Perinatal nicotine exposure impairs ability of newborn rats to autoresuscitate from apnea during hypoxia

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Fewell, James E., and Francine G. Smith. Perinatal nicotine exposure impairs ability of newborn rats to autoresuscitate from apnea during hypoxia. J. Appl. Physiol. 85(6): 2066–2074, 1998.—Failure to autoresuscitate by hypoxic gasping during prolonged sleep apnea has been suggested to play a role in sudden infant death. Furthermore, maternal smoking has been repeatedly shown to be a risk factor for sudden infant death. The present experiments were carried out on newborn rat pups to investigate the influence of perinatal exposure to nicotine (the primary pharmacological and addictive agent in tobacco) on their time to last gasp during a single hypoxic exposure and on their ability to autoresuscitate during repeated exposure to hypoxia. Pregnant rats received either nicotine (6 mg·kg⁻¹·24 h⁻¹) or vehicle continuously from day 6 of gestation to days 5 or 6 postpartum via an osmotic minipump. On days 5 or 6 postpartum, pups were exposed either to a single period of hypoxia (97% N₂-3% CO₂) and their time to last gasp was determined, or they were exposed repeatedly to hypoxia and their ability to autoresuscitate from primary apnea was determined. Perinatal exposure to nicotine did not alter the time to last gasp, but it did impair the ability of pups to autoresuscitate from primary apnea. After vehicle, the pups were able to autoresuscitate from 18 ± 1 (SD) periods of hypoxia, whereas, after nicotine, the pups were able to autoresuscitate from only 12 ± 2 periods (P < 0.001) of hypoxia. Thus our data provide evidence that perinatal exposure to nicotine impairs the ability of newborn rats to autoresuscitate from primary apnea during repeated exposure to hypoxia, such as may occur during episodes of prolonged sleep apnea.

THE SUDDEN INFANT DEATH SYNDROME (SIDS) is defined as “the sudden death of an infant under 1 yr of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history” (44). In North America, SIDS is a leading cause of death beyond the neonatal period during the first year of life. SIDS is thought to occur during sleep or during the transition from sleep to wakefulness, and it occurs primarily between 2 wk and 8 mo of age, with 90% of SIDS cases occurring in the first 6 mo of life (44). Numerous epidemiological studies indicate that smoking during pregnancy is a major and independent risk factor for SIDS (3, 14, 15, 25, 26, 28, 29, 32, 33); this risk increases in proportion to the number of cigarettes smoked (14). In the National Institute of Child Health and Human Development study of epidemiological factors for SIDS, the relative risk for SIDS associated with maternal smoking was 3.4; this was higher than any other maternal or newborn condition evaluated, with a frequency of smoking among SIDS mothers of 70% (15). It is not known, however, how maternal smoking places offspring at an increased risk for SIDS.

Cigarette smoke contains a wide variety of chemicals, including nicotine (43). Nicotine is a neurotoxin that easily crosses the placenta and is found in fetal cord blood in concentrations equal to or greater than those in maternal blood (23, 24, 39). Nicotine acts through highly selective and sensitive nicotinic cholinergic receptors and has the potential to influence the maturation of these receptors in fetal brain, autonomic ganglia, and the adrenal medulla (22, 34). Kinney et al. (19) have recently reported that human fetuses at midgestation have a heavy concentration of [³H]nicotine-binding sites in tegmental nuclei which govern cardiorespiratory integration, arousal, attention, rapid-eye-movement sleep, and somatic motor control. These [³H]nicotine-binding sites were found to decrease by 60–70% over the last half of gestation, leading the authors to suggest that mid-to-late gestation is a period when this region is most vulnerable to the harmful effects of nicotine from maternal smoking.

An inability to recover from prolonged sleep apnea has long been postulated as a possible factor in SIDS (12, 16, 17). In humans, recovery from sleep apnea is thought to occur early, as a result of arousal from sleep, or later, as a result of hypoxic gasping, when it is termed autoresuscitation (12, 41). Considering this and the aforementioned information on nicotine, one possible consequence of perinatal exposure to nicotine may be an alteration in protective responses that prevent severe hypoxia and death during prolonged sleep apnea. Our present experiments have been carried out to test the hypothesis that perinatal exposure to nicotine impairs the ability of newborn rats to autoresuscitate from primary apnea during repeated exposure to hypoxia, such as may occur during episodes of sleep apnea.

METHODS

Thirty-two Sprague-Dawley rat pups (5–6 days old) were studied. Each pup was born by spontaneous vaginal delivery and was housed with its mother and siblings (22 ± 1°C, 20–30% relative humidity, and 12:12-h light-dark cycle). Although 22°C is below the thermoneutral zone of newborn rats, each pup had the opportunity to select its ambient...
temperature between experiments by huddling with its siblings and/or mother (i.e., behavioral thermoregulation).

**Surgical Preparation**

In preparation for surgery, each pregnant rat was anesthetized on day 6 of gestation by inhalation of halothane (~2.0% for induction and maintenance) in O₂; the rat was placed in a prone position. A small incision was made over the scapulae, and a 28-day osmotic minipump (ALZET 2ML4, Alza) was inserted subcutaneously for continuous infusion of nicotine or vehicle. In this species, implantation of the embryo in the uterine wall begins on day 5 and is complete on day 7 (6).

All surgical and experimental procedures were carried out in accordance with the Guide to the Care and Use of Experimental Animals provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

**Experimental Protocols**

The respiratory response of both newborn (18) and adult (13) animals to acute hypoxia typically passes through four stages: hyperpnea, primary apnea, gasping, and terminal apnea.

**Experiment 1: Time to last gasp.** For an experiment, each 5- to 6-day-old pup was removed from its mother and siblings, weighed, and placed in a metabolic chamber regulated at 37°C into which flowed room air at a rate of 1 l/min. At the end of a 30-min stabilization period, the gas that flowed into the chamber was changed from room air to 97% N₂-3% CO₂ until primary apnea occurred. The gas was then changed

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![Polygraph Tracing](image-url)

**Fig. 1.** Three segments of a polygraph tracing showing respiratory response of 5-day-old rat pup to a single period of hypoxia. Variables shown are chamber CO₂ concentration, electrocardiogram, and respiratory pattern. Exposure to a single period of hypoxia resulted in a reproducible respiratory response that consisted of an initial period of hyperpnea (1a) and arousal (1b) that preceded primary apnea (1c). Primary apnea was followed by a period of rapid gasping that lasted 1–2 min (1d). This period of rapid gasping was followed by a period of slower gasping of 1 or 2 gasps/min that lasted for 6–8 min (1e). Finally, there was a period of rapid gasping, which eventually waned and gave way to terminal apnea and death (1f).
back to room air, and the ability of the pup to autoresuscitate by gasping was determined. This procedure was repeated at 5-min intervals until death occurred. Again, when the gas mixture was changed, the flow rate was increased until the gas concentrations in the chamber had stabilized; the flow rate was then lowered to 1 l/min. During an autoresuscitation experiment, primary apnea was detected by directly observing respiratory movements on the polygraph tracing. Autoresuscitation was deemed to occur when heart rate and respiratory rate returned to \( \geq \) 60% of control levels within 5 min. Eight pups of mothers that had received nicotine and eight pups of mothers that had received vehicle were studied.

Experimental Apparatus

The metabolic chamber used in our experiments consisted of a double-walled Plexiglas cylinder (30 cm long, 6 cm ID) into which flowed room air or 97% N\(_2\)-3% CO\(_2\). Chamber ambient temperature was controlled to 37.0 ± 0.1°C by circulating water from a temperature-controlled bath (Neslab-Endocal Refrigerated Circulating Bath RTE-8DD) through the space between the walls.

Experimental Measurements and Calculations

During an experiment, the electrocardiogram (ECG), respiratory movements and chamber CO\(_2\) levels were recorded on a model 7 polygraph (Grass Instruments) at a paper speed of 10 mm/s. The ECG was recorded from multistranded stainless steel wire electrodes (AS 633, Cooner Wire) sewn across the chest wall that were connected to a model 7HIP5 High Impedance Probe coupled to a model 7P5 Wide Band EEG A.C. Preamplifier (Grass Instruments). Respiratory movements were recorded from a model HgPC mercury-in-silicone rubber strain gauge (D. M. Davis) placed around the chest and connected to a bridge amplifier (Biomedical Technical Support Center, University of Calgary), which was coupled to a model 7P03 Adapter Panel (Grass Instruments). Chamber CO\(_2\) levels were measured by using an Applied Electrochemis-
try Carbon Dioxide Analyzer (Ametek) that was coupled to a model 7P03 Adapter Panel.

Nicotine

Nicotine (hydrogen tartrate salt, Sigma Chemical) was dissolved in sterile water at a concentration of 33 mg/ml and infused at a rate of 60 µl/day to give a dose of ~6 mg·kg⁻¹·24 h⁻¹ based on a final average weight of 330 g in the nicotine-exposed group of rat dams. This dosage regimen produces plasma levels of nicotine comparable to those observed in humans who smoke heavily (22, 30). Sterile water was used as vehicle.

Statistical Analysis

Statistical analysis was carried out by using an unpaired t-test to determine whether or not perinatal exposure to nicotine affected the time to last gasp or the number of successful autoresuscitations. A two-factor ANOVA for repeated measures, followed by a Newman-Keuls multiple-comparison test, was applied to determine whether time or drug affected gasping rate or heart rate after single hypoxic exposures and affected heart rates and respiratory rates during normoxemia after repeated hypoxic exposures. All results are reported as means ± SD, and P < 0.05 was considered to be of statistical significance.

RESULTS

Experiment 1: Time to Last Gasp

Perinatal exposure to nicotine did not significantly alter control respiratory rate (vehicle, 131 ± 44 breaths/min; nicotine, 140 ± 27 breaths/min) or heart rate (vehicle, 363 ± 33 beats/min; nicotine, 358 ± 23 beats/min) in the 5- to 6-day-old rat pups. Furthermore, there was no effect of perinatal exposure to nicotine on the time to last gasp (vehicle, 998 ± 98 s; nicotine, 1,095 ± 152 s). Exposure to a single period of hypoxia resulted in a reproducible respiratory response in both groups of

Fig. 4. Continuous polygraph tracing from a 5-day-old rat pup showing a successful autoresuscitation from primary apnea. During exposure to hypoxia, an initial period of hyperpnea (4a) and arousal (4b) preceded primary apnea and bradycardia (4c). Gasing was followed by an increase in heart rate (4d) and then restoration of normal respiratory pattern (4e).
animals, as illustrated in Fig. 1. Initially, there was a period of hyperpnea (1a) and arousal (1b) which preceded primary apnea (1c). Primary apnea was followed by a period of rapid gasping that lasted 1–2 min (1d). This period of rapid gasping was followed by a period of slower gasping of 1–2 gasps/min that lasted for 6–8 min (1e). Finally, there was a period of rapid gasping, which eventually waned and gave way to terminal apnea and death (1f). There were no significant effects of nicotine on the gasping or heart rate pattern in a single period of hypoxia (Figs. 2 and 3).

Experiment 2: Autoresuscitation

Perinatal exposure to nicotine impaired the ability of the rat pups to autoresuscitate from primary apnea. After perinatal exposure to vehicle, the pups were able to autoresuscitate from 18 ± 1 periods of hypoxia; however, after perinatal exposure to nicotine, the pups were able to autoresuscitate from only 12 ± 2 (SD) periods (P < 0.001) of hypoxia. Before autoresuscitation failure, all successful autoresuscitations exhibited the same cardiorespiratory pattern illustrated in Fig. 4. Initially, there was a period of hyperpnea (4a) and arousal (4b) that preceded primary apnea and bradycardia (4c). Gasping was followed by an increase in heart rate (4d) and then restoration of a normal respiratory pattern (4e). There was no effect of perinatal exposure to nicotine on heart rate or respiratory rate during normoxemia after repeated exposure to hypoxia (Figs. 5 and 6). However, the mechanism of autoresuscitation failure appeared to be different in the pups that were exposed to vehicle and in the pups that were exposed to nicotine. In all pups that were exposed to vehicle during the perinatal period, autoresuscitation failure appeared to result from atrial-ventricular (A-V) dissociation that followed early cardiac resuscitation as evidenced by an initial return of heart rate toward control. The A-V dissociation and ultimate loss of ventricular depolarization preceded the cessation of gasping (Fig. 7). In six of the eight pups that received nicotine during the perinatal period, however, gasping ceased before signs of cardiac resuscitation appeared on the ECG (Fig. 8).

**DISCUSSION**

Our experiments provide new information about factors that influence the newborn’s ability to survive hypoxia as may occur during prolonged sleep apnea. A novel finding in the present study was that, although perinatal exposure to nicotine did not alter the time to last gasp during a single hypoxic exposure, it did impair the ability of rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia. Furthermore, perinatal exposure to nicotine influenced the mechanism of autoresuscitation failure. In all pups that were exposed to vehicle during the perinatal period, autoresuscitation failure appeared to result from A-V dissociation that followed early cardiac resuscitation. The A-V dissociation and ultimate loss of ventricular depolarization preceded the cessation of gasping. In six of the eight pups that received nicotine during the perinatal period, however, gasping ceased before signs of cardiac resuscitation appeared on the ECG. Thus our data provide evidence that perinatal exposure to nicotine not only impairs the ability of newborn rats to autoresuscitate from primary apnea during repeated exposure to hypoxia but that nicotine exposure also alters the mechanism of autoresuscitation failure.

Exposure to a single period of hypoxia resulted in a reproducible respiratory response in rat pups that received nicotine well as in rat pups that received vehicle during the perinatal period. The respiratory response consisted of hyperpnea, primary apnea, gasping, and terminal apnea. In all animals, terminal apnea preceded the appearance of arrhythmias or an isoelectric pattern on the ECG. Of course, the lack of arrhythmias or an isoelectric pattern on the ECG does not ensure that an adequate blood pressure, which is essential for delivery of metabolic substrate (i.e., glucose) to the brain during lack of O₂, was maintained. However, previous experiments by Swann et al. (40) on 4-day-old dogs and experiments by Cassin et al. (4) on 1-day-old dogs, rabbits, and cats have shown that newborns of these species maintain their blood pressure at viable levels long after terminal apnea.
more, Stafford and Weatherall (37) have shown that neither liver glycogen or blood glucose levels determine survival time during exposure of normal newborn rats to nitrogen. In our present experiments, then, it appears that medullary loci critical for gasping [which in the rat are located in the lateral tegmental field and are most likely distinct from those responsible for eupnea (8)] fail before cardiovascular collapse occurs during acute hypoxia. Although it is well known that maturity at birth (1), postnatal age (2, 5, 11, 37), and body temperature (2, 7, 27, 37) influence the time to last gasp after exposure to a single period of hypoxia, little is known of the actual mechanisms that influence the pattern or duration of gasping. Regardless of the mechanism, our present experiments show that perinatal exposure to nicotine does not alter the gasping or heart rate pattern or the time to last gasp during a single hypoxic exposure.

Peiper (31), Stevens (38), and Thach (41) have emphasized the importance of gasping in self-resuscitation or autoresuscitation during apnea in human infants and that repeated episodes of apnea may lead to autoresuscitation failure and death. The process of recovery from hypoxia by gasping was first termed “self-resuscitation” in 1969 by Adolph (1) and then “autoresuscitation” in 1975 by Guntheroth (13). Gershan et al. (9) have recently defined the cardiorespiratory events that occur during successful autoresuscitation from hypoxic apnea in mice. These consisted of three sequential stages: 1) gasping with marked bradycardia, 2) cardiac resuscitation, and 3) full return of ventilation and heart rate.
tation with a rapid increase in heart rate to >60% of baseline, and 3) respiratory resuscitation with an increase in respiratory rate to >60% of baseline. We observed a similar sequence of events during successful autoresuscitation in our rat pups. Similarly, we found, as did Gershan et al. (10), that repeated exposure to hypoxia led to autoresuscitation failure that was associated with cardiac arrhythmia (i.e., A-V dissociation) that preceded cessation of gasping in normal animals. Another novel finding in our present experiments was that perinatal exposure to nicotine not only impaired the ability of rat pups to autoresuscitate after repeated exposure to hypoxia but also altered the sequence of events leading to autoresuscitation failure. Although our experiments were not designed to investigate the mechanism of the changes in the physiology of this protective response after perinatal administration of nicotine, there are a number of possibilities.

Nicotine is a neuroteratogen that easily crosses the placenta and is found in fetal cord blood in concentrations equal to or greater than those in maternal blood (23, 24, 39). Nicotine acts through highly selective and sensitive nicotinic cholinergic receptors and has the potential to influence the maturation of these receptors in fetal brain, autonomic ganglia, and in the adrenal medulla (22, 34). Slotkin et al. (35) have recently shown that prenatal exposure to nicotine produces a dose-dependent increase in mortality in 1-day-old rat pups during a 75-min exposure to 5% \( \text{O}_2 \). These investigators have also shown that, although prenatal exposure to nicotine (6 mg·kg\(^{-1}\)·day\(^{-1}\)) does not affect total adrenal catecholamine content, it does impair non-neurogenic-mediated catecholamine release, which plays a vital role in modulating cardiovascular, respiratory, and metabolic responses to hypoxia (20, 36). Early in postnatal life, rat adrenal chromaffin cells possess a developmentally regulated \( \text{O}_2 \)-sensing mechanism, similar to that of carotid body type I cells, that is responsible for this non-neurogenic-mediated catecholamine release (42). Thus it is possible that perinatal exposure to
nicotine altered non-neurogenic-mediated catecholamine release that impaired the ability of our rat pups to autoresuscitate during repeated exposure to hypoxia. This postulate requires further investigation, as do possible dose- and age-dependent effects of nicotine on the ability of rat pups to autoresuscitate during repeated exposure to hypoxia.

The results of our experiments provide insight into how maternal smoking may place offspring at an increased risk of SIDS. As previously discussed, an inability to recover from prolonged sleep apnea has long been postulated as a factor in SIDS (12, 16, 17), and recovery from sleep apnea is thought to occur either early, as a result of arousal from sleep, or later, as a result of hypoxic gasping, when it is known as autoresuscitation (12, 41). Given the recent results of Lewis and Bosque (21), who showed that infants of smoking mothers have an impaired arousal response to hypoxia, and our present findings, that perinatal exposure to nicotine impairs the ability of rat pups to autoresuscitate during repeated exposure to hypoxia, we speculate that maternal smoking places infants who have apnea from whatever cause at increased risk for severe hypoxia and death because of an impairment of protective responses that terminate apnea and restore normal tidal ventilation.

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