Expression of functional nicotinic acetylcholine receptors in neuroepithelial bodies of neonatal hamster lung

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Fu, Xiao Wen, Colin A. Nurse, Suzanne M. Farragher, and Ernest Cutz. Expression of functional nicotinic acetylcholine receptors (nAChR) in NEB cells of neonatal hamster lung. Am J Physiol Lung Cell Mol Physiol 285: L1203–L1212, 2003. First published June 20, 2003; 10.1152/ajplung.00105.2003.—Pulmonary neuroepithelial bodies (NEB) are presumed airway chemoreceptors involved in respiratory control, especially in the neonate. Nicotine is known to affect both lung development and control of breathing. We report expression of functional nicotinic acetylcholine receptors (nAChR) in NEB cells of neonatal hamster lung using a combination of morphological and electrophysiological techniques. Nonisotopic in situ hybridization method was used to localize mRNA for the β2-subunit of nAChR in NEB cells. Double-label immunofluorescence confirmed expression of α4-, α7-, and β2-subunits of nAChR in NEB cells. The electrophysiological characteristics of nAChR in NEB cells were studied using the whole cell patch-clamp technique on fresh lung slices. Application of nicotine (~0.1–100 μM) evoked inward currents that were concentration dependent (EC50 = 3.8 μM; Hill coefficient = 1.1). ACh (100 μM) and nicotine (50 μM) produced two types of currents. In most NEB cells, nicotine-induced currents had a single desensitizing component that was blocked by mecamylamine (50 μM) and dihydro-β-erythroidine (50 μM). In some NEB cells, nicotine-induced current had two components, with fast- and slow-desensitizing kinetics. The fast component was selectively blocked by methyllycaconitine (MLA, 10 nM), whereas both components were inhibited by mecamylamine. Choline (0.5 mM) also induced an inward current that was abolished by 10 nM MLA. These studies suggest that NEB cells in neonatal hamster lung express functional heteromeric α3β2, α4β2, and α7 nAChR and that cholinergic mechanisms could modulate NEB chemoreceptor function under normal and pathological conditions.

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proliferation is modulated by a serotonegic autocrine loop, inhibitable by α-bungarotoxin (α-BTXN) (39).

Pulmonary NEB are presumed airway oxygen sensors that may be involved in the autonomic regulation of breathing, especially during the neonatal period (16). Classic NEB cells are composed of innervated clusters of amine (serotonin, 5-HT) and neuropeptide-containing cells that respond to hypoxia by release of 5-HT (21). Studies on NEB cells in both culture and lung slices have shown that these cells express a membrane-delimited O2 sensor and that K+ channel activity plays an important role in hypoxia chemotransduction (20, 44, 47). We report here immunohistochemical, molecular, and electrophysiological evidence that NEB cells express a variety of nAChR composed of various subunits, including α3, α4, α7, and β2, raising the possibility of a heterogeneous population of ion channels. These findings underscore the importance of cholinergic mechanisms in NEB cell function under normal and pathological conditions.

MATERIALS AND METHODS

In all studies, lungs from neonatal (days 1–10) Syrian golden hamsters of both sexes were used. The hamsters were killed by an intraperitoneal Euthanily injection (100 mg/kg; Bimed-MTC, Cambridge, ON, Canada), and the lungs were removed. All experiments were carried out with the approval of the local ethics committee and in accordance with the Institutional Guidelines for Animal Care.

In Situ Hybridization for nAChR β2-Subunit

To cross-identify the cells expressing the mRNA signal and to identify cells expressing the nAChR β2-subunit, we used immunohistochemistry followed by nonisotopic in situ hybridization (NISH). Tissues were fixed in 10% buffered formalin and embedded in paraffin. First, anti-serotonin (5-HT) monoclonal antibody (Sera-Lab, Crawley Down, Sussex, United Kingdom) was used to localize NEB cells. The 5-HT antibody was diluted 1:100, and the reaction was detected with avidin-biotin complex. Subsequently, in situ hybridization was performed on the same sections using digoxigenin-labeled antisense or sense (used as control) RNA probes for the nAChR β2-subunit as described previously (45). The appropriate cRNA probes were generated from a cDNA for rat nAChR β2-subunit (a gift from Dr. J. Boulter, Univ. of California Los Angeles). Briefly, sections of hamster fetal lung were treated with protease VIII to unmask the mRNA signal. Detection of the signal was achieved with DIG Nucleic Acid Detection Kit (Roche Molecular Biochemicals, Boehringer Mannheim, Mannheim, Germany), giving a dark purple color.

Immunofluorescence for nAChR β2-, α7, and α4-Subunits

For immunohistochemical localization of α7 or β2 nAChR subunits in NEB cells, and to differentiate NEB cells from surrounding epithelial cells, we used double-label immunofluorescence on lung tissue fixed in 4% paraformaldehyde in 20% sucrose in PBS for 16 h at 4°C. The sections were washed in PBS before being exposed to blocking solution containing 10% normal goat and rabbit serum albumin in PBS for 30 min at room temperature, followed by overnight incubation at 4°C in a cocktail of the primary antisera. The primary antibodies used included 1) anti-AChR α7 (1:200 dilution), a rabbit polyclonal antibody raised against a recombinant protein corresponding to amino acids 367–502 of human origin (Santa Cruz Biotechnology, Santa Cruz, CA); 2) anti-AChR β2 (C-20; 1:200 dilution), a goat polyclonal antibody raised against a peptide mapping at the COOH terminus of the β2-subunit of nAChR of human origin (Santa Cruz Biotechnology); 3) rat anti-serotonin (5-HT) antibody IgG (1:100 dilution; Medicorp, Montreal, PQ, Canada) as a marker of NEB cells; 4) anti-AChR α4 (1:50 dilution), a rabbit polyclonal antibody raised against a recombinant protein corresponding to amino acids 342–474 of human origin (Santa Cruz Biotechnology); and 5) anti-calcitonin gene-related peptide (CGRP), a rabbit polyclonal antibody (1:200 dilution), against synthetic rat CGRP (Chemicon, Temecula, CA). The secondary antibodies consisted of FITC-conjugated rabbit anti-mouse IgG (1:300 dilution; Dako, Glostrup, Denmark), FITC-conjugated rabbit anti-goat IgG (1:400 dilution; Incstar, Stillwater, MA), and Texas red-conjugated goat anti-rabbit IgG (1:400 dilution; Jackson Immunoresearch Laboratories, West Grove, PA), respectively. Secondary antibodies were diluted in PBS containing 0.7% BSA. Samples were covered with Vectashield Mounting Medium (Vector Laboratories, Burlington, ON, Canada) before being viewed under an Olympus BX60 microscope (Carsen Group). RSimage software (Roper Scientific, Tuson, AZ) was used for image acquisition, and Adobe Photoshop 6.0 software was used to process the images. As a positive control for nAChR β2- and α7-subunits, we used paraffin-embedded sections of rat and hamster brain tissues fixed in 4% paraformaldehyde according to the immunostaining protocol recommended by the manufacturer. For localization of the α4-subunit of nAChR, we used paraffin-embedded sections of tissue fixed in methanol, as recommended by the manufacturer. Lung tissue fixed in methanol was found to be suitable for immunolocalization of CGRP but not for serotonin (unpublished observations). As a positive control, a section of rat brain fixed in methanol was used per the manufacturer’s instructions. As negative controls, the primary antisera were omitted.

Lung Slice Preparation

For electrophysiological studies, the lungs were cut into small pieces and embedded in 2% agarose (FMC Bioproducts, Rockland, ME). Sectioning was performed with tissue immersed in ice-cold Krebs solution that had the following composition (in mM): 140 NaCl, 3 KCl, 1.8 CaCl2, 1 MgCl2, 10 HEPES, and 5 glucose, pH 7.3, adjusted with NaOH (7). Transverse lung slices (~200–300 μm) were cut with a Vibratome (Ted Pella, Redding, CA).

Electrophysiological Techniques and Solutions

For electrophysiological recordings, the lung slices were transferred to a recording chamber mounted on the stage of a Nikon microscope (Optiphoto-2UD; Nikon, Tokyo, Japan). The perfusing Krebs solution had the following composition (in mM): 130 NaCl, 3 KCl, 2.5 CaCl2, 1 MgCl2, 10 NaHCO3, 5 HEPES, and 10 glucose, pH ~7.35–7.4. To identify NEB cells in fresh lung tissue, the slices were incubated with vital dye, neutral red (0.02 mg/ml) for 15 min at 37°C, as previously reported (20). The slices were then incubated in a pipette solution containing 0.1 mM Ca2+; with the following composition (in mM): 130 CsCl, 1 CaCl2, 2 MgCl2, 10 EGTA, 10 HEPES, and 4 MgATP, pH adjusted to 7.2 with CsOH. The chamber, which had a volume of 1 ml, was perfused continuously with Krebs solution at a rate of 6–7 ml/min. All recordings were included.
made from submerged lung slices at a temperature of 30 ± 2°C.

Drugs were applied to the perfusate, and their delivery to the cells was controlled by separate valves. The following drugs used in this study were obtained from Sigma (Oakville, ON, Canada): nicotine, ACh, atropine, choline, mecamylamine, 1,1-dimethyl-4-phenylpiperazinium (DMPP), and hexamethonium. The drugs obtained from RBI (Sigma-Aldrich Canada, Oakville, ON, Canada) included methyllycaconitine (MLA) and dihydroβ-erythroidine (DHβE). Stock solutions (1–10 mM) of all the drugs were prepared on the day of the experiment in twice-distilled water and diluted with Krebs solution to their final concentration before use.

An Axopatch 200B amplifier (Axon Instruments, Foster, CA) was used to record for whole cell currents (voltage clamp) or membrane potential (current clamp). Whole cell patch recordings were performed as described by Hamill et al. (25). The data were filtered at 5 KHz. The level of the fluid over the slices was kept low to minimize stray capacitance. Voltage and current clamp protocols, data acquisition, and analysis were performed using pClamp6 software and DigiData 1200B interface (Axon Instruments). All data values are given as means ± SE.

RESULTS

Localization of nAChR β2-, α7-, and α4-Subunits in NEB Cells

We combined immunostaining for 5-HT using the immunoperoxidase method with NISH to localize the β2-subunit of nAChR in NEB cells. Typically, 5-HT immunoreactive NEB formed small cell clusters localized within the airway mucosa (Fig. 1A, a). After NISH was applied using antisense probe for the β2-subunit of nAChR on the same section, a strong mRNA signal (dark purple) was localized in 5-HT immunoreactive NEB cells (Fig. 1A, b). Although the adjacent airway epithelial cells also expressed a weak signal for mRNA.
for nAChR β2-subunit (light purple signal), there was no 5-HT immunoreactivity in non-NEB cells (Fig. 1A, a).

For localization of the α7-subunit of nAChR in NEB cells, we used a dual-labeling immunohistochemistry method with antibodies against the α7-subunit (Texas red labeled) combined with anti-5-HT antibody (FITC labeled) as a marker of NEB cells. In accordance with a previous report (40), immunostaining for the α7-subunit was localized diffusely in airway epithelial cells (Fig. 1B, a). When the same section was examined for FITC-labeled 5-HT, positive immunoreactivity was restricted to NEB cell clusters (Fig. 1B, b). In merged images (yellow), α7 and 5-HT immunoreactivities were colocalized in NEB cells but not in adjacent epithelium (Fig. 1B, c). We also used a double-immunolabeling method to colocalize the α7- and β2-subunits of nAChR in the same lung tissue sections. Although there was diffuse positive labeling of airway epithelium with individual antibodies against either the α7- or β2-subunit (Fig. 1C, a and b), in merged images, strong colocalization of the two subunits was seen mostly in NEB cells, whereas adjacent epithelium was only weakly positive (Fig. 1C, c). Antibody against the α4-subunit of nAChR gave a positive reaction in NEB cells in lung tissue fixed in methanol but was negative in tissue fixed in 4% paraformaldehyde. Immunostaining for the α4-subunit was strongly positive in NEB cells, whereas adjacent epithelium appeared negative (Fig. 1D, a). We used CGRP as a marker for NEB cells in methanol-fixed tissue since immunostaining for serotonin was negative. Expression of CGRP in NEB cells is shown in Fig. 1D, b, and coexpression of the α4-subunit with CGRP appears as a yellow signal in NEB cell cytoplasm on a merged image (Fig. 1D, c).

**ACh-Induced Inward Current and Depolarization of Membrane Potential in NEB Cells**

The resting membrane potential of neonatal hamster NEB cells ranged from −40 to −60 mV (means = −50.4 ± 2.9 mV; n = 50), and the majority of experiments was performed on those with resting potentials more negative than −45 mV. Application of 100 μM ACh to a NEB cell voltage clamped at −60 mV elicited a transient inward current (Fig. 2A). Evoked current ranged from −100 to 450 pA, and the mean peak ACh-induced inward current (I_{ACh}) was 319.6 ± 90.8 pA (n = 10) at a holding potential of −60 mV. Desensitization of the nAChR is represented by the decay phase of I_{ACh} during prolonged application of the agonist (desensitization was determined by measuring from the peak amplitude of the currents evoked by ACh to after a desensitization of ACh); the decay was fitted with an exponential function, and the mean time constant (τ) was 32 ± 4.3 s (n = 7) (36). Under current clamp, mean ACh-induced (100 μM) depolarization was 10.3 ± 1.2 mV (n = 6; Fig. 2B).

![Fig. 2. Effects of ACh on whole cell current and membrane potential in NEB cells. A: ACh (100 μM) induced a rapid inward current. B: under current clamp, ACh evoked depolarization of membrane potential. Holding potential was −60 mV.](image)

**Effects of Nicotine on Inward Currents and Membrane Potential**

Nicotine activates two classes of currents in NEB cells. Rapid flow tube application of nicotine or other nicotinic agonists to voltage-clamped NEB cells evoked inward currents at negative potentials in 80% of cells studied (n = 135). The responses were usually one of two types (Fig. 3, A and B). In 88% of NEB cells (61 of 70 cells studied), nicotine (50 μM) induced a transient nicotine-induced inward current (I_{nic}) at the holding membrane potential of −60 mV, followed by a rapid desensitization of the response (Fig. 3A). The mean (± SE) peak amplitude of I_{nic} (50 μM) was 370.2 ± 31 pA (n = 25; range −200−600 pA), and τ was 33.2 ± 6 s (n = 15). In 11% of NEB cells (9 of 70 cells studied), a distinctly different response to nicotine (25 and 50 μM) was observed (Fig. 3). These currents had two components showing fast and slow desensitization, respectively. The mean peak inward current amplitude of NEB cells with two components was 412.2 ± 61 pA (n = 6), and τ was 42.2 ± 4 s (n = 6). The peak inward current amplitude evoked by bath application of 50 μM nicotine vs. holding potential is shown in Fig. 3D, based on cells with one desensitizing component. The nicotine-induced currents reversed near 0 mV and showed prominent inward rectification characteristic of neuronal AChR in other cell types (37, 46, 50, 51). Representative examples of the effect of 50 μM nicotine on membrane potential in NEB cells under current clamp conditions is shown in Fig. 3C. Nicotine consistently depolarized NEB cells, from a initial value of −45 ± 3.1 mV to −6.3 ± 2 mV (n = 6).

**Nicotine dose-response relationship.** The concentration dependence of inward currents evoked by bath application of nicotine is shown in Fig. 4A. At a holding potential of −60 mV, rapidly superfused nicotine (~0.1–100 μM) induced concentration-dependent cur-
rents that displayed desensitization in the continued presence of the agonist. The peak currents ranged from 2.8 to 500 pA for cells with a single desensitizing component. To obtain the dose-response relationship, the mean peak current at each nicotine concentration was normalized to that elicited by 50 μM nicotine. The EC₅₀ for receptor activation by nicotine was 3.8 ± 0.1 μM (n = 4), and the Hill coefficient was 1.1 ± 0.05 (n = 4) (Fig. 4B).

**Pharmacological Characterization of nAChR**

*Inward currents evoked by nAChR agonist DMPP.* For NEB cells, the inward rectifying current-voltage relationship obtained for nicotine responses was similar to that reported for other cells (7, 27, 46, 50). We also tested the effects of another selective nAChR agonist DMPP as shown in Fig. 5. Application of 50 μM nicotine evoked an inward current (Fig. 5, A and C), similar to that of 50 μM DMPP applied to the same cell (Fig. 5B). The mean current induced by 50 μM DMPP was -350 ± 17 pA at a holding potential of -60 mV, and τ was 35.4 ± 4 s (n = 8, Fig. 5B).

**Blockade of nicotine-induced currents by nAChR antagonists.** The current response induced by 50 μM nicotine was reversibly suppressed or abolished by 50 μM mecamylamine, an α₃β₂ and α₄β₂ nAChR antagonist (n = 12, Fig. 6, A and B), and by 50 μM DHβE, an α₄β₂ antagonist (n = 7, Fig. 6D) (4). In these experiments, the antagonists were applied to the cell for 5–10 min in Krebs solution, followed by exposure to the antagonist plus 50 μM nicotine. The blockade was reversible after a 10- to 20-min washout of the drug (Fig. 6, C and E). After nAChR expression in *Xenopus* oocytes, 50 μM mecamylamine completely blocked the α₃β₂ nAChR, whereas 5 μM mecamylamine completely blocked those containing α₃β₄-subunits (11). In NEB cells, the mean current induced by 50 μM nicotine was 444.5 ± 120.4 pA (n = 6) under control conditions and 78.2 ± 30.4 pA (n = 6) after 5 μM mecamylamine, corresponding to a reduction of ~82.5%. Application of 50 μM hexamethonium, another nicotinic ganglionic blocker, also reduced this I_sic from 383.5 ± 35 pA (n = 6) to 15.5 ± 5 pA (n = 6, see Fig. 9B), a reduction of ~97%. However, atropine (50 μM) only partially suppressed the nicotine-induced current responses in NEB cells (see Fig. 9B). Similar results were reported in petrosal neurons (50) and CB type I cells (43). In summary, these observations indicate expression of functional nAChR subunits in NEB cells and suggest the involvement of α₃β₂- and possibly α₃β₄-subunits. The nAChR receptors in NEB cells exhibit characteristics of ganglionic nAChR.

**MLA blocks nicotine-induced fast-desensitizing current and choline-induced current.** It has been reported that in cultured rat hippocampal neurons, ACh elicited both a rapidly desensitizing and more slowly desensitizing response. α-BTXN inhibited the fast-desensitizing current, suggesting the involvement of the nAChR α₇-subunit (51). Whole cell patch-clamp studies on cultured human bronchial epithelial cells demonstrated...
nicotine (25 or 50 μM) induced currents with two components consisting of both fast- and slow-desensitizing phases, as discussed above. To test whether or not the α7-subunit may be responsible for the fast-desensitizing current in NEB cells, we applied 10 nM MLA. Interestingly, this drug reversibly inhibited the fast-desensitizing current, whereas the slow-desensitizing current was unaffected (n = 6, Fig. 7, A and B). After a 10-min washout of MLA, 50 μM mecamylamine was found to block both fast- and slow-desensitizing currents induced by 50 μM nicotine (n = 3, Fig. 7, C and D). Application of 100 nM α-BTXN irreversibly blocked nicotine-induced current with fast component in NEB cells (data not shown). In addition, 50 μM DHβE also blocked both fast- and slow-desensitizing currents induced by 50 μM nicotine (data not shown). These findings are similar to those reported for hippocampal neurons (51) and midbrain dopaminergic neurons (24). The amplitude of MLA-sensitive fast current was 298.8 ± 40.2 pA, and τ was 4.8 ± 3.8 s (n = 6). The amplitude of MLA-insensitive slow current (that was blocked by 50 μM mecamylamine) was 111.3 ± 7.8 pA, and τ was 29.8 ± 3.8 s (n = 6). Choline (0.5 mM) induced a fast-desensitizing current in NEB cells (Fig. 8A). The mean amplitude of choline-induced current was 125.2 ± 8.7 pA (n = 11) at a holding potential of −60 mV, and τ was 25.5 ± 7.6 s (n = 11). The choline-induced fast-desensitizing currents were reversibly blocked by 10 nM MLA (Fig. 8, B and C; n = 5). Together, these data suggest that the α7-subunit of nAChR is responsible for the fast-desensitizing current, whereas the α3β2- and α4β2-subunits of nAChR most likely contribute to the slow-desensitizing current. The resulting coexpression of the α7- and β2-subunits raises the possibility of a further heterogeneity among nAChR channels (e.g., homomeric α7 and heteromeric α3β2 channels) expressed in individual NEB cells. Indeed, coexpression of the α7- and β2-subunits was reported recently in rat hippocampal interneurons (28).

A cumulative summary of inward currents in NEB cells induced by ACh, nicotine, DMPP, and choline is shown in Fig. 9A. These findings indicate that NEB cells express functional nAChR. The effects of blockade by selective antagonists on nicotine-induced current in

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**Fig. 4.** Dose-response curve is shown for nAChR on NEB cells. A: whole cell current in response to application of nicotine by perfusion at different concentrations (10, 50, and 100 μM). Holding potential was −60 mV. B: peak currents evoked at different concentrations (0.1, 0.5, 1, 10, 50, and 100 μM) are expressed relative to the peak current evoked by 50 μM of nicotine and plotted against the log [nicotine]; mean response was taken from 5 to 8 cells. Experimental data were fitted by the Hill equation with EC_{50} = 3.7 μM and Hill coefficient = 1.1.

**Fig. 5.** Inward currents were evoked by nAChR agonists. All traces were obtained from the same cell. Drugs were applied as follows. Nicotine, 50 μM (A) with a 120-s recovery period; 50 μM 1,1-dimethyl-4-phenylpiperazinium (DMPP, nAChR agonist) mimicked the nicotine response (B); 120-s recovery period; perfusion of 50 μM nicotine-induced inward current after washout of DMPP (C). Holding potential was −60 mV.
NEB cells are shown in Fig. 9B. Nicotine-induced inward current was reduced 96.1 ± 2% (n = 12) by 50 μM mecamylamine, 95.3 ± 10% (n = 7) by 50 μM DHβE, 86.5 ± 14.1% (n = 7) by 10 nM MLA, 93.5 ± 9% (n = 7) by 50 μM hexamethonium, and 65.7 ± 10% (n = 5) by 20 μM atropine.

DISCUSSION

Our studies demonstrate functional cholinergic mechanisms in NEB cells of neonatal hamster lung. The expression of nAChR subunits in NEB cells was demonstrated by a combination of molecular, immunohistochemical, and functional approaches. First, in situ hybridization experiments indicated that mRNA encoding the β2-subunit is expressed in NEB cells. Second, double-label immunofluorescence demonstrated that NEB cells coexpress proteins recognized by antibodies against α7-, α4-, and β2-subunits of nAChR. Third, whole cell patch-clamp studies on NEB cells demonstrated currents characteristic of nAChR composed of α3β2-, α4β2-, and α7-subunits. The component due to the α7-subunits was fast desensitizing (τ = 4.8 s, n = 6), whereas the component due to the β2-subunits was slow desensitizing (τ = 29.7 s, n = 6). The coexpression of α7- and β2-subunits in NEB cells raises the possibility of a heterogeneous population of channels containing α7 and possibly the combination of α7β2-subunits. It has been well documented that in heterologous expression systems or native tissues, the nAChR α7-subunits may coassemble with other nAChR subunits (28). Under current clamp, application of ACh and nicotine depolarized the membrane potential of the NEB cells. The responses of NEB cells to application of nicotine appear similar to neuronal nicotinic responses (13, 24, 51) since currents could be induced by ACh and nicotine and were sensitive to 50 μM mecamylamine. These responses are mediated by relatively high-affinity receptors, with EC50 ~ 3.8 μM for nicotine, and are inhibited by both mecamylamine and DHβE. The ion-gating properties of nAChR in NEB cells appear similar to those reported for nAChR in glomus cells of rat CB (46), nodose ganglia (12), and hippocampal neurons (5, 6, 28, 51), as well as human bronchial epithelial and endothelial cells that express α2-, α3-, α7-, and β2-subunits (31, 32, 45). With the use of immunohistochemistry and in situ α-BTXN binding, widespread distribution of α7 nAChR was reported in various components of developing lung, including airway epithelial cells, cells surrounding the large airways and blood vessels, alveolar type II cells, free alveolar macrophages, and pulmonary neuroendocrine cells (40). Our studies demonstrate similar expression of nAChR α7-
and β2-subunits in airway epithelial cells in neonatal hamster lung. However, except for NEB cells, we did not observe coexpression of nAChR α7- and β2-subunits in other lung cell types.

The two major subtypes of nAChR in the brain are composed of α4β2- and α7-subunits. The nAChR formed by α7-subunits are known to exhibit several unique properties. For example, homomeric α7 channels are more permeable to Ca\(^{2+}\) and desensitize more rapidly than channels formed by other nAChR subunits (3). It has been suggested that ACh and choline might function as local “cytotransmitters” and modulate cellular functions (45). The α7-subunit of nAChR is a good candidate for mediating long-lasting, “hormonal” functions of ACh, since it can be activated by choline long after cleavage of ACh by acetylcholinesterase, and the resulting change in intracellular Ca\(^{2+}\) could result in a variety of metabolic effects (45). In the central nervous system (CNS), α7-subunits are predominantly postsynaptic, suggesting that they modulate synaptic transmission in addition to their function in signal transduction (49). In embryonic muscle, α7 nAChR appear before the formation of synapses, and therefore, they may be involved in muscle development (19). Multiple functional subtypes of α7-containing nAChR have been reported in rat intracardiac ganglia, superior cervical ganglion neurons (14), and chick sympathetic neurons (46), suggesting the possibility for heteromeric α7-containing nAChR (28). Chronic exposure to nicotine related to the use of tobacco is known to upregulate the number of high-affinity α4β2 nicotine binding sites in the CNS and in heterologous expression systems (10). These findings implicate α4β2 nAChR in nicotine addiction (6, 10). The expression of postsynaptic or pre-
synaptic nAChR $\alpha_2\beta_2$, $\alpha_4\gamma$, and $\alpha_7$-subunits in NEB cells could indicate that ACh functions as an excitatory transmitter modulating the responses of NEB. There is now substantial evidence indicating that NEB function as airway chemoreceptors, possibly involved in the control of breathing (16, 20, 47). NEB possess complex innervation, although there is a considerable species variation. Recent immunohistochemical studies using a combination of confocal microscopy, vagotomy procedures, and neural tracing techniques revealed at least three distinct neural components innervating NEB in rat lungs (2, 8, 9, 42). The major component was represented by vagal afferents that originated in the nodose ganglion (8). The second component comprised CGRP-immunoreactive nerve fibers, originating in the spinal ganglia (8), whereas the third component exhibited immunoreactivity for nitric oxide synthase, with nerve fibers originating within the peribronchial ganglia (9). The evidence for cholinergic mechanisms in NEB and their possible involvement in hypoxia chemotransduction is, at present, mostly indirect. Previous ultrastructural studies on NEB in neonatal rabbit reported the presence of a sparse population of efferent-like (motor) nerve fibers containing small agranular vesicles (29, 30). Because these intracapriscal nerve endings survived after supranodose vagotomy, their origin was considered to be that of side branches of sensory nerve fibers rather than being the endings of separate motor nerve fibers. Histochemical studies demonstrated high levels of acetylcholinesterase in NEB cells of rabbit fetal/neonatal lungs (15). Recent immunohistochemical studies identified vesicular ACh transporter immunoreactivity in nerve fibers in contact with NEB in rat lungs (1). Together, these data support the notion of a cholinergic efferent-like component of NEB innervation involved in axon-like reflex affecting local neuroregulatory mechanisms (29, 30).

The potential role of nAChR in pulmonary pathophysiology is strongly linked to effects of smoking. Of particular relevance to the function of NEB are perinatal pulmonary disorders related to the effects of maternal smoking (35, 38). Recent studies have implicated cholinergic mechanisms in pulmonary development. Chronic exposure to nicotine in utero was reported to significantly increase the number of alveolar type II cells and NEB cells and also to increase expression of $\alpha_7$ nAChR in monkey fetal lungs (39). Thus NEB cells may act as a modulator of cell proliferation under conditions of chronic nicotine exposure. This could be critical during lung development since the release of 5-HT and peptides from NEB cells could affect adjacent bronchovascular structures by targeting airway and/or vascular smooth muscle cells and associated nerve endings (16). Epidemiological studies have identified a close relationship between maternal smoking and sudden infant death syndrome (SIDS) (32). Although the precise mechanism is not known, nicotine, a major component of cigarette smoke, may increase the vulnerability of infants to SIDS via its action on peripheral chemoreceptors. Increased size and number of NEB have been reported in the lungs of SIDS victims born to smoking mothers (17). By analogy with known effects of nicotine on CB (26), the responses of hyperplastic NEB to acute hypoxia may be blunted, making the infants of smoking mothers more susceptible to SIDS. The present study demonstrates expression and functional characterization of nAChR in NEB cells of neonatal hamster lungs, providing a useful model to further study the role of these receptors in NEB function as airway chemoreceptors under normal and pathological conditions.

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DISCLOSURES
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REFERENCES
14. Cueves J, Poth AL, and Berg DK. Two distinct classes of functional α7-containing nicotinic receptor on rat superior cervi-
15. Cutz E and Jackson A. Neuroepithelial bodies as airway
Maternal smoking and pulmonary neuroendocrine cells in sud-
17. Dinger BG, Hirano T, and Fidone SJ. Autoradio- graphic
localization of muscarinic receptors in rabbit carotid body. Brain
18. Fischer U, Reinhardt S, Albuquerque EX, and Maelicke A.
Expression of functional α7 nicotinic acetylcholine receptor dur-
ing mammalian muscle development and denervation. Eur J
19. Fu XW, Nurse CA, Wang YT, and Cutz E. Selective modula-
tion of membrane currents by hypoxia in intact airway chemo-
Paneth cell differentiation and functions. Lab Invest 82: 1647–
1659, 2002.
FJ. Improved patch-clamp techniques for high resolution cur-
cent from cells and cell-free membrane patches. Pflugers Arch
22. Holgert H, Hokfelt T, Hertzberg T, and Lagercantz H. Func-
tional and developmental studies of the peripheral chemo-
receptors in rat: effects of nicotine and possible relationship to
sudden infant death syndrome. Proc Natl Acad Sci USA 92:
23. Ifune CK and Steinbach JH. Rectification of acetylcholine-
elicted currents in PC12 pheochromocytoma cells. Proc Natl
24. Khiroug SS, Harkness PC, Lamb PW, Sudweeks SN, Khir-
oug L, Millar NS, and Takel JL. Rat nicotinic ACh receptor
α7 and β2 subunits co-assemble to form functional heteromeric
25. Lauwereyns JM and Van Lommel A. Effects of various vago-
try procedures on the reaction to hypoxia of rabbit neuroepi-
thelial bodies: modulation by intrapulmonary axon reflexes? Exp
26. Lauwereyns JM, Van Lommel AT, and Dom JR. Innervation of rabbit intrapulmonary neuroepithelial bodies: quantitative and qualitative ultrastructural study after vagotomy. J Neuro-
28. Maus AD, Pereira EF, Karachunski PI, Horton RM, Na-
vaneetham D, Macklin K, Cortes WS, Albuquerque EX, and Conti-
Fine BM. Human and rodent bronchial epithelial cells express functional nicotinic acetylcholine receptors. Mol Pharmaco-
29. Mitchell EA and Milred J. Smoking and sudden infant death
syndrome. In: International Consultation on Environmental To-
acco Smoke and Child Health. Geneva: World Health Organiza-
32. Reh J, Bertrand D, Galizi JL, Devillers-Thiry A, Mule
C, Hussy N, Bertrand S, Ballivet M, and Changeux J-P.
Mutations in the channel domain alter desensitization of a
33. Sargent PB. The diversity of neuronal nicotinic acetylcholine
34. Schuller HM, Jull BA, Sheppard BJ, and Plummer HK.
Interaction of tobacco-specific toxicants with neuronal α7 ni-
ocinic acetylcholine receptor and its associated mitogenic signal
transduction pathway: potential role in lung carcinogenesis and
35. Schuller HM, Plummer HK, and Jull BA. Receptor-mediated
effects of nicotine and its nitrosated derivative NNK on pulmo-
36. Sekhon HS, Jia Y, Raab R, Kuryatov A, Pankow JP, Whit-
sett JA, Lindstrom J, and Spindel ER. Prenatal nicotine
increases pulmonary α7 nicotinic receptor expression and alters
tenal lung development in monkeys. J Clin Invest 103: 637–647,
1999.
37. Shirahata M, Ishizawa Y, Rudisill M, Schofield B, and
Fitzgerald RS. Presence of nicotinic acetylcholine receptors in
38. Van Lommel A, Lauwereyns JM, and Berthoud HR. Pulmo-
nary neuroepithelial bodies are innervated by vagal afferent
nerves: an investigation with in vivo anterograde DiI tracing and
39. Wang D and Cutz E. Simultaneous detection of messenger
ribonucleic acids for bombesin/gastrin-releasing peptide and its
receptor in rat brain by nonradiolabeled double in situ hybrid-
40. Wang D, Youngson C, Wang V, Yeger H, Dinauer MC,
Vega-Saenz ME, Rudy B, and Cutz E. NADPH-oxidase and a
hydrogen peroxide-sensitive K+ channel may function as an
oxygen sensor complex in airway chemoreceptors and small cell
lung carcinoma cell lines. Proc Natl Acad Sci USA 93: 13182–
13187, 1996.
41. Wang Y, Pereira EFR, Maus ADJ, Ostile NS, Navaneetham
D, Lei S, Albuquerque EX, and Conti-Fine BM. Human
bronchial epithelial and endothelial cells express α7 nicotinic
42. Wyat CN and Peers C. Nicotinic acetylcholine receptors in
isolated type I cells of the neonatal rat carotid body. Neuro-
43. Youngson C, Nurse CA, Yeger H, and Cutz E. Oxygen sens-