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Enhanced nicotinic acetylcholine receptor-mediated [^{3}H]norepinephrine release from neonatal rat hypothalamus

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Abstract

Nicotinic acetylcholine receptor (nAChR)-evoked release of norepinephrine (NE) has been demonstrated in a number of brain regions that receive sole noradrenergic innervation from the locus coeruleus (LC). Many of these structures display enhanced nicotine-stimulated NE release in the neonate. We have examined the hypothalamus in order to determine if this region, which receives NE projections from both the LC and medullary catecholaminergic nuclei, also demonstrates maturational changes in nAChR-mediated NE release. Quantification of radiolabeled-NE release from rat hypothalamus slices by a maximally effective dose of nicotine revealed a peak response during the first postnatal week. This was followed by a decrease at postnatal day (P) 14, and a second peak at P21. Thereafter, release was equivalent to that observed at P14. Comparison of the pharmacological properties of nAChRs mediating NE release in neonatal (P7) and mature hypothalamus suggested involvement of different nAChR subtypes at the two ages. Using the selective toxin, DSP-4, nAChR-mediated NE release in the neonatal hypothalamus was shown to be from LC terminals. Our findings demonstrate an early sensitivity of hypothalamic LC terminals to nAChR regulation that may be associated with development of systems controlling critical homeostatic functions such as stress, feeding and cardiovascular regulation.

Keywords: Catecholamine; Locus coeruleus; Neurotransmitter release; Nicotine; Ontogeny

1. Introduction

Maternal smoking remains one of the leading causes of preventable perinatal death, and of numerous persistent cognitive and behavioral abnormalities in human offspring (Andres and Day, 2000; Cnattingius, 2004; Weitzman et al., 2002). Animal studies suggest that many of these deleterious effects result from the actions of nicotine, the major psychoactive component of tobacco (Ernst et al., 2001; Lichtensteiger et al., 1988). Selective alterations in both central and peripheral norepinephrine (NE) systems following gestational nicotine exposure, observed in clinical and animal studies (Lavezzi et al., 2005; Navarro et al., 1988; Oncken et al., 2003; Slotkin et al., 1987), may contribute to the etiology of a number of disorders associated with maternal smoking, including sudden infant death syndrome (SIDS) and attention deficit hyperactivity disorder (ADHD) (Linnet et al., 2003; MacDorman et al., 1997).

1.1. Development

We have previously shown that nicotine activates NE release from fetal locus coeruleus (LC) cells (Gallardo and Leslie, 1998b). The LC, which supplies the sole NE innervation to the forebrain (Loughlin et al., 1986), develops prenatally and exerts a neurotrophic influence on various maturational processes (Felten et al., 1982; Nakamura and Sakaguchi, 1990;

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Parnavelas and Blue, 1982). It is also important for functional development of a number of systems that are critical for neonatal survival, including early olfactory learning and the neural control of respiration (Sullivan, 2003; Viemari et al., 2004).

nAChRs are highly expressed in fetal brain, and are transiently upregulated in many regions, often during periods that are key to neuronal maturation and synaptogenesis (Broide et al., 1995). During these ''critical periods'' presynaptic nAChR activation elevates neurotransmitter release, which is necessary for neuronal development and formation of proper connections (Metherate, 2004). We have previously demonstrated nicotine-stimulated [^{3}H]NE release from LC terminal regions throughout development (Gallardo and Leslie, 1998a; Leslie et al., 2002; O'Leary and Leslie, 2003). This effect is age-dependent, and NE terminals within the cerebellum and parietal cortex are hypersensitive to nicotine during the early postnatal period. The pharmacological properties of the nAChRs that mediate NE release vary between the neonate and the adult, suggesting regulation by different nAChR subtypes (O'Leary and Leslie, 2003). Thus far, however, nicotine-stimulated [^{3}H]NE release during development has only been studied in brain regions that receive NE input exclusively from the LC. It remains to be determined whether or not nicotine-stimulated [^{3}H]NE release from the hypothalamus, which receives mixed innervation from both the LC and medullary NE nuclei, is also developmentally regulated.

1.2. Hypothalamus

The hypothalamus is one of the few brain regions that receive input from noradrenergic neurons located in both the pons and medulla. The medullary catecholamine neurons of the nucleus tractus solitarius and the ventrolateral medulla supply the main noradrenergic input to the hypothalamus (Ter Horst et al., 1989; Tucker et al., 1987), whereas the LC provides a lesser input (Loughlin et al., 1986). NE neurons in each of these cell groups receive distinct peripheral input (Andresen and Kunze, 1994; Berridge and Waterhouse, 2003; Swanson et al., 1981) and therefore control different hypothalamic functions, including stress response, food intake, baroreceptor reflex, and other autonomic systems (Leibowitz et al., 1989; Leibowitz et al., 1983).

The hypothalamus has extensive cholinergic innervation, from both intrinsic and extrinsic neurons (Tago et al., 1987; Woolf and Butcher, 1986). Interaction of cholinergic and noradrenergic systems has been implicated in regulating several hypothalamic functions, including sleep and feeding behavior (Bayer et al., 2005; Saint-Mleux et al., 2004). nAChRs are expressed in both pontine and medullary NE cell groups that project into the hypothalamus, as demonstrated through detection of nAChR subunit mRNAs, receptor-binding sites, and immunohistochemical localization of receptors (Lena et al., 1999; Wada et al., 1989). Nicotine influences several functions that are mediated by NE in the hypothalamus, including the stress response, feeding behavior, and the sleep–wake cycle (Jo et al., 2002; Matta et al., 1998; Saint-Mleux et al., 2004). In the adult, nicotine induces NE release from hypothalamic medullary CA terminals by activation of cell soma nAChRs, whereas nicotine stimulation of the hypothalamus evokes NE release solely from LC terminals (Matta et al., 1998; Sperlagh et al., 1998).

It is not presently known whether hypothalamic noradrenergic afferents respond to nicotine in the neonate. In order to address this issue, we have examined nicotine-stimulated [^{3}H]NE release from rat hypothalamic slices throughout postnatal development and have evaluated the underlying mechanisms. By ablating the LC with the selective neurotoxin, DSP-4 (Cornwell-Jones et al., 1989; Fritschy and Grzanna, 1991), we have also examined the origin of the terminals that release NE in response to nicotine in the neonatal hypothalamus.

2. Materials and methods

2.1. Materials

Drugs used in the experimental procedures were acquired from the following companies: acetylcholine HCl (ACh), atropine, bisindolylmaleimide I HCl (BIS), *N*-(2-chlorethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4), (−)-cytisine, dihydro-β-erythroidine hydrobromide (DHβE), diisopropyl flourophosphate (DFP), EGTA, mecamylamine HCl, methyllycaconitine citrate (MLA), (−)-nicotine hydrogen tartrate salt, nisoxetine HCl, *N*ω-nitro-L-arginine methyl ester HCL (L-NAME), (+)-tubocurarine chloride hydrate, zimelidine dihydrochloride (Sigma Chemical Co., St. Louis, MO). [^{3}H]NE (specific activity = 51 Ci/mmol) was purchased from Dupont NEN (Boston, MA) and Ecolume scintillation fluid from ICN (Irvine, CA).

2.2. Animals

All experiments were carried out in accordance with the Institutional Animal Care and Use Committee at the University of California, Irvine and Federal Guidelines. Animals were placed in climate-controlled housing (temperature 21 °C and humidity 50%) with a 24-h light–dark cycle (12 light:12 dark), and were provided food and water ad libitum. Animals used were Sprague–Dawley rats obtained from Harlan, San Diego, CA. Pups aged postnatal day (P) 1–40 were obtained from pregnant dams that were purchased prior to parturition. The day of birth was defined as P0. At birth, litters were culled to 10 pups to ensure adequate nutrition. Animals were weaned at P21 and separated by gender. Adult animals were purchased by weight (240 g–260 g) equivalent to approximately P60, and upon arrival were allowed to habituate to the facilities for at least 2 days prior to experimentation.

2.3. Neurotransmitter release

The superperfusion release assay protocol, as described previously (Leslie et al., 2002; O'Leary and Leslie, 2003), was used for all release studies. Animals were decapitated and brains were quickly removed and chilled in 4 °C Krebs–HEPES buffer (KHB: 127 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 15 mM HEPES acid, 10 mM glucose, and 1 mM ascorbic acid) for 30 s. Hypothalamus, which was defined by the optic chiasm rostrally, the median eminence caudally, immediately above the lateral fissure dorsally, and laterally by the point at which the neocortex meets the hypothalamus on the ventral surface of the brain, was dissected on an ice-chilled platform, cross-chopped into 250 μm square slices using a McIlwain Chopper, and placed in 5 ml ice-cold KHB. The number of animals needed to obtain enough tissue for 1 experiment varied with age from 10 animals at P1–P3 adult animals. Tissue was pre-incubated at 37 °C for 30 min, with buffer changes after each 10-min period. Slices were incubated for 20 min at 37 °C in KHB containing 15 nM [^{3}H]NE, then rinsed in fresh buffer for 2 × 5 min and 2 × 10 min at 37 °C. Following the last wash, slices were resuspended in 5 ml buffer and 15 μl aliquots of gravity-packed tissue