Increased nicotinic receptor desensitization in hypoglossal motor neurons following chronic developmental nicotine exposure

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Pilarski JQ, Wakefield HE, Fuglevand AJ, Levine RB, Fregosi RF. Increased nicotinic receptor desensitization in hypoglossal motor neurons following chronic developmental nicotine exposure. J Neurophysiol 107: 257–264, 2012. First published October 19, 2011; doi:10.1152/jn.00623.2011.—Neuronal nicotinic acetylcholine receptors (nAChRs) are expressed on hypoglossal motor neurons (XII MNs) that innervate muscles of the tongue. Activation of XII MN nAChRs evokes depolarizing currents, which are important for regulating the size and stiffness of the upper airway. Although data show that chronic developmental nicotine exposure (DNE) blunts cholinergic neurotransmission in the XII motor nucleus, it is unclear how nAChRs are involved. Therefore, XII MN nAChR desensitization and recovery were examined in tissues from DNE or control pups using a medullary slice preparation and tight-seal whole cell patch-clamp recordings. nAChR-mediated inward currents were evoked by brief pressure pulses of nicotine or the α4β2 nAChR agonist RJR-2403. We found that, regardless of treatment, activatable nAChRs underwent desensitization, but, following DNE, nAChRs exhibited increased desensitization and delayed recovery. Similar results were produced using RJR-2403, showing that DNE influences primarily the α4β2 nAChR subtype. These results show that while some nAChRs preserve their responsiveness to acute nicotine following DNE, they more readily desensitize and recover more slowly from the desensitized state. These data provide new evidence that chronic DNE modulates XII MN nAChR function, and suggests an explanation for the association between DNE and the incidence of central and obstructive apneas.

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NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) trace back to the earliest nervous systems (Tsunoyama and Gojobori 1998) and were the first ligand-gated ion channel to be isolated (Langley 1907). Accordingly, membrane properties of nAChRs, especially activation and desensitization, have been extensively studied using receptors expressed at the neuromuscular junction (Colquhoun and Sakmann 1998; Fatt and Katz 1951; Galzi and Changeux 1995; Katz and Thesleff 1957) and in culture (Buisson and Bertrand 2001; Lukas 1991; Peng et al. 1997; Sokolova et al. 2005; Vallejo et al. 2005). Despite their widespread presence in the CNS, much less is known about nAChR behavior in functionally viable brain tissue where the target neurons maintain their in vivo anatomical location, density, number, and subunit composition (but see Calabresi et al. 1989; Guo and Lester 2007; Lester and Dani 1995; Wooltorton et al. 2003). Moreover, we do not know how chronic activation of neuronal nAChRs during embryonic development alters receptor properties, despite substantial data showing an association between DNE and breathing and cardiovascular abnormalities. For example, animals exposed to nicotine in utero have been shown to have reduced ventilatory output (Huang et al. 2004a; St-John and Leiter 1999), altered breathing patterns (Fewell and Smith 1998; Fewell et al. 2001a; Hafstrom et al. 2002a; Huang et al. 2004a), increased apnea frequency (Fewell and Smith 1998; Huang et al. 2004a) and duration (Froen et al. 2002), delayed arousal in response to hypoxia (Hafstrom et al. 2000; Lewis and Bosque 1995), diminished sensitivity to hypoxia (Bamford and Carroll 1999; Bamford et al. 1996; Fewell and Smith 1998; Fewell et al. 2001a and 2001b; Froen et al. 2002; Hafstrom et al. 2005; St-John and Leiter 1999), diminished capacity for autoresuscitation following severe hypoxic exposure (Fewell and Smith 1998; Froen et al. 2000), and altered control of preganglionic parasympathetic neurons innervating the heart (Huang et al. 2006; Huang et al. 2004b; Neff et al. 2004).

In an effort to address these issues, Robinson et al. (2002), Eugenin et al. (2008), Kamendj et al. (2009), Pilarski and Fregosi (2009), and Pilarski et al. (2010) showed that chronic developmental nicotine exposure (DNE) is associated with abnormal nicotinic cholinergic neurotransmission in brainstem respiratory neurons. Yet, it is not clear whether these results are due to agonist-induced nAChR desensitization, secondary effects of nicotine exposure, or both. Nonetheless, the work of others demonstrates that chronic nicotine exposure evokes long-lasting desensitization of nAChRs in many brain regions (reviewed in Gentry and Lukas 2002), suggesting that brainstem respiratory neurons will respond similarly in the face of chronic developmental nicotine exposure.

Thus, we examined the effects of chronic DNE on nAChRs that are expressed on hypoglossal motoneurons (XII MNs) using a medullary slice preparation, and we tested the hypotheses that chronic DNE evokes a more profound desensitization in response to repetitive acute nicotine applications. XII MNs innervate tongue muscles and are important for regulating the size and stiffness of the upper airway (Fregosi et al. 2003; Fuller et al. 1999). XII MN nAChRs come in two main structural forms, the homopentameric α4β2 subtype and the homomeric α7 subtype, and their activation evokes depolarizing currents (Chamberlin et al. 2002; Quitadamo et al. 2005; Zaninetti et al. 1999). nAChRs found on rodent XII MNs also exhibit classical, acute receptor desensitization, which has been observed as a blunting of the whole cell current response following pretreatment with nicotine (Quitadamo et al. 2005). Our results show that XII MNs from DNE animals indeed exhibit increased desensitization and a slower recovery from...
the desensitized state. We also show that α4β2 nAChR subtypes contribute to the observed nAChR desensitization hypersensitivity following DNE. These results provide new evidence about the association between DNE, XII MNs, and breathing-related problems during early life and suggest that alterations in nAChR function are a contributing factor.

MATERIALS AND METHODS

Animals and developmental nicotine exposure. Studies were done using neonatal rats that were exposed to either saline or nicotine throughout gestation, and for the first week of life (via breast milk), as described previously (Fregosi and Pilarski 2008; Pilarski and Fregosi 2009; Pilarski et al. 2010). We studied neonates of either sex that ranged in developmental age from 1 to 4 postnatal (P) days (i.e., P1-P4) with a body weight range of 7–12 g. Wherever possible we minimized the number of animals necessary to assess treatment effects. All procedures were approved and in accordance with guidelines provided by the Institutional Animal Care and Use Committee at the University of Arizona. DNE was achieved by subcutaneous implantation of an Alzet 1007D mini-osmotic pump (Alzet, CA) into the pregnant dams. Implantation was performed on gestational day 5 under aseptic conditions, as described previously (Fregosi et al. 2004; Fregosi and Pilarski 2008; Huang et al. 2004a; Luo et al. 2007). This nicotine dose (6 mg·kg⁻¹·day⁻¹) has no effect on body weight in age-matched neonates (Fregosi and Pilarski 2008).

Brain slice preparation. Transverse medullary slices are a widely accepted experimental model for understanding breathing control (for review, see Rekling and Feldman 1998) and were produced as described previously (Pilarski et al. 2010). Briefly, the brainstem was isolated and sectioned in a rostral to caudal orientation in ice-cold artificial cerebral spinal fluid (aCSF). Upon visualization of the appropriate brainstem landmarks, a single 500- to 600-μm slice that included the semi-compact division of the nucleus ambiguous, the preBötzinger Complex, the rostral portion of the hypoglossal (XII) motor nucleus, XII premotor neurons, and XII nerve tracts and their ventral rootlets were collected (Fig. 1A). After incubation for a minimum of 15 min in a holding bath containing aCSF, the slice was placed into a 1-ml fixed-stage recording chamber for another 15–30 min. The chamber was continuously superfused at 2–3 ml/min with thermoregulated (27°C) aCSF, gassed with 95% O₂ and 5% CO₂ (pH 7.3–7.4). The aCSF contained (in mM): 124 NaCl, 5 KCl, 2.4 CaCl₂, 1.3 MgSO₄, 26 NaCO₃, 1.2 KH₂PO₄, 3 0 D-glucose, with osmolarity adjusted to 300–325 mOsm.

Electrophysiology. Individual XII MNs (Fig. 1B) were visualized using an Olympus BX-50WI fixed-stage microscope (40× objective) equipped with differential interference contrast optics and an infrared video camera (C25400-07; Hamamatsu, Schüpfen, Switzerland). Whole cell recordings of XII MNs were made with glass pipettes (3–5 MΩ) pulled from thick-walled borosilicate glass capillary tubes (OD 1.5 mm, ID 0.75 mm; Sutter Instruments). The patch pipettes were loaded with a solution containing (in mM): 140 K-gluconate, 5 NaCl, 0.1 CaCl₂, 1.1 EGTA, 10 HEPES, 2 ATP (Mg²⁺ salt), with pH adjusted to 7.2 and osmolarity adjusted to 250–275 mOsm. For experiments using a specific nAChR subtype agonist (described below), a Cs-based intracellular solution was used, which included (in mM): 130 CsCl, 5 NaCl, 2 MgCl₂, 1 CaCl₂, 10 HEPES, 2 ATP-Mg, 2 sucrose, with pH adjusted to 7.2 using CsOH.

Continuous bath application of nicotine (0.5 μM) for 15 min reduces the response to pressure pulses (20 psi) of nicotine (2 mM), documenting desensitization of the nAChRs. Note that the nAChR-mediated inward current amplitude slowly recovers during the washout period. Arrows indicate pulse timing. Horizontal white bar indicates normal aCSF and the black bar indicates aCSF + nicotine (0.5 μM). The pressure pipette was positioned 10 μm from the XII MN. psi, pounds per square inch; V-clamp, voltage clamp.

Fig. 1. A: cartoon of the medullary slice preparation and experimental configuration showing the rostral surface, including the XII motor nucleus, the spinal tract of the trigeminal nerve (5SP), preBotzinger complex (preBotC), nucleus ambiguous (NA), inferior olivary nucleus (IO), and the intermediate reticular formation (IRt). B: high resolution image (×40) of an isolated XII MN along with a recording pipette and a pressure ejection “puffing” pipette. C: nicotine-activated inward current showing nAChR desensitization current fade during a 1-s pressure pulse. D: example of slow nicotinic acetylcholine receptor (nAChR) desensitization. Continuous bath application of nicotine (0.5 μM) for 15 min reduces the response to pressure pulses (20 psi) of nicotine (2 mM), documenting desensitization of the nAChRs. Note that the nAChR-mediated inward current amplitude slowly recovers during the washout period. Arrows indicate pulse timing. Horizontal white bar indicates normal aCSF and the black bar indicates aCSF + nicotine (0.5 μM). The pressure pipette was positioned 10 μm from the XII MN. psi, pounds per square inch; V-clamp, voltage clamp.
and osmolarity adjusted to 250–275 mOsm. Cells were voltage clamped at −70 mV, and series resistance (R_s) was monitored regularly without applying compensation. Cells exhibiting R_s > 10% of the whole cell input resistance (R_in) were not studied.

Drugs and protocols. Drugs were obtained from Sigma (St. Louis, MO) or Tocris Bioscience (Ellisville, MO) and mixed daily from stock solutions. To study nAChR currents in isolation, the superfusate always contained tetrodotoxin (1 μM). We also added bicuculline methiodide (10 μM) and strychnine (0.4 μM) to block gamma-aminobutyric acid-A receptors (GABA_A) and glycine-mediated inhibitory neurotransmission, and (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801; 1 μM) and 6-cyano-7-nitroquinoline-2,3-dione (10 μM) to block glutamatergic neurotransmission. From a holding potential of −70 mV, we used pressure pulses (Picospritzer II) to deliver nAChR agonists to XII MNs as described by others (Chamberlin et al. 2002; Quitadamo et al. 2005; Zaninetti et al. 1999). The pressure pulse delivered either the broad nAChR agonist nicotine bitartate (2 mM) or the nicotinic receptor ε4β2 subtype agonist [E]-N-methyl-4-[3-pyridinyl]-3-buten-1-amine monofumarate or RJR-2403 (300 μM). Nicotine was used instead of acetylcholine to target the nAChR without simultaneously activating muscarinic AChRs. Compounds used for pressure ejection were dissolved in the external recording solution, loaded into a pipette that was identical to a patch pipette, and positioned between 50 and 200 μm from the XII MN of interest. When testing for nAChR desensitization and recovery, agonists were pressure injected at predetermined interpulse intervals, for example, at 5 s, 30 s, 60 s, 120 s, and 240 s following a control pulse. Although the drug concentration, pressure, and pulse duration remained constant for each experiment, the distance from the MN soma to the pressure pipette varied for each cell examined.

Last, one or all of the following controls were employed for each cell studied: 1) In 12 cells, nicotine-evoked currents were fully suppressed by nAChR antagonists dihydro-β-erythroidine hydrobromide (DH-β-E; 0.2 μM) and/or mecamylamine (1 μM), as described previously (Chamberlin et al. 2002; Quitadamo et al. 2005); 2) In all 45 cells, nicotine-mediated currents were eliminated at a holding potential near 0 mV, the approximate reversal potential for these nonspecific cation channels (Sokolova et al. 2005); 3) In seven cells, we used a 1-s pulse of nicotine to produce a sustained plateau current, which eventually desensitized and decayed back to baseline (Fig. 1C) (Di Angelantonio and Nistri 2001; Quitadamo et al. 2005); 4) In three cells, nicotine (0.5 μM) was added to the superfusate for ≥15 min (n = 3) to evoke nAChR desensitization, which was documented by showing that the acute nicotine-evoked current evoked by pressure pulses was reduced (Fig. 1D) (Quitadamo et al. 2005; Wooltorton et al. 2003). After agonists or blockers were added to the bath, only a single XII MN from each slice was studied to avoid pharmacological problems associated with drug washout and/or receptor sensitization.

Data analysis and statistics. Current and voltage signals were amplified and acquired as previously described (Piakersi et al. 2010). R_in was calculated for each cell by measuring the current response evoked by rapid 10-mV depolarizing steps from a holding potential of −70 mV. Inward currents from XII MNs were analyzed with Clampfit (Molecular Devices) and using a custom software program written with Spike2 software (CED, Cambridge, UK). Recovery from desensitization was quantified as the ratio of the peak amplitude for nicotine-evoked responses 2–5, each divided by the first response (see Figs. 2 and 3). The recovery time course was calculated as the time constant (τ) of a single parameter exponential equation (Sigma Plot, Systat Software). For statistical analysis, data were subjected to unpaired Student’s t-tests or ANOVA (main effects: time and treatment), followed by Tukey’s post hoc analysis when significant differences were found (JMP software; SAS, Cary, NC). All data are presented as means ± SE. Data are considered statistically significant if P ≤ 0.05.

RESULTS

Properties of synaptically isolated XII MNs. We studied a total of 76 XII MNs from neonatal Sprague-Dawley rats of either sex, from their first day through the first 4 days of life, i.e., postnatal day 1 through postnatal day 4. Neither sex nor age had any impact on the measured variables, so cells were combined across age and gender, leaving DNE or saline exposure as the only comparison. XII MNs were large (20–60 μm) and were localized in thick transverse brainstem slices by their anatomy, location, and physiology, as previously reported (Fig. 1, A and B) (Funk et al. 1993; Piakersi et al. 2010; Tan et al. 2010). In these thick slices, XII MNs receive rhythm
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Fig. 3. DNE affects desensitization and recovery of α4β2 nAChR currents. A: examples of inward currents induced by pressure pulses of the α4β2 agonist RJR-2403 (300 μM; 20 ms) in CON and DNE animals during a 4-min pressure-pulse protocol. Pressure pulses occurred at 5 s, 30 s, 60 s, 120 s, and 240 s following a control pulse at time 0. B: RJR-2403-induced currents are almost abolished by the α4β2 antagonist DH-β-E (0.1 μM) delivered to the bath superfuse (n = 3). C: summary data showing DNE-induced differences in the desensitization and recovery of α4β2 nAChR-mediated peak currents. All data are means ± SE. *P < 0.05. D: quantification of time-dependent nAChR recovery using a single exponential fit to the average data shown in C. DNE greatly slows α4β2 nAChR recovery (n = 5, CON; n = 7, DNE).

DISCUSSION

Our goal was to better understand how chronic DNE alters the functional expression of nAChRs found on XII MNs that control muscles of the tongue. DNE is a powerful model to study the consequences of persistent in utero and early postnatal activation of nAChRs, which are both critical periods for nAChR maturation in the brain (for review, see Dwyer et al. 2008). Importantly, chronic DNE has been linked to deficits in respiratory oscillations that originate in the preBötzinger complex (Smith et al. 1991). XII MNs were isolated from synaptic input as described above. Of the 76 XII MNs studied, 59% or 45 cells (22 control and 23 DNE) responded to short (20–200 ms) pulses of nicotine (2 mM) with a sharp inward current. The average peak current amplitude was 131 ± 52 pA in control animals and 146 ± 58 pA in DNE preparations. These values are not different (P > 0.8), suggesting that the effective dose was similar for control and DNE cells. The rest of the motoneurons were unresponsive to nicotine application. XII MNs was similar for control and DNE cells. The duration of the interpulse interval was increased, the peak current amplitude progressively recovered. Figure 2, A and B, show that both treatment groups displayed significant desensitization at interpulse intervals ranging from 5 to 120 s (P < 0.01), but that cells from both treatment groups also fully recover from desensitization if 120 s or longer is allowed to elapse between pulses of nicotine. However, the magnitude of nAChR desensitization was greater in XII MNs from DNE compared with control animals at 5 s (P < 0.04) and 30 s (P < 0.03) (Fig. 2B). Furthermore, the rate of recovery from desensitization was also slowed by DNE compared with controls as the recovery time constant (τ) for control cells was 42 s vs. 82 s for DNE cells (Fig. 2C).

We also tested the hypothesis that XII MN nAChR desensitization is mediated by the α4β2 nAChR subtype, which is broadly expressed on XII MNs (Chamberlin et al. 2002; Quitadamo et al. 2005; Zaninetti et al. 1999). In the present study, 12 out of 14 XII MNs (86%), regardless of treatment, responded to the α4β2 agonist RJR-2403 with a small, rapid inward current (Fig. 3A), which was nearly abolished by the α4β2 antagonist DH-β-E (0.2 μM; Fig. 3B). Using the same protocol presented in Fig. 2, we quantified the degree of time-dependent α4β2 nAChR subtype desensitization in each treatment group by administering an initial conditioning pulse of RJR-2403 (300 μM), followed by additional pulses at successively longer interpulse intervals (Fig. 3A). Figure 3C shows that both treatment groups displayed significant desensitization at interpulse intervals up through 120 s (P < 0.0001, but that cells from both groups fully recovered from desensitization by 240 s following the initial stimulus. The magnitude of α4β2 nAChR desensitization in XII MNs from DNE animals was greater at interpulse intervals of 5 s (P < 0.0002), 30 s (P < 0.0002), and 60 s (P < 0.00005) compared with cells from control neonates (Fig. 3C). Similar to the results with nicotine application, the recovery from desensitization evoked by RJR-2403 was slower in XII MNs from DNE animals compared with controls (τ = 10 s in control cells and τ = 109 s in DNE cells).
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breathing in neonatal humans and animals, presenting itself as inappropriate apnoeic episodes (Robinson et al. 2002; Huang et al. 2004a), blunted chemosensitivity (Bamford et al. 1996; Eugenin et al. 2008; Fewell and Smith 1998; Hafstrom et al. 2002b; Huang et al. 2010; Slotkin et al. 1995), and obstructive sleep apnea (Toubas et al. 1986) among other conditions (for reviews, see Hafstrom et al. 2005; Kinney et al. 2009). Despite some evidence that suggests nAChRs play a role in these deficits (Kamendi et al. 2009; Kinney et al. 2009; Pilarski and Fregosi 2009), there is no definitive evidence showing that DNE alters nAChR structure and/or function.

It has long been observed that the continuous presence of nicotine or nicotinic agonists results in nAChR desensitization (Katz and Thesleff 1957). However, the time course and the extent to which nAChR properties are affected in various experimental preparations are still unclear (for reviews, see Gentry and Lukas 2002; Quick and Lester 2002). Depending on the experimental details (e.g., duration and timing of exposure), chronic activations can increase, decrease, or have no effect on nAChR expression and/or function (Rowell and Wonnacott 1990; Lukas 1991; Marks et al. 1993; Peng et al. 1994; Yu and Wecker 1994; Buisson and Bertrand 2001; Sokolova et al. 2005). Here, we used a thick medullary slice preparation, which preserves nAChR location, density, number, and subtype composition, and tested whether DNE increases the magnitude of receptor desensitization and recovery. We found that DNE produced a significant loss of nAChR current responsiveness following repetitive acute applications of nicotine, indicative of receptor desensitization, and we also found that nicotine-evoked nAChR currents from DNE cells recovered more slowly with time constants that were more than twice as long as controls.

At present no electrophysiological data exist in which nAChR desensitization and recovery have been examined in the context of chronic DNE. Nonetheless, our results are consistent with data showing a strong relationship between the length of nAChR agonist application and the degree of the functional deficit (Lukas 1991; Peng et al. 1994; Paradiso and Steinbach 2003). Conformation states of nAChR activation, desensitization, and recovery have been modeled and suggest that nAChRs cycle through functional and nonfunctional states depending on the concentration of the agonist and the properties of the specific receptors (Katz and Thesleff 1957; Quick and Lester 2002). These models also show that the longer nAChRs are in the presence of an agonist, the more likely that they will enter the nonfunctional desensitization state (Changeux et al. 1984; Giniatullin et al. 2005). In the present experiments, we found that despite chronic DNE, there was still a population of nAChRs that was capable of being activated by brief nicotine pulses. More work is needed to determine whether activatable nAChRs represent receptors that had recovered from desensitization during tissue preparation or whether the high concentration of nicotine activated some receptors that were otherwise desensitized. Future studies are also needed to determine how DNE modulates nAChR desensitization and recovery. For example, similar to acute nicotine exposures, DNE may contribute to the onset of nAChR desensitization directly via its role in Ca2+ influx (Fenster et al. 1997; Guo and Lester 2007; Miledi 1980; Shen and Yakel 2009) and/or by indirectly influencing recovery from desensitization through receptor phosphorylation (Fenster et al. 1999; Huganir and Greengard 1990; Paradiso and Brehm 1998).

In a separate set of experiments, we also tested whether whole cell inward currents mediated by the α4β2 nAChR subtype were altered by DNE. Although different populations of nAChRs have been identified on hypoglossal motoneurons, it is generally accepted that the most abundant nAChR is the heteropentamer α4β2 subtype (Flores et al. 1992; Marubio et al. 1999; Picciotto et al. 1995; Schoepfer et al. 1988; Whiting et al. 1987). Thus, we used the α4β2 nAChR subtype agonist RJR-2403 to generate nicotinic currents in XII MNs. RJR-2403 has been used in similar brain preparations for this purpose as it exhibits relatively low nonspecific binding to other nicotinic subtypes, especially the α7 subtype (Bencherif et al. 1996; Papke et al. 2000; Shao and Feldman 2002; Wu et al. 2006). Moreover, the α4β2 nAChR subtype has a high affinity for nicotine and a vulnerability to desensitization at low nicotine concentrations (Barik and Wonnacott 2009; Gentry and Lukas 2002; Quick and Lester 2002). Similar to nicotine, RJR-2403 applications produced an inward current that desensitized in a time-dependent manner. Unlike nicotine pulses, recovery from RJR-2403-evoked desensitization was more complex, containing both fast and slow components in control cells (Fig. 3D). Subsequent to DNE, RJR-2403-evoked currents exhibited greater desensitization and slower recovery compared with control cells. Interestingly, recovery from RJR-2403-induced desensitization was more than 10 times slower for DNE cells compared with control cells (Fig. 3D). Although more work is needed to precisely characterize nAChR subunit combinations that may contribute to these results, it is likely that a heterogeneous population of α4β2 nAChRs is responsible for the observed response. Previous reports describe a least two populations of α4β2 nAChRs expressed in human embryonic kidney (HEK) cells, so-called high and low affinity α4β2 nAChRs, which desensitize and recover at different rates (Buissen et al. 2000; Covernton and Connolly 2000; Paradiso and Steinbach 2003). Accordingly, we show that RJR-2403-evoked currents from control animals exhibit a fast component during recovery that is absent in the DNE group (Fig. 3D). Because DNE eliminated this fast component of recovery, these data imply that DNE preferentially affects a population of XII MN α4β2 nAChRs that may be more sensitive to desensitization.

Despite clear evidence that DNE produces XII MN nAChRs that are more sensitive to desensitization and slower to recover, other studies often report enhanced function demonstrated by increases in the nAChR current response after chronic nicotine exposure (for an example, see Sokolova et al. 2005). We show no evidence that nAChR function is enhanced; rather, nAChR function was significantly compromised following DNE. It is hard to interpret why such discrepancies are so common, but the variable effects of chronic nicotine exposure on nAChRs are likely due to a dependence on exposure timing (pre- or postnatal exposure), dose and duration (minutes to weeks), experimental preparation (brain tissue vs. cultured cells), and the properties of the specific nAChR subtypes involved (Gentry et al. 1999; Huganir and Greengard 1990; Paradiso and Brehm 1998).
and Lukas 2002). In the present study, we attempted to mimic as closely as possible a clinical situation in which a developing embryo and newborn are exposed to nicotine for extended periods under conditions where brainstem neurons are developing rapidly. Using this model in combination with the medullary slice preparation, it seems clear that XII MN nAChR function is compromised by DNE.

While the significance of nAChR desensitization has also been widely debated, it is generally thought that desensitization functions in life as a form of short-term plasticity, which can shape synaptic transmission and regulate neuronal excitability (Giniatullin et al. 2005). However, when prolonged periods of neurotransmitter or agonist binding occur, such as during volitional nicotine exposure (e.g., cholinomimetic therapy and cigarette smoking), desensitization can lead to pathological signaling, especially during ontogeny. Consistent with these proposals, we and others showed previously that nAChR-mediated activation of respiratory motor outflow was blunted following DNE (Robinson et al. 2002; Eugenin et al. 2008; Pilarski and Fregosi 2009), and, in a separate study, we showed that rhythmically active XII MNs received less excitatory synaptic input after DNE (Pilarski et al. 2010). Here, we extend these observations and show that DNE has a direct effect on the function of nAChRs expressed on XII MNs. These results suggest that decrements in cholinergic neurotransmission following chronic nicotine exposure in human and non-human animals can be explained in part by direct effects on postsynaptic nAChRs.

As reviewed in the Introduction, many laboratories have reported breathing abnormalities in awake neonates that were exposed to nicotine in utero. A recent paper from our group demonstrated that the ventilatory response to hypercapnia or combined hypoxia/hypercapnia was consistently and significantly blunted in animals that were nicotine exposed using the same approach used here (Huang et al. 2010). The blunted ventilatory response to chemoreceptor stimulation was present on P1 and persisted through P18. How DNE leads to the blunting of the ventilatory response to postnatal ventilatory responses to hypoxia and hypercapnia. Respir Physiol 106: 1–11, 1996.


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