but
these responses were completely blocked by DH-β-E or HMT
and partially blocked by v-TC. This antagonist profile resembles
that seen in rat retinal ganglion cells (Lipton et al. 1987)
and that of α4β2 nAChRs expressed in mouse fibroblasts
(Whiting et al. 1991). Thus our data suggest that the predom-
inate preBoC nAChR involved in respiratory modulation by
low concentrations of nicotine is an assembly of α4 β2 sub-
units and not a homomeric assembly of α7. Our data are
consistent with the observations that the ACh-induced increase
in respiratory frequency in the en bloc brain stem-spiral cord
preparation can be completely abolished by a combination of
DH-β-E and atropine (Murakoshi et al. 1985); the excitatory
effects of iontophoresically applied nicotine on respiratory-
related and nonrespiratory neurons can be blocked by DH-β-E
(Bradley and Lucy 1983); and nAChR subunits α4 and β2 are
present in the reticular formation in ventrolateral medulla
including the preBoC (Wada et al. 1989).

Effects of RJR-2403

The newly developed nicotinic agonist RJR-2403 is selec-
tive for α4β2 nAChR subtypes (Bencherif et al. 1996; Lip-
piello et al. 1996; Papke et al. 2000). Here, RJR-2403 evoked
changes in respiratory-related rhythmic activity pattern and in
preBoC inspiratory neurons similar to those induced by nico-
tine, and MLA had little effect while DH-β-E completely
blocked these responses. These data suggest the nAChR sub-
type mediating the modulatory effects of nicotine/ACh on
respiratory pattern is α4β2. Bencherif et al. (1996) showed that
the potency and efficacy of RJR-2403 in activating rat thalamic
synaptosomes was comparable to nicotine. Papke et al. (2000)
showed that RJR-2403 was more potent and more efficacious
than nicotine in activating human α4β2 nAChR expressed in
 Xenopus oocytes. In this study, the effects induced by RJR-
2403 were smaller than those induced by nicotine (e.g., 186 vs.
276% change in frequency) in a similar concentration range.
There could be two possible explanations for the smaller ef-
effects of RJR-2403: RJR-2403 is a less potent agonist for α4β2
nAChRs in neonatal rat preBoC that are somehow different
from those in adult thalamus and from human α4β2 nAChRs
expressed in oocytes or RJR-2403 is selective for α4β2
nAChR compared with nicotine, but nicotine acts on wider
range of nAChR subtypes in preBoC that may also be in-
volved in modulation of respiratory pattern. We are not able to
exclude the latter possibility because, besides α4β2 and α7,
pairwise combinations of α2-α4 with β2 or β4 as well as some
type subunit combinations such as α3β4β2 and α3β2α5 can
form functional nAChRs when expressed in Xenopus oocytes
or other expression systems (Chavez-Noriega et al. 1997;
Colquhoun and Patrick 1997; Gopalakrishnan et al. 1996;
Luetje and Patrick 1991; McGehee and Role 1995; Papke et al.
2000). Native functional nAChRs containing subunits other
than α4, β2, and α7 are found in a limited number of brain
regions (Klink et al. 2001; le Novère et al. 1999; Zoli et al. 1998) and are much less well characterized.

Functional implications

nAChR subtypes are linked to a wide variety of brain functions such as cognition, addiction, locomotion, and pain sensitivity as well as pathological conditions such as Parkinson’s disease, Alzheimer’s disease, epilepsy, and schizophrenia (Jones et al. 1999; Nomikos et al. 2000; Picciotto et al. 2000). In anesthetized animals in vivo, ACh enhances ventilation; these effects are potentiated by the cholinesterase inhibitor physostigmine (Gesell et al. 1943). Iontophoretic administration of ACh excites some medullary respiratory neurons, while it inhibits or has no effect on others (Böhmer et al. 1987, 1989; Bradley and Lucy 1983; Haji et al. 1996; Jordan and Spyer 1981; Kirsten et al. 1978; Salmoiraghi and Steiner 1963). These studies are difficult to interpret due to the confounding effects of anesthesia, the lack of precise anatomical, and/or physiological characterization of neurons. Using the more reduced medullary slice preparation combined with IR-DIC microscopy, we determined the differential effects of nicotine on the preBoC and the hypoglossal nucleus as well as the underlying cellular mechanisms (Shao and Feldman 2001). In the current study, we demonstrated that α4β2 nAChRs in the preBoC played a role in respiratory modulation. In contrast, Neff et al. (1998) showed that, in a medullary area adjacent to preBoC, nicotine modulated presynaptic glutamate release onto cardiac vagal neurons by actions at α7 containing nAChRs. These results are physiologically significant as two different nAChR subtypes are linked to distinct respiratory and cardiac modulatory functions (although probably not exclusively) in the medulla. Given that many brain stem respiratory-related areas are excluded in our slices, ACh/nicotine could also affect ventilation by acting on neurons in these other respiratory-related areas.

nAChRs are classified by their molecular composition (Lukas et al. 1999). To identify the subunit combinations of native functional nAChRs in the brain has been difficult because of lack of specific agonists or antagonists for most nAChR subtypes. One approach to gain insight into the molecular composition of native nAChRs is to compare their functional and pharmacological profiles with those obtained with recombinant receptors. Although with the available pharmacological tools the precise molecular composition of the preBoC nAChRs mediating the modulatory effects of nicotine on respiratory pattern cannot be unambiguously identified, our pharmacological characterization of these receptors provides useful information relevant for the therapeutic use of nicotinic agents (Gothe et al. 1985; Levin et al. 1999; Rezvani and Levin 2001; Rusted et al. 2000). With the preparation described in this study, the effects of various drugs on respiratory neurons, on neurotransmitter systems in the preBoC, and on respiratory-related motor activity can be easily examined, where the respiratory rhythm generation circuits are highly accessible for pharmacological manipulation. These results provide a basis for the investigation of nicotinic agonists in the treatment of obstructive sleep apnea (Gothe et al. 1985; discussion in Shao and Feldman 2001) and of antagonists for central respiratory failure resulting from nerve gas exposure (Rickett et al. 1986). This preparation can be used to screen for potential respiratory side effects of nicotinic agents developed to treat nonrespiratory neurological disorders, e.g., for Parkinson’s disease or Alzheimer’s disease.

Low concentrations of nicotine enhance tonic excitatory input to and inhibits excitatory coupling between preBoC inspiratory neurons. Based on computational models of respiratory rhythm generation (Butera et al. 1999), these cellular effects of nicotine can account for the cholinergic modulation of respiratory frequency and pattern (Shao and Feldman 2001). Here, we observed that whenever a nicotinic agonist induced a tonic inward current associated with an increase of membrane noise, increased the frequency and amplitude of sEPSCs in the preBoC inspiratory neurons (indicating an enhancement of tonic excitatory input to these neurons) as well as decreased the phasic inspiratory drive current (indicating an inhibition of excitatory coupling between these neurons), we concurrently observed an increase in frequency and a decrease in amplitude of the respiratory-related rhythmic activity in the XIlN (Figs. 4–6). When the nicotinic agonist-induced responses at the cellular level were blocked by an antagonist, the increase in frequency and decrease in amplitude of the respiratory-related motor activity in XIlN were blocked and vice versa (Figs. 1–3). These parallel changes in preBoC inspiratory neurons and in the respiratory motor output induced by various nicotinic agonists and antagonists suggest that the modulation of excitatory neurotransmission affecting preBoC inspiratory neurons is a mechanism underlying the regulation of respiratory frequency and pattern by nicotine/ACh.

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