Effect of prenatal nicotine exposure on biphasic hypoxic ventilatory response and protein kinase C expression in caudal brain stem of developing rats

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Simakajornboon, Narong, Vukmir Vlasic, Hong Li, and Hemant Sawnani. Effect of prenatal nicotine exposure on biphasic hypoxic ventilatory response and protein kinase C expression in caudal brain stem of developing rats. J Appl Physiol 96: 2213–2219, 2004. First published January 29, 2004; 10.1152/japplphysiol.00935.2003.—Current evidence suggests that maternal smoking is associated with decreased respiratory drive and blunted hypoxic ventilatory response (HVR) in the newborn. The effect of prenatal nicotine exposure on overall changes in HVR has been studied; however, there is limited data on the effect of nicotine exposure on each component of biphasic HVR. To examine this issue, 5-day timed-pregnant Sprague-Dawley rats underwent surgical implantation of an osmotic minipump containing either normal saline (Con) or a solution of nicotine tartrate (Nic) to continuously deliver free nicotine at 6 mg·kg of maternal weight−1·day−1. Rat pups at postnatal days 5, 10, 15, and 20 underwent hypoxic challenges with 10% O2 for 20 min using whole body plethysmography. At postnatal day 5, Nic was associated with attenuation of peak HVR; peak minute ventilator increased 44.0 ± 6.8% (SE) from baseline in Nic pups, whereas that of Con pups increased 62.9 ± 5.1% (P < 0.05). Nic pups also had a reduction in the magnitude of ventilatory roll-off; minute ventilation at 15 min decreased 7.3 ± 7.1% in Nic pups compared with 27.3 ± 4.0% in Con pups (P < 0.05). No significant difference in HVR was noted at postnatal days 10, 15, and 20. Hypercapnic response was similar at all ages. We further investigated the effect of prenatal nicotine exposure on PKC expression in the caudal brain stem (CB) of developing rats. At postnatal day 5, Nic was associated with increased expression of PKC-β and PKC-δ in CB, whereas other PKC isoforms were not affected. It is concluded that prenatal nicotine exposure is associated with modulation of biphasic HVR and a selective increase in the expression of PKC-β and PKC-δ within the CB of developing rats.

respiratory control; maternal smoking; sudden infant death syndrome; signal transduction; prenatal exposure to cigarette smoke

SMOKING DURING PREGNANCY is the important preventable risk factor for sudden infant death syndrome (SIDS) (18, 31). The mechanism underlying the correlation between prenatal exposure to cigarette smoke and SIDS is currently unknown. SIDS has been attributed to a variety of pathophysiological mechanism, including respiratory control abnormalities (24). The effect of prenatal nicotine exposure on breathing pattern and hypoxic ventilatory response (HVR) in offspring has been previously studied in both human and animal models with conflicting results. In humans, Lewis and Bosque (27) found that infants of smoking mothers had no change in HVR or hypercapnic response but had attenuated hypoxic arousal response. Later study suggested that infants born to smoking mothers had reduced ventilatory drive and blunted HVR (42). In an animal model, nicotine infusion in developing lambs attenuated HVR and augmented ventilatory response to hyperoxia, suggesting that nicotine may alter peripheral chemoreceptor oxygen sensitivity (29). Bamford and coworkers (2), however, demonstrated no change in the hypoxic or hypercapnic ventilatory response in nicotine-exposed developing rats. Subsequent study by the same laboratory revealed that prenatal nicotine exposure resulted in abnormal ventilatory response to hyperoxia, suggesting an abnormal ventilatory response to withdrawal of baseline peripheral chemoreceptor drive (1). However, these studies in animal models assessed only changes in overall minute ventilation (Ve) during hypoxia without evaluation of various components of HVR. The ventilatory response to acute hypoxia in mammalian animals is biphasic. There is an initial increase in ventilation followed by a subsequent decrease in ventilation, with the later being termed hypoxic ventilatory roll-off (34). In developing animals, the reduction in ventilation during hypoxic ventilatory roll-off is more prominent (8).

The neuronal molecular mechanisms underlying the effect of nicotine on the peripheral chemoreceptor component of ventilatory response has been reported. Holgert et al. (22) studied the effect of postnatal nicotine exposure on signal transduction in carotid body and found that nicotine may interfere with postnatal oxygen sensitivity resetting of the peripheral chemoreceptor by increasing tyrosine hydroxylase mRNA and dopamine content in the carotid body. Later study by Gauda et al. (10) demonstrated that prenatal nicotine exposure upregulated tyrosine hydroxylase mRNA levels in the peripheral chemoreceptors of newborn rats. However, the effect of nicotine on signal transduction mechanisms mediating ventilatory response in the caudal brain stem (CB) is currently unknown. Previous experimentation has demonstrated that protein kinase C (PKC) activation mediates critical components of HVR, and selective translocation of certain PKC isoforms occurs in the dorsocaudal brain stem during hypoxia (12, 13, 15). Of the 12 PKC isoforms currently known, PKC-β, PKC-δ, and PKC-ε are the most likely candidates underlying functional roles in generation of respiratory drive and in modulation of HVR (15, 37). In addition, PKC isoforms are developmentally regulated, and overall ventilatory output may be more critically dependent on PKC activity in immature animals (3). Prenatal nicotine exposure could lead to alteration of HVR through modulation of PKC expression in the respiratory control area of the CB. In this study, the effect of prenatal nicotine exposure on...
various components of biphasic HVR and PKC expression in the CB was evaluated.

METHODS

The experimental protocols were approved by the Institutional Animal Use and Care Committee, Tulane University Health Science Center. All efforts were made to minimize animal suffering and to reduce the number of animals used. Time-pregnant Sprague-Dawley rats were purchased from a commercial breeder (Charles River, Wilmington, MA), and delivery times were recorded.

Preparation of Pregnant Rats

Surgical implantation of osmotic pumps. Forty pregnant Sprague-Dawley rats at 5 days of gestation underwent surgical implantation with osmotic minipump (ALZET, 2ML4 model) under anesthesia with intraperitoneal ketamine injection (20 mg/kg ketamine + 8 mg/kg xylazine). In this rat species, embryo implantation started on day 5 and was completed on day 7. The osmotic minipump was filled with normal saline (control) or a solution of nicotine tartrate at a concentration to deliver free nicotine of 6 mg/kg maternal weight⁻¹ day⁻¹. The dose was calculated on the estimated mean weight during pregnancy. This implantable minipump has been shown to allow for a steady-state infusion of nicotine at the dose mimicking the plasma levels found in smokers and users of transdermal nicotine patches (39). A small incision was performed over the scapulae, an osmotic minipump was inserted subcutaneously for continuous delivery of nicotine or vehicle, and this minipump can deliver for up to 28 days, so nicotine delivery continued until postnatal day 9–10.

Ventilatory Response in Rat Pups

Rat pups in each group were delivered by spontaneous vaginal delivery. Each animal was weighed and kept with the mother until the experiment. For hypoxic ventilatory studies, rat pups were randomly selected from every litter at postnatal days 5–6 (n = 21–22), 10–11 (n = 13–15), 15–16 (n = 11–13), and 20–21 (n = 10–12). These age groups have been previously shown as representative of maturational changes in the biphasic ventilatory response to hypoxia (8). Additional rat pups (n = 15 per age group per condition) underwent hypercapnic ventilatory challenges. For all experiments, similar numbers of male and female pups were used.

HVR and hypercapnic ventilatory response in rat pups. Respiratory measurement was continuously acquired in the freely behaving, unrestrained animal placed in a previously calibrated 0.5-liter barometric chamber (Buxco Electronics, Troy, NY) using the methods described by Bartlett and Tenney (4) and Pappenheimer (33). To minimize the long-term effect of signal drift due to temperature and pressure changes outside the chamber, a reference chamber of equal size in which temperature was measured with a T-type thermocouple was used. In addition, as previously recommended by Epstein et al. (9), a correction factor was incorporated into the software routine to account for inspiratory and expiratory barometric asymmetries. Environmental temperature was maintained within 29–32°C, which corresponds to usual temperatures recorded in the dam. A calibration volume of 0.1 ml of air was repeatedly introduced into the chamber before and on completion of recordings. At least 30 min before the start of each protocol, animals were allowed to acclimate to the chamber, in which humidified air (90% relative humidity) warmed to 30°C was passed through at a rate of 2 l/min by using a precision flow pump-reservoir system. Pressure changes in the chamber caused by the inspiratory and expiratory temperature changes were measured using a high-gain differential pressure transducer (model MP45-1; Validyne, Northridge, CA). Analog signals were simultaneously digitized and analyzed online by a microcomputer software program (Buxco Electronics). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. Tidal volume (VT), inspiratory duration, expiratory duration, and VE were computed and stored for subsequent offline analysis. The baseline ventilatory parameters were established for 30 min while animals were in room air. Ventilatory challenge was performed with 10% O2 in N2 lasting for 20 min in whole body plethysmography. Gas switches were performed by rapidly bleeding the premixed gas mixture into the recording chamber. For hypercapnic ventilatory challenges, 5% CO2-balance room air was administered for 20 min.

Immunoblots for PKC Isoform Expression

To quantitatively assess the expression of PKC isoforms, immunoblot was performed in nicotine-exposed and control rat pups at 5 days, 10 days, 15 days, and 20 days. Six to seven separate experiments corresponding to a total of 35–40 animals per postnatal age per group were conducted.

Brain tissue preparation. Normoxic rat pups at days 5, 10, 15, and 20 from each treatment and control group were euthanized with a pentobarbital overdose. The skull was rapidly opened, and the brain was removed, immediately frozen in liquid nitrogen, and stored at −70°C. For dissection, brain was warmed to −5°C in cold artificial cerebrospinal fluid. The obex was visually identified, and a coronal section 1.5 mm caudal to and 1.5 mm rostral to the obex was performed. The CB was carefully removed and stored at −70°C.

Immunoblot analysis. Tissues corresponding to the CB from four to five animals were pooled and homogenized at 4°C with a tissue blender in 20 mM Tris-HCl buffer, pH 7.5, containing 2 mM EDTA, 0.5 mM EGTA, 25 μg/ml leupeptin, 25 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. The homogenates were then centrifuged for 10 min at 1,000 g at 4°C to remove cell debris. Protein content was measured in each soluble fraction using the Bradford method (DC-Biobead protein assay, Hercules, CA), and samples were frozen at −70°C until analysis. Proteins (75 μg/sample) were subjected to SDS-PAGE with 8% Tris-glycine gel (Novex, San Diego, CA). The proteins from the gel were then transferred to 0.2 μM nitrocellulose membrane. Membranes were blocked for 1 h in 5% nonfat dry milk solution in Tris-buffered saline-Tween. These membranes were then incubated overnight with antibodies to PKC-α, -β, -γ, -δ, -ε, -ι, and -μ isoforms (Transduction Laboratories, Lexington, KY). Next, membranes were washed and incubated for 1 h with a horseradish peroxidase-labeled goat anti-mouse (1:30,000; Kirkegaard & Perry Laboratories, Gaithersburg, MD). In all experiments, a control lysate provided by Transduction Laboratories for each PKC isoform was included. The concentration of the PKC isoforms were as follows: PKC-α, 1:2,000; PKC-β, 1:100; PKC-γ, 1:500; PKC-δ, 1:100; PKC-ε, 1:250; PKC-ζ, 1:250; and PKC-μ, 1:800. Proteins were visualized by enhanced chemiluminescence (Amersham). At least six to seven different experiments, each consisting of pooled tissue obtained from four to five animals, were conducted for each postnatal age.

Data Analysis

Values are reported as means ± SE unless indicated otherwise. Baseline ventilation before each hypoxic challenge was defined as the average of ventilatory measures during the 5-min period immediately preceding the gas switch. For ventilatory challenge, mean minute-by-minute ventilatory measurements were calculated throughout the 20-min hypoxic exposure. To normalize across the various postnatal ages, the ventilatory measurement during hypoxia was expressed as percentage change from baseline. For Western blot experiments, semiquantitative analysis of the bands was performed by scanning densitometry. Comparisons between nicotine and control at various age groups were performed by using either paired t-test or two-way ANOVA followed by Newman-Keuls post hoc tests, as appropriate. A P value of <0.05 was considered significant.
Table 1. Body weight at each age group

<table>
<thead>
<tr>
<th>Age</th>
<th>Control, g</th>
<th>Nicotine, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–6 days</td>
<td>12.6±1.9</td>
<td>10.9±1.6*</td>
</tr>
<tr>
<td>10–11 days</td>
<td>19.9±2.5</td>
<td>20.2±2.1</td>
</tr>
<tr>
<td>15–16 days</td>
<td>28.5±2.2</td>
<td>29.1±2.3</td>
</tr>
<tr>
<td>20–21 days</td>
<td>45.3±3.3</td>
<td>46.2±2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 compared with control.

RESULTS

Mean weights for each postnatal age are shown in Table 1. Nicotine exposure during pregnancy was associated with a decrease in the weight at 5 days postnatal age. No significant difference in the weight at days 10, 15, and 20 was noted. The average body temperature was similar in all postnatal ages.

Ventilatory Measurements

The baseline ventilatory parameters are shown in Table 2. At postnatal day 5, nicotine-treated pups had slighter higher respiratory rate compared with control pups (165 ± 23 vs. 144 ± 17 breaths/min nicotine-treated vs. control pups; P = 0.05); however, VT and VE at baseline were similar. No significant difference in VT, respiratory frequency (f), or VE was noted at postnatal days 10, 15, and 20.

Prenatal nicotine exposure was associated with a decrease in peak HVR and significant attenuation of hypoxic ventilatory roll-off in 5-day-old rat pups. In nicotine-treated pups, peak VE increased 44.2 ± 6.5% from baseline, whereas that of control pups increased by 62.9 ± 5.1% (P < 0.05). VE at 15 min of hypoxic challenge decreased by 7.3 ± 7.0% in nicotine-treated pups compared with 27.3 ± 4.2% in control pups (Figs. 1 and 2; P < 0.05). The modulation of initial HVR at postnatal day 5 in the prenatal nicotine-exposed group was due to alteration in both VT and f (Figs. 3 and 4). At 1 min of hypoxic exposure, f increased 19.5 ± 3.6% in the nicotine-treated group and 41.2 ± 5.1% in the control group (P < 0.05). At peak HVR (2 min), VT increased 5.0 ± 3.0% in the nicotine-treated group and 15.2 ± 3.6% in the control group (P < 0.05). The attenuation of hypoxic ventilatory roll-off is primarily due to an alteration in VT (Figs. 3 and 4). VT at 15 min of hypoxic challenge decreased 19.3 ± 4.9% in the nicotine-treated group and 33.2 ± 4.7% in the control group (P < 0.05). No significant difference in f at 15 min of hypoxic exposure was noted between the two groups (14.9 ± 4.0 vs. 8.8 ± 8.6% nicotine-treated vs. control groups; P = not significant).

Table 2. Ventilatory parameter measurements at baseline in control and prenatal nicotine-exposed rat pups at 5 days, 10 days, 15 days and 20 days

<table>
<thead>
<tr>
<th></th>
<th>5–6 days</th>
<th>10–11 days</th>
<th>15–16 days</th>
<th>20–21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT, ml</td>
<td>0.090±0.031</td>
<td>0.123±0.009</td>
<td>0.176±0.025</td>
<td>0.275±0.011</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>144±17</td>
<td>183±13</td>
<td>179±15</td>
<td>163±18</td>
</tr>
<tr>
<td>VE, ml/min</td>
<td>13.1±2.3</td>
<td>23.2±1.7</td>
<td>23.5±1.5</td>
<td>43.7±1.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. VT, tidal volume; f, breathing frequency; VE, minute ventilation. *P = 0.05 compared with control.

DISCUSSION

Our study revealed that prenatal nicotine exposure was associated with modulation of both components of biphasic HVR in 5-day-old rat pups. Nicotine-treated pups had attenuation in peak HVR and reduction in the magnitude of hypoxic ventilatory roll-off. In addition, nicotine exposure led to selective changes in specific PKC isoforms, which were previously identified as playing a major role in ventilatory control.

Previous studies have shown the effect of prenatal nicotine exposure on baseline VE. St. John et al. (40) have demonstrated that prenatal nicotine exposure is associated with a decrease in baseline VE in developing rats. Hafstrom and coworkers (16) have found that nicotine-exposed developing lambs have similar VE but have lower VT and higher f. Our study did not reveal any difference in VE or VT, but nicotine-exposed pups had slightly higher baseline f at 5 days of age. The disparity

![Fig. 1. Average percent change in minute ventilation (VE) in 5 days postnatal age.](http://jap.physiology.org/doi/10.2252/jappl.2004.96.5.381/fig/1)
between the studies could be due to the difference in the animal model or the dose of nicotine exposure.

The effect of prenatal nicotine exposure on overall changes in HVR has been previously investigated with discrepant results. Both direct nicotine infusion and prenatal nicotine exposure have been shown to attenuate HVR in developing lambs (17, 29). Bamford and colleagues (2) revealed no significant effect of prenatal nicotine exposure on HVR and hypercapnic ventilatory response in developing rats, but subsequent study by the same laboratory has showed an alteration in dynamic ventilatory response to hyperoxia in nicotine-exposed rat pups (1). However, these studies focused on the overall changes in $V\dot{E}$ without assessment of different components of HVR. A recent study (35) has demonstrated that prenatal nicotine exposure is associated with increased frequency of apnea during hypoxia leading to alteration in hypoxic ventilatory roll-off in developing mice. Our study is the first to demonstrate that prenatal nicotine exposure has an effect on both components of biphasic HVR in developing rats. Nicotine-exposed pups at 5 days of age had an attenuated peak HVR as previously shown by former investigators. Interestingly, nicotine also modulated hypoxic ventilatory roll-off by decreasing the magnitude of this response. Because the hypoxic ventilatory roll-off is the prominent feature in developing animals (8), the effect of prenatal nicotine exposure on this specific component of HVR may lead to significant adverse effect in immature animals. The effect of prenatal nicotine exposure on biphasic HVR was transient, such that no such difference in HVR pattern was observed in postnatal days 10, 15, and 20.

The modulation of peak HVR and hypoxic ventilatory roll-off in the prenatal nicotine-exposed group in the present study was primarily due to changes in $Vt$. Hafstrom and coworkers (16) have demonstrated that nicotine-exposed rat pups have a decrease in $Vt$ at baseline and further speculated that prenatal nicotine-exposed developing animals may have a diminished ability to increase alveolar ventilation through augmented $Vt$. Our experiment revealed that the capability to increase $Vt$ during initial hypoxic challenge was indeed limited in nicotine-exposed pups, even though we did not find any difference in baseline $Vt$. The mechanism underlying the effect of nicotine on the ability to mount $Vt$ during ventilatory challenge is
unknown. Prenatal nicotine exposure has been shown to alter lung function in newborn rhesus monkeys, which may limit the ability to increase Vt during respiratory challenge (36). In fact, we found that nicotine-exposed rat pups had significantly less weight than control animals only at 5 days postnatal age. This difference in weight may account for differences in lung and chest wall compliance, thereby limiting the animal’s ability to increase Vt. The other alternative mechanism is that nicotine exposure may interfere with development of neural cells in respiratory control areas that control Vt.

The effect of prenatal nicotine exposure on the hypoxic ventilatory roll-off component of HVR may have a significant functional implication. Previous study from our laboratory has shown that activation of platelet-derived growth factor (PDGF)-β receptor occurs in the brain stem regions, such as the nucleus of solitary tract during hypoxic ventilatory roll-off. This activation may underlie a crucial role in neuroprotection and survival strategies during oxygen deprivation (14). In fact, hypoxia-induced activation of the PDGF-β receptor in the CB is temporally associated with activation of an antiapoptotic mechanism via phosphatidylinositol 3-kinase-dependent phosphorylation of both Akt and BAD pathway (38). In addition, activation of PDGF-β receptor is an important contributor to hypoxic ventilatory roll-off at all postnatal ages but more critical in developing animals (45). Because prenatal nicotine exposure is associated with modulation of hypoxic ventilatory roll-off, it could lead to perturbation of this important protective mechanism, which may lead to decreased hypoxic tolerance of brain stem neurons. In fact, nicotine exposure has been previously shown to increase neuronal cell death in the medulla (26). Additional study is required to assess the effect of prenatal nicotine exposure on hypoxia-induced PDGF-β receptor phosphorylation and subsequent activation of downstream anti-apoptotic pathway. This perturbation in the anti-apoptotic mechanism may underlie the association between prenatal smoke exposure and SIDS.

Our study also revealed that prenatal nicotine exposure resulted in modulation of expression of specific PKC isoforms
in the CB of rat pups. Previous studies indicated the important role of PKC in the CB on both excitatory and inhibitory respiratory neurons. Haji et al. (19) showed that PKC activation in the CB increased tonic activity and excitability of expiratory neurons, whereas Morin-Surun et al. (32) demonstrated that activation of phospholipase C and subsequent PKC activation was associated with a reduction of hypoglossal activity. In the adult rat, certain PKC isoforms are expressed within the dorsocaudal brain stem nuclei, where they play an important role in hypoxic chemotransduction (12, 15). In addition, developing animals demonstrate increased dependency on PKC-mediated signal transduction pathways for generation and maintenance of respiratory timing mechanisms, and expression of certain PKC isoforms is more prominent in young pups (3). In this experiment, only PKC-β and PKC-δ expression were increased in prenatal nicotine exposure. Both PKC isoforms have been previously shown to be most likely PKC candidates mediating HVR (37). Furthermore, the onset of hypoxic ventilatory roll-off has been shown to correspond with the decline in overall PKC activity, particularly the reduction in PKC-β and PKC-δ (11). The increased expression of both PKC isoforms in the nicotine-exposed group may account for the decrease in the magnitude of the hypoxic ventilatory roll-off in these developing animals.

The present study has revealed different changes in PKC isoform expression pattern than our laboratory’s previous report on the effect of prenatal exposure to cigarette smoke (20). Because cigarette contains several complex chemical ingredients with >4,000 compounds, including nicotine, carbon monoxide, etc. (7, 43). Interaction between different substances in cigarettes may lead to different changes in the expression of PKC isoforms. The dose of nicotine in this study was 6 mg/kg maternal wt−1day−1. This dose has been previously shown to yield blood levels of nicotine corresponding to two to four times the baseline present in smokers (5, 6, 21).

Our study did not elucidate the mechanism underlying the effect of nicotine on PKC activity. PKC expression is more prominent in respiratory control areas in the brain stem, especially in the dorsocaudal brain stem (15). In human fetuses, a high concentration of nicotine binding sites is found in the respiratory control area of the CB (25). The effect of nicotine on PKC has been previously studied in cell lines. Stimulation of nicotinic receptor leads to activation and translocation of PKC (28, 41). Administration of cotinine, a slowly eliminated metabolite of nicotine, is associated with stimulation of nicotinic receptor and subsequent PKC activation (44). These studies suggest that nicotine exposure may directly affect PKC activity through the nicotinic receptor. The other putative mechanism may involve the indirect effect of nicotine on PKC through N-methyl-D-aspartate glutamate receptor. In fact, nicotine exposure has been shown to modulate N-methyl-D-aspartate glutamate receptor in certain areas of the developing brain (23). Furthermore, nicotine specifically alters the channel property of glutamate receptors in neurons mediating inspiratory drive (35).

The present study has certain limitations. First, postnatal nicotine exposures through breast milk cannot be excluded because the osmotic pumps used in this experiment can deliver nicotine solution for 28 days. Because the pump was implanted at 5 days of pregnancy, nicotine delivery could continue up to 10 days postnatal age. Even though the modulation of HVR was noted only at postnatal day 5 but not at postnatal day 10, the dose of nicotine per body weight through the milk was much greater in postnatal day 5 than in postnatal day 10. Thus the changes in nicotine-exposed animals could still be secondary to postnatal exposures, and the difference between the two groups was based on the dose effect. Second, whole body plethysmography may have some limitations in quantification of very small V˙t and V˙E (2). However, we analyzed the data by using the percent increases from baseline, which may eliminate the problem with absolute value. Third, the PKC expression was assessed only in the CB; other areas of the brain were not evaluated. This is based on our laboratory’s previous study, which indicated the important role of specific PKC isoforms in the CB on respiratory pattern and HVR in developing animals (3). The function of PKC in other areas of the brain, e.g., pons or midbrain, on the control of breathing has not been previously examined. Finally, our study did not provide specific information regarding the particular area within the CB involved in these PKC changes.

In conclusion, prenatal nicotine exposure is associated with modulation of biphasic HVR and modification of specific PKC isoforms previously identified as playing an important role in respiratory control. The alteration in biphasic HVR and PKC isoforms may underlie the association between maternal cigarette smoking and SIDS. The result also suggests that substitution of smoking during pregnancy with a nicotine patch may not be a safe alternative. Further study is essential to evaluate the impact of alteration in hypoxic ventilatory roll-off in prenatal nicotine exposure on the antiapoptotic mechanism in the CB of developing animals.

ACKNOWLEDGMENTS

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