Adverse Effects of Nicotine and Interleukin-1β on Autoresuscitation After Apnea in Piglets: Implications for Sudden Infant Death Syndrome

J. Frederik Frøen, MD*‡; Harriet Akre, MD§; Babill Stray-Pedersen, MD, PhD‡; and Ola Didrik Saugstad, MD, PhD*

ABSTRACT. Objectives. Maternal cigarette smoking is established as a major dose-dependent risk factor for sudden infant death syndrome (SIDS). Both prenatal and postnatal exposures to constituents of tobacco smoke are associated with SIDS, but no mechanism of death attributable to nicotine has been found. Breastfeeding gives a substantial increase in absorbed nicotine compared with only environmental tobacco smoke when the mother smokes, because the milk/plasma concentration ratio of nicotine is 2.9 in smoking mothers. Furthermore, many SIDS victims have a slight infection and a triggered immune system before their death, thus experiencing a release of cytokines like interleukin-1β (IL-1β) that may depress respiration. Because apneas in infancy are associated with SIDS, we have tested the hypothesis that postnatal exposure to tobacco constituents and infections might adversely affect an infant’s ability to cope with an apneic episode. This is performed by investigating the acute effects of nicotine and IL-1β on apnea by laryngeal reflex stimulation and on the subsequent autoresuscitation.

Design. Thirty 1-week-old piglets (±1 day) were sedated with azaperone. A tracheal and an arterial catheter were inserted during a short halothane anesthesia. The piglets were allowed a 30-minute stabilization period before baseline values were recorded and they were randomized to 4 pretreatment groups (avoiding siblings in the same group): 1) immediate infusion of 10 pmol IL-1β intravenously/kg (IL-1β group; n = 8); 2) slow infusion of 5 μg nicotine intravenously/kg 5 minutes later (NIC group; n = 8); 3) both IL-1β and NIC combined (NIC + IL-1β group; n = 6); or 4) placebo by infusion of 1 ml 9% NaCl (CTR group; n = 8). Fifteen minutes later, apnea was induced by insufflation of .1 ml of acidified saline (pH = 2) in the subglottic space 5 times with 5-minute intervals, and variables of respiration, heart rate, blood pressure, and blood gasses were recorded.

Results. Stimulation of the laryngeal chemoreflex by insufflation of acidified saline in the subglottic space produced apneas, primarily of central origin. This was followed by a decrease in heart rate, a fall in blood pressure, swallowing, occasional coughs, and finally autoresuscitation with gasping followed by rapid increase in heart rate, rise in blood pressure, and (in the CTR group) an increase of respiratory rate. Piglets pretreated with nicotine had more spontaneous apneas, and repeated spontaneous apneas caused an inability to perform a compensatory increase of the respiratory rate after induced apnea. This resulted in a lower SaO2 than did CTR at 2 minutes after apnea (data shown as median [interquartile range]: 91% [91–94] vs 97% [94–98]). The pretreatment with IL-1β caused prolonged apneas in piglets and an inability to hyperventilate causing a postapneic respiratory rate similar to the NIC. When nicotine and IL-1β were combined, additive adverse effects on respiratory control and autoresuscitation compared with CTR were observed: NIC + IL-1β had significantly more spontaneous apneas the last 5 minutes before induction of apnea (2 [3–6] vs 0 [0–0]). Apneas were prolonged (46 seconds [39–51] vs 26 seconds [22–31]) and followed by far more spontaneous apneas the following 5 minutes (6.6 [4.0–7.9] vs .5 [1.2–9]). Instead of normal hyperventilation after apnea, a dramatic decrease in respiratory rate was seen (at 20 seconds: −45% [−28 to −53] vs +29% [±24–±50], and at 60 seconds: −27% [−23 to −32] vs +3% [2 to 6], leading to SaO2 below 90% 3 minutes after end of apnea: 89% (87–93) versus 97% (95–98). These prolonged adverse effects on ventilation were reflected in lowered PaO2, elevated PaCO2 and lowered pH 2 minutes, and even 5 minutes, after induction of apnea.

Conclusions. Nicotine interferes with normal autoresuscitation after apnea when given in doses within the range of what the child of a smoking mother could receive through environmental tobacco smoke and breast milk. This is seriously aggravated when combined with the presence of IL-1β that is released during infections. This experimental model with piglets may shed light on important mechanisms involved in the cause of SIDS.

ABBREVIATIONS. SIDS, sudden infant death syndrome; IL-1β, interleukin-1β; CRP, C-reactive protein; IL-6, interleukin-6; IL-1β group, 10 pmol IL-1β intravenously/kg; NIC group, 5 μg nicotine intravenously/kg; NIC + IL-1β group, both IL-1β and NIC combined; CTR group, placebo.

Maternal cigarette smoking is established as a major dose-dependent risk factor for sudden infant death syndrome (SIDS). For obvious reasons, most studies on pathology and epidemiology in SIDS cannot differentiate between the effects of maternal smoking during pregnancy and infancy. A few studies found both prenatal and postnatal exposure to be important separate and additive risk factors, and the father’s smoking was found to

From the *Departments of Pediatric Research and JObstetrics and Gynecology, Institute of Surgical Research, The National Hospital, University of Oslo; and §Department of Otorhinolaryngology, Sleep Related Breathing Disorders Unit, Ullevål University Hospital, Oslo, Norway. Received for publication Apr 27, 1999; accepted Nov 29, 1999. Reprint requests to (J.F.F.) Department of Pediatric Research, The National Hospital, University of Oslo, Pilestredet 32, N-0027, Oslo, Norway. E-mail: j.f.froen@klinmed.uio.no PEDIATRICS (ISSN 0031 4005). Copyright © 2000 by the American Academy of Pediatrics.
be a risk factor as well. However, the mechanisms involved are unknown.

Investigators have emphasized the chronic fetal hypoxia with low birth weight and brainstem gliosis seen in SIDS. Nonetheless, SIDS victims of non-smoking mothers do not have low birth weight, and they have little, if any, brainstem gliosis. Thus, although prenatal exposure to tobacco constituents has a causal association both to these 2 conditions and SIDS, the hypothesized causal association between them and SIDS remains to be demonstrated.

Infants can be exposed to nicotine both through environmental tobacco smoke and breastfeeding. There have been few investigations studying the possibility that the depressive effect on respiration of postnatal exposure to nicotine could affect respiratory regulation in infants, in addition to the increased susceptibility to airway infections. Interleukines are released during infections, and interleukin-1β (IL-1β) has been shown to prolong apnea and interfere with the subsequent autoresuscitation.

We have tested the hypothesis that postnatal exposure to tobacco constituents and infections might adversely affect an infant’s ability to cope with an apneic episode by investigating the acute effects of nicotine and IL-1β on apnea by laryngeal reflex stimulation, and on the subsequent autoresuscitation.

METHODS

The experimental protocol was approved by the hospital’s ethics committee for animal studies under the surveillance of the Norwegian Animal Research Authority, and performed by certified category C researchers of the Federation of European Laboratory Animal Science Associations.

Forty-two 1-week-old piglets (±1 days) were transported (1 hour) from a local farm on the day of the experiment. Thirty minutes before surgery, they were premedicated with the butyrophenone azaperone (5 mg/kg intramuscular) that exerts an α-adrenergic blocking action. Halothane was administered up to 2% in 40% oxygen during surgery (avg). Local analgesia was achieved with a total of 2 mL 5% lidocaine/2.5% upivacaine on the throat and inside of the left hind leg before skin incisions for cannulation of the trachea for insertion of a 0.45-mm (outer diameter) catheter into the immediate subglottic space and insertion of a Portex 0.58-mm (inner diameter) catheter in the left femoral artery. The piglets were put in the prone position in a sling with their legs hanging freely. Rectal temperature was maintained between 38.0 and 39.5°C by means of a heating lamp. A peripheral ear vein was cannulated for infusion of a solution containing 7.5% NaCl and 1.25% glucose at a rate of 10 mL/kg/hour throughout the experiment. A pulse oximeter was attached to the right foreleg. Heart rate was monitored via skin electrodes. The arterial catheter was connected to a strain gauge transducer for continuous recording of blood pressure on a Gould recorder 2600S (Gould Inc Recording Systems, Cleveland, OH). Airflow was monitored by external thermistors placed in front of the nostrils, and strain gauge belts around abdomen and thorax monitored breath movements (Alice 3, Healthdyne International, Brussels, Belgium). Data were recorded using Alice 3 software.

Blood samples were taken postoperatively for analysis of blood glucose, blood gas analysis, and hematocrit. (.75 mL); 1.5 mL was sampled in precooled ethylenediamine-tetraacetic acid tubes and immediately centrifuged at 3000 rpm for 10 minutes at 4°C, and .5 mL plasma was used for immediate analysis of plasma (CRP). All extracted blood was replaced by 3 times the volume of 9% NaCl.

Eight piglets were used in a pilot study and were not included in the sample study. Thirty piglets (16 female and 14 male; mean weight 2131 g ± 146 95% confidence interval) were included with clinical signs of disease (Tp: 38.0–39.5°C; Hb: 5.0–13.0 g/dL; Hct: 20%–40%; white blood cell count: 3.5–11.0 × 10⁹/L; CRP <5 mg/L; blood glucose: 4–10 mg/L; SaO₂ >90%; PaO₂: 10–15 kPa; PacO₂: 4.5–6.0 kPa and pH: 7.35–7.45). Four piglets were excluded, respectively, attributable to anemia, elevated CRP, diarrhea, and sudden arousal after apnea necessitating acute administration of an overdose pentobarbital intravenously to avoid unnecessary distress.

The included piglets were allowed 30 minutes stabilization before recording of baseline values and randomization (avoiding siblings in the same group) to 4 pretreatment groups: 1) immediate infusion of 10 pmol IL-1β intravenously/kg (IL-1β group; n = 8); 2) slow infusion of 5 μg nicotine intravenously/kg 5 minutes later (NIC group; n = 8); 3) both IL-1β and NIC combined (NIC + IL-1β group; n = 6); or 4) placebo by infusion of 1 mL 9% NaCl (CTR group; n = 8). Fifteen minutes later, apnea was induced by insufflation of 1 mL 9% NaCl acidified with HCl to a pH of 2 through the tracheal catheter. Apnea was induced 2 times with 5-minute intervals, and arterial blood gas analysis (25 mL) performed at the start, 2 and 5 minutes after each apnea. Experiments were ended 15 minutes after the last apnea by halothane anesthetia and an overdose of pentobarbital intravenously.

Apnea was defined as no airflow for >5 seconds, and end of apnea was defined as the start of a respiratory movement producing airflow not followed by continued apnea >5 seconds.

Nicotine hydrogen tartrate was purchased from Sigma-Aldrich, Oslo, Norway. Recombinant IL-1β (Escherichia coli, human sequence) was purchased from R&D Systems Europe Ltd, Oxon, UK.

Statistics were performed on StatView for Windows, Version 5.0 (SAS Institute Inc, Cary, NC). Differences among the 5 consecutive apneas were insignificant, and data are based on the means of all 5 apneas, except baseline values recorded before the first apnea. Mann–Whitney U test was applied when comparing 2 groups, Kruskal–Wallis test when comparing all 4 groups, and paired sign test when comparing changes within a group. An exception is the SaO₂ values, which are presented with results of repeated measurement analysis of variance and 2-sample t tests. Mann–Whitney U test was also applied and yielded similar results. Post hoc Bonferroni corrections for repeated comparisons were applied when appropriate.

RESULTS

Insufflation of acidified saline in the subglottic space produced apneas, primarily of central origin, followed by decrease in heart rate, a fall in blood pressure, swallowing, occasional coughs, and finally autoresuscitation with gasping followed by rapid increase in heart rate, rise in blood pressure, and (in CTR) increase of respiratory rate (Table 1 and Fig 1). Pretreatment with nicotine caused more spontaneous apneas (P < .04; Fig 2), and repeated spontaneous apneas caused an inability to perform a compen-

| TABLE 1. BP and HR at Baseline Before Apnea, the Lowest Value During Apnea, and the Highest Value After Apnea* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Groups                          | Before Apnea    | During Apnea    | After Apnea     | Before Apnea    | During Apnea    | After Apnea     | Before Apnea    |
|                                 | BP              | HR              | BP              | HR              | BP              | HR              | BP              |
| NIC + IL-1β                     | 50 ± 6          | 120 ± 19        | 44 ± 4          | 89 ± 14         | 58 ± 6          | 220 ± 31        | 50 ± 6          |
| IL-1β                           | 56 ± 8          | 137 ± 23        | 46 ± 8          | 90 ± 16         | 68 ± 6          | 173 ± 26        | 56 ± 8          |
| NIC                             | 60 ± 4          | 157 ± 14        | 50 ± 6          | 97 ± 25         | 66 ± 6          | 205 ± 42        | 60 ± 4          |
| CTR                             | 58 ± 6          | 132 ± 21        | 50 ± 6          | 78 ± 14         | 68 ± 6          | 181 ± 14        | 58 ± 6          |

* Data are mean ± 95% confidence interval. BP indicates blood pressure; HR, heart rate.
satory increase of the respiratory rate after induced apnea ($P < .03$; Fig 1). This resulted in a lower $\text{SaO}_2$ than CTR at 2 minutes after apnea (data shown as median [interquartile range]: 91% [91–94] vs 97% [94–98]; $P < .05$; Fig 3). In contrast, IL-1$\beta$ caused prolonged apneas ($P < .03$; Fig 2) and an inability to hyperventilate causing a postapneic respiratory rate similar to NIC ($P < .03$; Fig 1), but regaining normal $\text{SaO}_2$ after induced apnea like CTR (Fig 3). When nicotine and IL-1$\beta$ were combined, additive adverse effects on respiratory control and autoresuscitation compared with CTR were observed. NIC + IL-1$\beta$ had significantly more spontaneous apneas the last 5 minutes before induction of apnea (2 [3–3] vs 0 [0–0]; $P < .03$; Fig 2). Apneas were prolonged (46 seconds [39–51] vs 26 seconds [22–31]; $P < .01$; Fig 2) with greater fall in $\text{SaO}_2$ (Fig 3), and followed by far more spontaneous apneas the following 5 minutes (6.6 [4.0–7.9] vs 5 [2–9]; $P < .05$). The ability to hyperventilate normally after apnea as seen in CTR was abolished; and instead, a dramatic decrease in respiratory rate was seen at 20 seconds: $-45\%$ [-28 to -53] vs $+29\%$ [+24–+50], and at 60 seconds: $-27\%$ [-23 to -32] vs $+3\%$ [-2–+6]; $P < .01$; Fig 1), leading to $\text{SaO}_2$ below 90% 3 minutes after the end of apnea (89% [87–93] vs 97% [95–98]; $P < .03$; Fig 3). These prolonged adverse effects on ventilation were reflected in lowered $\text{Pao}_2$, elevated $\text{Paco}_2$ and lowered pH 2, and even 5, minutes after induction of apnea (all $P < .04$; Table 2).

**DISCUSSION**

Apnea mediated by laryngeal reflex has been implicated in several kinds of prolonged infantile apnea and has been proposed to play an important role in the apnea associated with SIDS, although it is probably stimulated frequently without adverse effects in healthy infants. Our results clearly demonstrate that nicotine combined with IL-1$\beta$ has serious additive adverse effects on this type of normally occurring apnea and the following autoresuscitation in piglets.

The sudden unexplained death in SIDS is probably attributable to a diversity of causes rather than a single syndrome as the name would indicate, and many victims have none of the risk factors associated with it. Nonetheless, our findings should be linked to SIDS as many characteristics of typical SIDS infants seem to point in the direction of a vicious circle that includes the presence of apneas: periodic breathing.

---

**Fig 1.** Change in respiratory rate (in percent) after end of induced apnea versus baseline values. Values are mean, and bars represent the standard error of the mean. *$P < .03$; and †$P < .01$ versus CTR.

**Fig 2.** The first group of columns indicates the last 5 minutes preceding the first induced apnea. The third group of columns indicates the 5 minutes after an induced apnea. Columns are median values, and bars represent the interquartile range. *$P < .03$; †$P < .01$; and ‡$P < .04$ versus CTR.
and apneas, sleep, vomiting, low gestational age, overheating, hypoxia, infection, and release of interleukines.12–16 These characteristics are separately known to enhance, produce, and prolong apneas, and interfere with normal autoresuscitation after apnea.9,11,17–19 Early in life, the infant is more susceptible to apnea by laryngeal stimulation11 and hypoxia during apnea,20 and probably responds inadequately to mild hypoxia by increased periodic breathing.21–23 Potential additive adverse effects when combined with nicotine as demonstrated in this study, could prove detrimental, and SIDS victims have often been exposed to nicotine before death.24,25

Neonatal rats exposed prenatally to nicotine had increased mortality during hypoxic challenge.26 Nonetheless, subsequent experimental studies in the neonatal rat subjected to hypoxia,27 anoxia,28 or hypercapnia,29 showed unaffected respiratory response after maternal nicotine exposure during gestation. In contrast, lambs subjected to acute infusion of nicotine showed decreased ventilation during hypoxia.30 This might indicate that postnatal exposure to nicotine has more effects on respiration during hypoxia than prenatal exposure, but how the combined effects of prenatal and postnatal exposure to tobacco constituents affect respiration has not been explored. None of these studies addressed the effects on apnea, whereas results in our study should be related both to the hypoxic event and the apnoeic reflex and autoresuscitation in the piglet sedated by azaperone.

Breastfeeding gives a substantial increase in absorbed nicotine compared with only environmental tobacco smoke when the mother smokes.6–8 In smoking mothers, the milk:plasma concentration ratio of nicotine is 2.9, whereas that of the primary metabolite cotinine is 1.2.7 Infants of smoking mothers have higher levels of cotinine excretion in urine than do adult passive smokers, and when nursed, it reaches levels in the range of adult smokers.6 These infants have altered respiration and oxygen saturation after nursing.31

The bioavailability of ingested nicotine in infants is probably not lower than in adults, where it is ~25% to 30% attributable to a first pass metabolism in the liver. Assuming adult metabolic capacity, a breastfed infant of 5 to 6 kg ingesting a meal of 120 to 220 mL and breathing environmental tobacco smoke ~1L/minute for 30 minutes, could theoretically receive a wide variation of doses of bioavailable nicotine (1–6.5 µg/kg body weight) dependent on the mothers smoking habits and time from smoking to nursing.6–8 Thus, being in the range of our dosage but less than the mean of 16 µg/kg body weight/cigarette in white adults.32 The time from administration of nic-
otine to the first induced apnea in our study equals the half-life of distribution of nicotine in plasma. The reduced risk for SIDS associated with breastfeeding disappears if the mother smokes, but a clear negative effect of nursing by a smoking mother has not been demonstrated. IL-1β is the prototypic proinflammatory and alarm cytokine that is released in the inflammatory response. Its effects on apnea have been demonstrated and discussed previously. IL-1β has been found elevated in SIDS, although elevated interleukin-6 (IL-6) is a more common finding. This is probably caused by an earlier peak in release and rapid return to baseline values of IL-1β during immune reaction, while IL-6 has a more delayed and sustained release. In pilots for this study, we did not find acute effects of IL-6 similar to those found with IL-1β.

We attempted to demonstrate successive prolongation of apnea after repeated stimulation of the laryngeal reflex, but this was not seen. This may be attributable to too long intervals, because hypoxia primarily resolved within 5 minutes, combined with a possible habituation to the stimulus and decreasing levels of nicotine and IL-1β. The possibility of entry into a vicious circle by repeated laryngeal stimulation should not be excluded.

CONCLUSION

The acute adverse effects of nicotine and IL-1β on induced apnea and the following autorecuperation in piglets may shed light on important mechanisms involved in the causation of SIDS.

ACKNOWLEDGMENTS

Dr Frøen is a fellow with the Norwegian Women’s Public Health Association.

We thank Dr T. A. Hayge for the analysis of hematology and CRP at the Institute of Clinical Chemistry, The National Hospital, Oslo; Dr O. Skatvedt, A. K. Borgersen, and B. Øverland, Ullevål University Hospital, for their skilled technical assistance, and Professor I. W. S. Mair, University of Oslo, for helpful comments on the final version of the manuscript.
Adverse Effects of Nicotine and Interleukin-1β on Autoresuscitation After Apnea in Piglets: Implications for Sudden Infant Death Syndrome

J. Frederik Frøen, Harriet Akre, Babill Stray-Pedersen and Ola Didrik Saugstad

*Pediatrics* 2000;105:e52

Updated Information & Services

Including high resolution figures, can be found at:
/content/105/4/e52.full.html

References

This article cites 32 articles, 5 of which can be accessed free at:
/content/105/4/e52.full.html#ref-list-1

Citations

This article has been cited by 1 HighWire-hosted articles:
/content/105/4/e52.full.html#related-urls

Subspecialty Collections

This article, along with others on similar topics, appears in the following collection(s):
- Fetus/Newborn Infant
  /cgi/collection/fetus:newborn_infant_sub
- SIDS
  /cgi/collection/sids_sub

Permissions & Licensing

Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
/site/misc/Permissions.xhtml

Reprints

Information about ordering reprints can be found online:
/site/misc/reprints.xhtml
Adverse Effects of Nicotine and Interleukin-1β on Autoresuscitation After Apnea in Piglets: Implications for Sudden Infant Death Syndrome
J. Frederik Frøen, Harriet Akre, Babill Stray-Pedersen and Ola Didrik Saugstad

Pediatrics 2000;105;e52

The online version of this article, along with updated information and services, is located on the World Wide Web at:
/content/105/4/e52.full.html