THE EFFECTS OF NICOTINE EXPOSURE ON THE RESPIRATORY RHYTHM

GENERATOR OF NEONATAL RATS

By:

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Abstract

Many children are exposed to environmental tobacco smoke prenatally and postnatally, which can have adverse health affects throughout life, including an increased risk of low birth weight, Sudden Infant Death Syndrome (SIDS), obstructive lung disease, cancers, childhood infections, and altered neurodevelopment. The goal of this study was to determine whether postnatal nicotine exposure affects the respiratory rhythm generator in neonatal rats. Brainstem spinal cord preparations from neonatal rats will be exposed to small amounts of nicotine. Spontaneous respiratory activity from C4-C5 ventral roots were recorded with glass suction electrodes. C4-C5 ventral roots contain the phrenic motor axons that supply the diaphragm muscle. The results indicate that nicotine exposure affects the respiratory rhythm generator and causes an increase in respiratory frequency.
Introduction

In the United States, approximately 38% of children are exposed to environmental tobacco smoke, also known as secondhand smoke, in the home (Gergen, 1988). Prenatal and early postnatal exposure to tobacco smoke has a wide range of adverse health effects, including an increased risk of low birth weight, Sudden Infant Death Syndrome (SIDS), obstructive lung disease, cancers, childhood infections, and altered neurodevelopment (Hofhuis, 2003).

It is well established that nicotine, a main constituent of tobacco smoke, can affect multiple organs in the fetus and newborn, potentially with life-long consequences for the rest of the newborns life (Maritz, 2011). Exposure to nicotine during gestation and lactation is associated with reducing lung function of the offspring, which can lead to accelerating lung aging and putting the lungs at a higher chance of obstructive lung disease (Maritz, 2011).

In North America 20-25% of pregnant women smoke tobacco even though studies show smoking during pregnancy is the leading cause of fetal morbidity and mortality (Jiménez, 2006). Experiments show that when neonatal rats are exposed to nicotine in milk during lactation, their circulating catecholamine concentrations were higher than those of controls. These high levels of catecholamine levels or early adrenal medullary dysfunction caused by nicotine exposure in the milk, may have later impact on the cardiovascular function (Maritz, 2011).

From birth to death, respiration is essential to survive. Respiratory dysfunction is the primary factor determining morbidity and mortality (Wang,
Acetylcholine plays a role in central respiratory control system (Burton et al. 1995). Nicotine acts similar to Acetylcholine by docking to Acetylcholine receptors and producing similar effects (Shao, 2000). Nicotine, a neurotetratogen present in tobacco, is the main ingredient linking cigarette smoking to cardiorespiratory dysfunctions (Hafstrom et al., 2005). One respiratory dysfunction that is commonly studied is Sudden Infant Death Syndrome (SIDS); SIDS is correlated with maternal smoking and nicotine exposure from the breastmilk, which causes higher levels of circulating levels of nicotine in the infants (Haglund and Cnattingius 1990). Although the exact cause of SIDS is not precisely known, most studies have concluded that it results from a failure in the respiratory rhythm generator (Nattie and Kinney, 2002).

Activation of nicotinic Acetylcholine receptors from exposure to Acetylcholine evokes fast cation influx and causes membrane depolarization (Chamberline et al. 2002). In an experiment using neonatal rat brain stem-spinal cord preparations, an injection with acetylcholine into a bath chamber increased the respiratory frequency (Shao, 2001). Consequently, the question remains: what effect does nicotine have on the respiratory rhythm of neonatal rats? Based on previous studies, our hypothesis is that nicotine, will act similar to Acetylcholine, and will increase the respiratory frequency of neonatal rats. We will test our hypothesis by recording spontaneous respiratory activity from C4-C5 ventral roots using a glass suction electrode. C4-C5 ventral roots contain the phrenic motor axons that supply the diaphragm muscle.
**Methods**

Experiments were performed on Sprague-Dawley rats of either sex between the ages of 1-5 days of life. All procedures were in accordance with guidelines provided by the Institutional Animal Care and Use Committee (IACUC) at the University of Arizona. All neonates were born by vaginal delivery from unexposed pregnant mother rats. Neonates were housed together with their mothers and siblings until the day of study. Mothers had unrestricted access to food and water. All animals were kept in a quiet room at 21-23°C, 20-30% humidity, on a 12/12 hour light/dark cycle.

Neonatal rats were cooled rapidly on ice. Following hypothermic analgesia, the brain stem and spinal cord were removed while immersed in artificial cerebrospinal fluid. The artificial cerebrospinal fluid is composed of (in mM): 124 NaCl, 5 KCl, 2.4 CaCl₂, 1.3 21.3 MgSO₄, 26 NaHCO₃, 1.2 KH₂PO₄, and 30 D-glucose. The solution was equilibrated with 95% Oxygen- 5% Carbon Dioxide (pH 7.4).
The isolated brainstem/spinal-cord preparation (figure 1) was transferred to a bath chamber containing artificial cerebrospinal fluid and positioned ventral side up. The extracellular K+ concentration of the artificial cerebrospinal fluid was raised by 6 mM from 6 to 9 mM to facilitate spontaneous respiratory network activity (Smith et al. 1991). Elevated K+ has been shown to strengthen the frequency and magnitude of synaptic input onto respiratory neurons (Okada et al. 2005).

Spontaneous activity from C4-C5 ventral roots was recorded with glass suction electrodes. Electrical signals were recorded and analyzed. Experiments were conducted at room temperature. Glass pipettes were pulled from glass capillary tubes. The tip of the glass electrode was placed in contact with the ventral surface of the brain stem. The recordings were band-pass filtered (30-1,000 Hz), and captured using Spike Analysis software. Fifteen-20 minute recordings were obtained after stable ventral root activity was established.

Two hundred µl of 0.5 mM nicotine solution was added to 200 mL of artificial cerebrospinal fluid. Therefore, the solution consisted of 200 mL of artificial cerebral spinal fluid with 0.5µM nicotine. The stopcock was then switched so that only the nicotine artificial cerebrospinal fluid was flowing into the bath chamber containing the brain stem and spinal cord. Fifteen-20 minute recordings were obtained.

The stopcock was then switched back to the original artificial cerebrospinal fluid, and 15-20 minute recordings were obtained. All data were recorded using Spike Analysis software.
Results

The respiratory rhythm of neonatal rats was recorded when the artificial cerebrospinal fluid either contained or did not contain nicotine. When the bath chamber contained nicotine the respiratory frequency increased as shown in Figure 2. Figure 2A represents a control recording, when the brainstem-spinal cord was submerged in artificial cerebral spinal fluid. Figure 2B shows the increase of spike frequency with 0.5μM of nicotine. A concentration of 0.5μM nicotine was chosen because it is equivalent to the arterial blood nicotine concentration shortly after a cigarette has been smoked (Henningfield et al. 1993). Figure 2C illustrates how the frequency returned to normal after the nicotine was removed. Time scales (X-axis) for all scenarios are identical (160 seconds).
Under control conditions, when the brain stem-spinal cord was in artificial cerebral spinal fluid, the average time between spikes was 11.43 seconds with a standard deviation of ±1.044. When the brainstem-spinal cord was immersed in 0.5μM nicotine, the average time between spikes was decreased to 6.4 seconds with a standard deviation of ±0.983. Therefore, the frequency increased and there were more spikes per minute. Finally, after removing the nicotine, the average time between spikes returned to approximately 12.3 seconds with a standard deviation of ±1.138. Table 1 summarizes these conclusions.

Table 1:

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<th>Time (sec.)</th>
<th>Amount of Spikes in time frame</th>
<th>Average time between spikes (sec)</th>
<th>Standard Deviation</th>
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<tbody>
<tr>
<td>Artificial Cerebrospinal fluid</td>
<td>160</td>
<td>14</td>
<td>11.43</td>
<td>±1.044</td>
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<td>Artificial Cerebrospinal fluid with Nicotine</td>
<td>160</td>
<td>25</td>
<td>6.4</td>
<td>±0.983</td>
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<td>Artificial Cerebrospinal fluid</td>
<td>160</td>
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<td>12.3</td>
<td>±1.138</td>
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The hypothesis of the current study states that: nicotine, will act similarly to Acetylcholine, and will increase the respiratory frequency of neonatal rats. Consistent with the hypothesis, the presence of nicotine in the bath chamber increased the respiratory frequency.

Discussion

Throughout this experiment, we found that like Acetylcholine, the presence of nicotine in the bath chamber with artificial cerebrospinal fluid increases the respiratory frequency in neonatal rats. Respiratory rhythm is generated in the
brainstem; in particular, the primary site of respiratory rhythm generation is the preBotzinger Complex (Shao, 2009). In fact, the preBotzinger Complex is the critical area where Acetylcholine acts to affect the respiratory rhythm (Smith, 1991). Experiments show that microinjection of nicotine into the preBotzinger Complex in brainstem slice preparations increases respiratory frequency (Shao, 2009). Therefore, when either Acetylcholine or Nicotine are added to the bath chamber of brainstem-spinal cord preparations in neonatal rats, there will be an increase in frequency of the respiratory activity.

This study is of great importance because nicotine can be very harmful prenatally and postnatally to all mammals. Nicotine is like Acetylcholine, in which it causes an increase in respiratory frequency. However, nicotine is a neurotoxin that is not naturally produced or regulated by the body, while Acetylcholine is regulated by the body. This up regulation can cause a wide range of serious health affects, including an increased risk of low birth weight, Sudden Infant Death Syndrome (SIDS), obstructive lung disease, cancers, childhood infections, and altered neurodevelopment (Hofhuis, 2003). In many animal studies, prenatal exposure to nicotine affects neuronal development and up regulates the nicotinic acetylcholine receptors in the brain (Frank et al. 2001). Nicotinic acetylcholine receptors are present in the preBotzinger Complex (Shao 2009); therefore, there can be detrimental side affects to the respiratory rhythm generator later in life.

One limitation in this study was the small sample size. Few experiments were completed due to a short time frame and technical difficulties with the
electrical wiring. However, the experiments that were completed showed strong results and agreed with past research experiments.

There are many neurological disorders related to the respiratory control in the brainstem-spinal cord. This experiment has led to further knowledge between molecular events and respiratory behavior in neonatal rats. Since exposure to environmental tobacco smoke is so common among children, research on the effects of nicotine are of great importance. Research on the exact effects nicotine plays on the respiratory rhythm generator can provide a pharmalogical basis for treatment and prevention.
References:


Shao, X. M. & Feldman, J. L. 2000 Acetylcholine modulates respiratory pattern:
