Prenatal nicotine affects catecholamine gene expression in newborn rat carotid body and petrosal ganglion

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Gauda, Estelle B., Reed Cooper, Patrice K. Akins, and Guimei Wu. Prenatal nicotine affects catecholamine gene expression in newborn rat carotid body and petrosal ganglion. J Appl Physiol 91: 2157–2165, 2001.—Nicotine exposure modifies the expression of catecholamine and opioid neurotransmitter systems involved in attenuation of hypoxic chemosensitivity. We used in situ hybridization histochemistry to determine the effect of prenatal and early postnatal nicotine exposure on tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), preproenkephalin (PPE), and D2-dopa-mine receptor mRNA levels in the rat carotid body and petrosal ganglion during postnatal development. In the carotid body, nicotine increased TH mRNA expression in animals at 0 and 3 postnatal days (both, P < 0.05 vs. control) without affecting TH mRNA levels at 6 and 15 days. At 15 postnatal days, DBH mRNA levels were increased in the carotid body of nicotine-exposed animals. Dopamine D2-receptor mRNA levels in the carotid body increased with postnatal age but were unaffected by nicotine exposure. PPE was not expressed in the carotid body at any of the ages studied in control or treated animals. In the petrosal ganglion, nicotine increased the number of ganglion cells expressing TH mRNA in animals at 3 days (P < 0.01 vs. control). DBH mRNA expression was not induced nor was PPE mRNA expression increased in the petrosal ganglion in treated animals. Prenatal nicotine exposure upregulates mRNAs involved in the synthesis of two inhibitory neuromodulators, dopamine and norepinephrine, in peripheral arterial chemoreceptors, which may contribute to abnormalities in cardiorespiratory control observed in nicotine-exposed animals.

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TO BETTER UNDERSTAND the biological mechanisms that help explain the increased association between sudden infant death syndrome and exposure to tobacco smoke (19), several physiology studies have investigated the effect of prenatal nicotine exposure, a major component of tobacco smoke, on ventilatory and cardiovascular control in newborn animals. In newborn rats exposed prenatally to nicotine, abnormalities in ventilation (46), autoresuscitation (11), and cardiovascular responses (32) have been reported. Specifically, prenatal exposure to nicotine blunts the ventilatory responses to short exposures to hypoxia (46) and hyperoxia (2), manipulations that are typically used as a test of peripheral chemoreceptor function in unanesthetized animals and newborn infants.

Nicotine binds to nicotinic cholinergic receptors on catecholamine-containing neurons and affects neurotransmitter expression in the peripheral and central nervous systems (31, 51). Nicotine increases dopamine, norepinephrine, and opioid content and upregulates catecholaminergic synthesizing enzymes and peptide expression (8). The peripheral arterial chemoreceptors express nicotinic receptors (1), contain catecholamines and opioids, and are involved in modulating respiratory and arousal responses to hypoxia (for review, see Ref. 20). In the peripheral arterial chemoreceptors, catecholamines are the most abundant neuromodulators in the carotid body. Dopamine, through binding to dopaminergic D2 receptors, and norepinephrine, through binding to α2-adrenergic receptors on carotid sinus nerve fibers, attenuate hypoxic chemosensitivity (for review, see Ref. 20). Opioids, specifically, Met-enkephalins through binding to δ-opioid receptors, also attenuate hypoxic chemosensitivity (35).

Hypoxic chemosensitivity of peripheral arterial chemoreceptors increases with postnatal age (for review, see Refs. 17 and 10). Associated with maturation of hypoxic chemosensitivity are changes in neurotransmitter profiles and changes in the biochemical and electrical properties of cells in the carotid body (for review, see Ref. 17). Specifically, dopamine content is elevated in the carotid body of newborn rats, and this is associated with blunted chemoreceptor responses (25). Similarly, our laboratory has shown that mRNA levels for tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine synthesis, are significantly elevated in newborn animals, whereas mRNA levels for dopamine D2 receptors are decreased in the same cells in the carotid body (14). However, with increasing maturation, TH mRNA levels decreased, whereas dopamine D2-receptor mRNA levels increased. Less is known about the effect of maturation on the enkephalin content in the carotid body. In addition, our laboratory has previously demonstrated that preproenkephalin (PPE) mRNA is present in petrosal ganglion cells but not in the carotid body of 14-day-old rats (16).

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Acute nicotine exposure affects catecholaminergic and opioid neurotransmitters in peripheral and central nervous systems. However, how prenatal nicotine exposure affects the expression of these inhibitory neurotransmitter systems in the carotid body and sensory ganglion is not known. Thus the purpose of this study was to elucidate molecular mechanisms involved in alterations in the expression of critical neurotransmitters that may explain alterations in chemosensitivity observed in newborn rats exposed prenatally to nicotine. We determined the effect of prenatal and early postnatal nicotine exposure on TH, PPE, and dopamine D2-receptor gene expression in the carotid body and petrosal ganglion in newborn rat pups during the first 2 wk of postnatal development. We also determined the effect of prenatal nicotine exposure on dopamine β-hydroxylase (DβH) gene expression in the carotid body and petrosal ganglion in animals at 2 wk postnatal age. Because nicotine exposure upregulates catecholaminergic and opioid traits in central and peripheral nervous systems, we hypothesized that prenatal nicotine exposure would affect neurotransmitters by upregulating mRNAs encoding proteins involved in inhibitory catecholaminergic and opioid systems in peripheral arterial chemoreceptors during early postnatal ages.

**METHODS**

Time-dated, pregnant Sprague-Dawley rats were used. On days 2–3 of gestation, before implantation of the embryo in the uterine wall, an osmotic minipump (type 2ML4, Alza, Palo Alto, CA) containing nicotine bitartrate or sterile saline (0.9%) was inserted subcutaneously. The dose of nicotine delivered by the osmotic pump (6 mg·kg⁻¹·day⁻¹ of free base nicotine) produces plasma nicotine levels that are seen in heavy smokers (3 packs/day) (31, 38). Dams received nicotine or vehicle until parturition on day 22 and then for 1 wk after delivery, i.e., for a total of 4 wk. After spontaneous delivery, the rat pups were cross-fostered to maintain equal litter sizes.

Tissues were taken from Sprague-Dawley rats on postnatal days 0, 3, 6, and 15 (n = 7, each age). All animals were briefly anesthetized with 3% methoxyflurane and decapitated. The bifurcation of the carotid artery with the carotid body and the superior cervical, nodose, petrosal, and jugular ganglia was quickly removed en bloc, placed in embedding media (Fisher), and quickly frozen on dry ice. The right and left tissue blocs were removed from the animals within 5 min of decapitation. The tissues were stored at −70°C until further processing.

*In situ* hybridization histochemistry. Tissue blocs were cut in 12-μm sections on a cryostat. Sections were thaw-mounted onto gelatin-chrome, alum-subbed slides. Slide-mounted sections were then fixed in 4% paraformaldehyde, acetylated in fresh 0.25% acetic anhydride in 0.1 M triethanolamine, dehydrated in ascending series of alcohols, delipidated in chloroform, and then rehydrated in a descending series of alcohols. Slides were air dried and then stored at −20°C.

Antisense ribonucleotide probes were used for detection of TH, DβH, PPE, and dopamine D2-receptor mRNAs. The antisense probes were constructed from cDNAs for each of these genes by in vitro transcription. The cDNA for the TH, PPE, and dopamine D2 receptors was complementary to base pairs 1,120–1,488 (22), 51–987 (52), and 372–1,174 (3) of the rat genes, respectively. cDNA for DβH contained a 575-base pair fragment corresponding to the nucleotides 205–780 of the rat DβH cDNA described by McMahon et al. (34). The cDNA fragment was obtained by PCR amplification of rat striatum cDNA with sense strand oligonucleotide corresponding to base pairs 205–225 and antisense oligonucleotide strand corresponding to bases 759–780 of the published sequence (34). The PCR products, along with control samples, were electrophoresed through an agarose gel. A single band of ~500 base pairs was excised from the gel and subsequently subcloned into pCR4-TOPO TA cloning vector (Invitrogen, Carlsbad, CA). This cloning vector allows for direct cloning of PCR products into an EcoRI (restriction enzyme) cloning site and contains T3 and T7 RNA polymerase sites for generating sense and antisense ribonucleotide probes for in situ hybridization experiments and direct sequencing. The subcloned cDNA fragment was partially sequenced to determine orientation of the cloned fragment.

To verify specificity of the ribonucleotide probes, coronal brain sections from adult animals corresponding to the following bregma coordinates (bregma = −10.04, −5.80, and 0.20) were used as controls. Control brain slides were hybridized, exposed, and developed simultaneously with the experimental slides. Uniformly, these control slides demonstrated the known patterns of gene expression in striatopallidal neurons for dopamine D2-receptor and (18) PPE mRNAs (44), nigrostriatal neurons for TH (40), and locus coeruleus neurons for DβH (data not shown). All control brain slides showed discrete and specific hybridization signal in the neuronal cell groups known to express these genes without any background or nonspecific hybridization signal. In addition, the patterns of TH, PPE, DβH, and dopamine D2-receptor mRNA expression differed in the tissue bloc of the carotid body and petrosal and superior cervical ganglia, further demonstrating probe specificity.

Probes were labeled with [35S]UTP via in vitro transcription as outlined by Chesselet et al. (5). Labeled probes of 1.2–1.5 × 10⁶ dpm were added to 100 μl of hybridization buffer (50% formamide, 600 mM NaCl, 300 mM NaCl, 20 mM Tris–HCl, pH 7.5, 1 mM EDTA, 10% dextran sulfate, 1× Denhardt’s solution, 100 μg/ml salmon sperm DNA, 250 μg/ml yeast total RNA (type XI), 250 μg/ml yeast tRNA, and 100 mM dithiothreitol), which was applied to slides containing 8–10 sections per slide. Hybridization was performed at 55°C overnight. The slides were then washed in 1× SSC (0.15 M sodium chloride–0.015 M sodium citrate, pH 7.2) at room temperature. After treatment with RNAase A (20 mg/ml), slides were washed at 60°C in 0.2× SSC, rinsed in deionized water, and air dried. Slides were then dipped in Kodak photographic emulsion, dried, and exposed in the dark at −20°C for 4–8 wk. After exposure, the slides were thawed at room temperature, developed with Dektol (Kodak), and counterstained with thionin, and coverslips were applied with Permount.

*Data analysis.* Comparisons were only made between data obtained from slides that were processed for *in situ* hybridization, hybridized, exposed, and developed together under the same conditions. Silver grains generated by [35S] in the emulsion were analyzed by using a microscope and Macintosh image analysis program [National Institutes of Health (NIH) image, W. Rasband, NIH]. Semiquantitation of silver grains was performed by 10.04, 10.220.33.6 on July 11, 2017 http://jap.physiology.org/ Downloaded from
eter circles was counted, and a mean was obtained for each section of the carotid body. These numbers were combined from two to three sections of the carotid body to obtain an average count per animal. The population of values of average counts per animal was compared between control and treated groups with ANOVA and post hoc analysis via Student’s unpaired t-test. Statistical significance was set at \( P < 0.05 \). Because of the additional tissue sections that were available from 15-day-old animals, we determined whether prenatal nicotine exposure altered \( \beta \)H gene expression in the carotid body and petrosal ganglion in this age only.

Semiquantitation of the number of ganglion cells in the petrosaljugular ganglion (PG/JG) complex expressing TH mRNA was determined by counting the number of positive cells per tissue section with dark-field microscopy at \( \times 400 \). A mean was obtained from each animal after determining the mean number of TH-positive ganglion cells from five to seven tissue sections per animal. The mean number of TH-positive ganglion cells per PG/JG section between control and nicotine-exposed animals was determined by unpaired \( t \)-test in animals at 3 and 15 postnatal days of age.

**RESULTS**

Prenatal nicotine exposure did not affect litter sizes nor did it affect dam or rat pup mortality. However, rat pups exposed to nicotine were smaller than control pups at each of the postnatal ages studied (Table 1). Prenatal and early postnatal nicotine exposure significantly increased the expression of TH mRNA in the carotid bodies of newborn rats \( (P = 0.02, \text{ANOVA}) \). TH mRNA levels were greater in animals exposed to nicotine at 0 and 3 postnatal days, as shown in representative photomicrographs from four animals in Fig. 1 and in the histogram of composite data from all animals in Fig. 2. Although TH mRNA levels at days 6 and 15 did not differ between control and treated animals (Fig. 2), nicotine exposure did upregulate \( \beta \)H mRNA expression in the carotid body of 15-day-old animals \( (P = 0.03, \text{control vs. nicotine}) \), as shown in the photomicrographs of one control and one prenatally nicotine-exposed animal (Fig. 3, A and B), along with composite data (bar graph) from all animals (Fig. 3, right). Similar to TH mRNA, \( \beta \)H mRNA was moderately expressed in many cells in the superior cervical ganglion in control animals, as shown in the low- (Fig. 4A) and high-power (Fig. 4B) dark-field photomicrographs from one control animal at day 15. However, prenatal nicotine exposure did not significantly change the level of \( \beta \)H mRNA levels in the superior cervical ganglion (data not shown). Prenatal nicotine exposure also increased the number of ganglion cells in the PG/JG complex expressing TH mRNA at 3 days (Fig. 5) but not at 15 postnatal days \( (P = 0.2, \text{control vs. nicotine at 3 days}) \). \( \beta \)H mRNA was not detected in the PG/JG complex in either control or treated animals at day 15.

Dopamine \( D_2 \)-receptor mRNA was expressed in the petrosal ganglion and carotid body in both control and treated animals. The low-power, bright-field photomicrograph of the nissl-stained tissue section (Fig. 6A) depicts the anatomy of the petrosal ganglion, IX cranial nerve, carotid sinus nerve, and carotid body. The high-power, dark-field photomicrograph (Fig. 6B) of the same tissue section shown in Fig. 6A shows clusters of silver grains representing dopamine \( D_2 \)-receptor mRNA within ganglion cells of the petrosal ganglion. As previously described (14) and shown in this study, dopamine \( D_2 \)-receptor mRNA levels in the carotid body increased with postnatal age. However, nicotine exposure did not alter the level of dopamine \( D_2 \) expression in the carotid body or the petrosal ganglion at any of the four postnatal ages studied (Fig. 7).

PPE mRNA was not expressed in the carotid body at any of the ages studied in either control or nicotine-exposed animals. Although PPE was not expressed in the carotid body, it was expressed in the superior cervical and petrosal ganglia as previously described (16). Nicotine exposure did not induce PPE gene expression in the carotid body nor did it change PPE mRNA levels in the superior cervical or petrosal ganglia (data not shown).

**DISCUSSION**

Experimental models investigating neurotransmitter expression in the peripheral and central nervous system strongly suggest that alterations in cholinergic neurotransmission affect neurotransmitter phenotypes in immature and mature neurons (30, 31, 39, 51). This study is the first to show that continuous prenatal nicotine exposure during fetal development and early postnatal exposure modifies catecholaminergic gene expression in the peripheral arterial chemoreceptors. Nicotine exposure increases TH mRNA expression in both the carotid body and PG/JG complex in animals within the first 3 days of postnatal life and upregulates \( \beta \)H mRNA levels in the carotid body of 15-day-old animals. However, prenatal nicotine exposure does not alter dopamine \( D_2 \)-receptor mRNA levels in the carotid body or petrosal ganglion. In addition, we have shown that PPE mRNA is not expressed in the carotid body but is expressed in the superior cervical and petrosal ganglion, and the level of expression is not altered by prenatal exposure to nicotine. Thus alterations in transynaptic neurotransmission affected by prenatal and early postnatal nicotine exposure induce catecholaminergic neurotransmitter expression in peripheral arterial chemoreceptors in newborn rat pups.

We observed an increase in TH mRNA expression in the carotid body of newborn animals at 0 and 3 postnatal days and in the petrosal ganglion in 3-day-old animals exposed to nicotine. However, this effect was no longer seen at 6 and 15 postnatal days. The most

**Table 1. Effect of prenatal nicotine exposure on body weight**

<table>
<thead>
<tr>
<th>Postnatal Day</th>
<th>Nicotine, g</th>
<th>Control, g</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.7 ± 0.3</td>
<td>6.1 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>7.8 ± 0.5</td>
<td>8.1 ± 0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6</td>
<td>13.9 ± 0.7</td>
<td>14.8 ± 0.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>15</td>
<td>34.2 ± 1.4</td>
<td>39.6 ± 0.9</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 7 \) at each age.
plausible explanation for the loss of effect is that the 6- and 15-day-old animals were no longer being exposed to nicotine. The mini-osmotic pump containing nicotine or sterile saline was not removed from the nursing dam, and the pump delivered nicotine or sterile water for ~1 wk postpartum. The 6-day-old animals may have received some nicotine via the milk; however, the relative dose per weight was considerably less than that of 0- and 3-day-old animals.

Cardiorespiratory physiology is altered in rats exposed prenatally to nicotine. Peripheral chemoreceptors are involved in multiple cardiorespiratory functions that include, but are not limited to, modulating respiration in response to changes in temperature, chemical stimuli, and recovery from asphyxial apnea associated with upper airway obstruction (12). Intact peripheral chemoreceptors are not essential for establishment of rhythmic breathing at birth. However, intact peripheral chemoreceptors at birth appear to provide essential trophic influences for maintaining rhythmic breathing during maturation (for review, see Ref. 17). Animals exposed prenatally to nicotine have 1) reduced ventilation during air breathing and in response to hypoxia (46), 2) less reduction in ventilation in response to hyperoxia (test of peripheral chemoreceptor function) (2), 3) reduced ability to autoresuscitate from repeated episodes of hypoxia (11), and 4) increased mortality from severe, continuous hypoxic exposure (45). Alterations in these cardiorespiratory reflexes may involve adverse molecular and cellular changes in peripheral arterial chemoreceptors induced by prenatal nicotine exposure during prenatal development. Bamford and Carroll (2) reported altered peripheral chemoreceptor function (Dejours’ test) in awake 3-day-old newborn animals that were exposed prenatally to nicotine. However, these investigators, using a superfused ex vivo preparation, were unable to detect a difference between control and treated animals when measuring activity from the whole carotid sinus nerve in response to a severe hypoxic challenge (5% O₂-5% CO₂). Yet the preponderance of physiological studies suggest that peripheral arterial chemoreceptor responses could be affected by prenatal nicotine exposure in awake 3-day-old newborn animals that were exposed prenatally to nicotine. One explanation for the observed discrepancy between the whole animal and the isolated carotid body preparations might be secondary to the isolated preparation used. The severe hypoxia stimulus may have saturated the whole nerve response, making it difficult to distinguish differences in the carotid sinus nerve activity in the preparations between control and prenatally treated animals. Our study is the first to demonstrate that changes in gene expression in the peripheral chemoreceptors are induced by prenatal and perinatal exposure to nicotine. Increased expression of inhibitory catecholaminergic traits may in part explain the alterations in cardiorespiratory responses observed in animals treated prenatally with nicotine.

Nicotine exposure affects catecholaminergic traits in the peripheral and central nervous system: comparison

![Fig. 1: Dark-field photomicrographs of the carotid body showing the expression of tyrosine hydroxylase (TH) mRNAs in control animals at 0 (A) and 3 postnatal days (C) and animals treated prenatally with nicotine at 0 (B) and 3 postnatal days (D). Nicotine exposure increased the expression of TH mRNA in the carotid body in animals at 0 and 3 postnatal days (B and D). Silver grains appear as white dots. Scale bars = 25 μm.](http://jap.physiology.org/)
to and extension of previous literature. Acute nicotine exposure induces upregulation of catecholaminergic synthesizing enzymes in several dopaminergic and noradrenergic cell groups in the central and peripheral nervous system (43, 47). Furthermore, nicotine significantly increases TH mRNA levels in PC-12 cells (27), an immortalized cell line that is frequently used as a model of chemosensitive type I cells in the carotid body (36). Type I cells in the carotid body contain TH, dopamine, and norepinephrine, and acute nicotine exposure increases dopamine and norepinephrine release from dissociated type I cells (48). In 3-day-old rat pups, acute postnatal nicotine exposure reduced dopamine content and increased TH mRNA expression in the carotid bodies (28). Furthermore, chemoreceptor function was diminished in these 3-day-old rat pups treated acutely with nicotine (28). We have extended the findings of Holgert et al. (28) by showing that chronic prenatal and early postnatal exposure to nicotine is associated with increased TH mRNA expression in the carotid body and petrosal ganglion of newborn rat pups at 0 and 3 postnatal days. Although the effect of nicotine exposure on TH mRNA levels in the carotid body of 15-day-old animals was no longer apparent, we did observe an increase in DβH gene expression in the carotid body in these animals. Because of the limitation of available tissue, we do not know if nicotine exposure also increased DβH mRNA levels in the younger age groups. Similar to the findings of Holgert et al., we report no significant effect of nicotine exposure on dopamine D2-receptor mRNA levels in the carotid body.

Possible mechanisms for nicotine-induced upregulation of TH and DβH mRNAs in peripheral chemoreceptors. Several mechanisms, either indirectly or directly, may account for the upregulation of TH mRNA levels in the carotid body and petrosal ganglion induced by prenatal nicotine. The most plausible indirect mechanism is by inducing hypoxemia in the fetus. It is possible that prenatal nicotine exposure in the dose used in this experiment may have induced vasoconstriction of uterine vessels with subsequent decreased oxygen delivery to the rat pups. The rat pups exposed prenatally to nicotine were smaller than the control animals, an observation that has also been reported by other investigators using the same paradigm of nicotine exposure (2, 45). Litter sizes were matched between nicotine and control animals, although our animals were not pair-fed. We chose the dose of nicotine used in this study because equivalent dosing and exposure para-

Fig. 3. Photomicrographs (A and B) and bar graph (right) showing effect of prenatal nicotine exposure on dopamine β-hydroxylase (DβH) mRNA expression in the carotid body of 15-day-old animals. Representative photomicrographs of a dark-field image of the carotid body (CB) in two 15-day-old animals show the expression of DβH mRNA in a control animal (A) and an animal treated prenatally with nicotine (B). Nicotine exposure increased the expression of DβH mRNA in the carotid body. Arrows are pointing to clusters of silver grains that represent DβH mRNA expression. Scale bars = 25 μm. Right: bar graph showing DβH mRNA levels in the carotid body control animals (open bar) and animals treated prenatally with nicotine (solid bar) at 15 days postnatal age. Values are means ± SE; n = 5. *P = 0.02 vs. control.

Fig. 4. Representative low- (A) and high-power (B) dark-field photomicrograph showing the specificity and cellular localization of DβH mRNA expression in the superior cervical ganglion (SCG) of a 15-day-old animal. Scale bars = 50 μm.
digms have been used in studies showing abnormalities in hypoxic or hyperoxic chemosensitivity in newborn rat pups (2, 45, 46). Nevertheless, transcription, regulation, and enzymatic function of TH are significantly altered by the partial pressure of oxygen. TH gene expression within the hypoxic exposure is induced in the carotid body of adult rats (6). The increase in TH mRNA levels appears to be secondary to an increase in transcription and stabilization of the mRNA transcripts, as suggested by experiments exposing PC-12 cells to hypoxia (7). Although our laboratory has also shown that hypoxia significantly upregulates TH mRNA levels in the carotid body, it did not increase the grains per cell or the number of cells expressing TH mRNA in the PG/JG ganglion complex (15). In contrast, in our present study, prenatal exposure to nicotine did increase the number of ganglion cells expressing TH mRNA.

Alternatively, nicotine may have directly increased TH and DβH mRNA levels in the carotid body and petrosal ganglion by a cascade of intracellular events, thus leading to an increase in gene transcription similar to its effects in PC-12 cells (23) and in the rat.
adrenal medulla (27). In addition, nicotine may have
induced upregulation of TH-positive neurons in
the petrosal ganglion via an autocrine or paracrine effect
involving the induction of neuropeptides such as brain-
derived neurotrophic factor (BDNF). BDNF is released
from the carotid body and is essential for the develop-
ment of full expression of catecholaminergic neurons in
the petrosal ganglion (24). Of interest, nicotine has
been shown to induce BDNF in the striatum of rodents
(33), and BDNF has been implicated as providing a
protective effect induced by nicotine on the survival of
dopamine-containing nigrostriatal neurons in rodent
models of Parkinson’s disease (33). Although not ad-
dressed by this study, these alternative mechanisms
may be operative in the upregulation of TH mRNA and
DβH in the carotid body and TH-positive neurons in
the petrosal ganglion in animals exposed to nicotine.

Relationship between TH and DβH mRNA levels and
changes in dopamine and/or norepinephrine levels.

Hypoxia increases dopamine levels, dopamine release,
and TH enzyme activity in the carotid body of many
mammalian species (for review, see Ref. 20). Furth-
more, acute exposure to nicotine increases TH mRNA
levels and dopamine release in the carotid body of
newborn rats (28). TH is the rate-limiting enzyme for
catecholamine synthesis. We do not know if the in-
crease in TH mRNA levels observed in the animals
 treated prenatally with nicotine represents an increase
in dopamine, epinephrine, or norepinephrine levels at
any of the ages studied, because levels were not mea-
sured. Nicotine did increase DβH mRNA expression in
the carotid body of 15-day-old animals.

Nicotine exposure increases norepinephrine levels in
the noradrenergic cell groups in the brain (13) and
induces DβH gene transcription and DβH enzyme lev-
eels. Furthermore, acute nicotine exposure preferen-
tially released norepinephrine vs. dopamine from rat
(48) and rabbit (4) carotid bodies. One interpretation of
our data is that prenatal and early postnatal exposure
to nicotine modifies the ratio of dopamine to norepi-
nephrine protein levels in the rat carotid body, which
may be longer lasting than the duration of the expo-
sure. Norepinephrine, through binding to the α2-adre-
nergic receptors, inhibits output from the carotid body
(42). Thus whether the elevation in TH mRNA levels
represents an increase in dopamine or norepinephrine
levels at the younger age groups or if the elevation of
DβH represents an elevation in norepinephrine levels
in the older animals is unclear. However, both neuro-
transmitters may contribute to reduction in peripheral
arterial chemoreceptor activity in newborn animals
exposed to nicotine.

Prenatal and early postnatal nicotine exposure does
not induce PPE mRNA in the carotid body of newborn
animals. Enkephalins have been measured in the car-
rotid body, are coreleased with dopamine from the
rabbit carotid body (21), and are involved in reduction
in hypoxic chemosensitivity. Immunoreactivity for
Met-enkephalin has been localized to type I cells in the
carotid body of several mammalian species (50). How-
ever, in the rat, enkephalin immunoreactivity has only
been localized to nerve fibers innervating the carotid
body (26). Our previous and present findings show that
PPE mRNA was abundantly expressed in the cell bod-
ies in the petrosal ganglion but was not detected in the
carotid body, corroborating the immunohistochemical
studies in the rat. Similar cellular mechanisms are
involved in the nicotine-induced upregulation of PPE
gene transcription as they are in TH and DβH gene
transcription. PPE mRNA is present in the striatum
and adrenal chromaffin cells, and acute nicotine in-
duces the expression of PPE in bovine adrenal chro-
maffin cells in culture (49) and in the striatum (9, 29)
and hippocampus of adult rats (29). However, prenatal
exposure to nicotine at the dose used in this study did
not induce PPE gene expression in the carotid body nor
does it upregulate PPE mRNA in the cell bodies of the
petrosal ganglion. Similar to our findings, chronic vs.
acute nicotine exposure does not upregulate PPE
mRNA expression in the striatum or hippocampus of
the adult rat (30).

In summary, prenatal nicotine exposure is associ-
ated with blunted peripheral chemoreceptor function
(46) and reduced ability to autoresuscitate (11) in new-
born animals. We have found that prenatal nicotine
exposure upregulates TH mRNA levels in both the
carotid body and petrosal ganglion in rat pups at 0 and
3 postnatal days and upregulates DβH mRNA levels in
the carotid body of 15-day-old animals. However, do-
pamine D2-receptor and PPE mRNA levels in the
carotid body or petrosal ganglion were unaltered in ani-
imals exposed prenatally to nicotine during the first 2
wk of postnatal life. TH and DβH are the rate-limiting
enzymes for catecholamine and norepinephrine syn-
thesis, respectively. Dopamine and norepinephrine,
through binding to inhibitory dopamine D2- and α2-
adrenergic receptors, respectively, on postsynaptic re-
cipients within the carotid body, are associated with
reduced hypoxic chemosensitivity. Our data support a
possible role for upregulation of catecholamines in
peripheral arterial chemoreceptors as one possible cellu-
lar mechanism that could explain the reduced physio-
logical responses involving peripheral chemoreceptors
in newborn rat pups exposed prenatally to nicotine.

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