Recurrent Hypoxia in Rats during Development Increases Subsequent Respiratory Sensitivity to Fentanyl

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Background: In children with a history of significant obstructive sleep apnea who undergo adenotonsillectomy, postsurgical administration of opiates has been alleged to be associated with an increased risk for respiratory complications, including respiratory depression. The authors hypothesize that this association is due to an effect of recurrent hypoxemia that accompanies more severe obstructive sleep apnea on altered responsiveness to subsequent exogenous opiates.

Methods: The current study was designed to test the effect of recurrent hypoxia in the developing rat on respiratory responses to subsequent administration of the μ-opioid agonist fentanyl. Rats were exposed to 12% oxygen balance nitrogen for 7 h daily for 17 days, from postnatal day 17 to 33, a period equivalent to human childhood. After 17 additional days in room air, rats were given a fentanyl dose and tested for their respiratory response to fentanyl using a whole body plethysmograph. Rats undergoing similar protocols without recurrent hypoxia served as controls.

Results: As compared with controls, rats preexposed to recurrent hypoxia displayed a more profound depression with fentanyl in minute ventilation, respiratory frequency, tidal volume, and tidal volume divided by inspiratory time that represents respiratory drive. These results indicated an increased respiratory sensitivity to fentanyl after recurrent hypoxia.

Conclusions: Previous recurrent hypoxia increases respiratory sensitivity to subsequent opiate agonists. If these findings are applicable to humans, opiate dosing in children must be adjusted depending on history of recurrent hypoxemia to avoid respiratory depression.

In a prospective clinical study published in this issue of Anesthesiology,1 we have shown that children with a history of obstructive sleep apnea associated with recurrent hypoxemia display a reduced opiate requirement for analgesia. We hypothesized that previous recurrent hypoxemia also increases respiratory sensitivity to subsequent administration of opiates. The link between recurrent hypoxemia and respiratory responsiveness to opiates is important because of the high incidence of respiratory complications after surgery reported in children with severe obstructive sleep apnea, in which it was the degree of previous oxygen desaturation that foretold the subsequent respiratory complications, including respiratory depression.2–4

Because the postsurgical conditions in the clinical study5 did not permit direct measurement of ventilatory responses to opiates, the current study was designed to assess such responses directly in an animal model. To this end, recurrent hypoxia was imposed on developing rats throughout a period presumed to simulate human childhood.5 Subsequently, ventilatory responses to a dose of the μ-opioid agonist fentanyl were determined and compared with responses obtained from control rats that had not been exposed to recurrent hypoxia. The study showed that a history of recurrent hypoxemia during development in rats increases respiratory sensitivity to subsequent administration of fentanyl.

Materials and Methods

The experimental design, procedures, and protocol were similar to those previously used by our laboratory in reports describing a rat model for recurrent hypoxia5 and effects of fentanyl on respiratory control in rats.6 All experiments included measures to minimize pain and discomfort and were performed in compliance with the local institutional animal care committee (Montreal Children’s Hospital, McGill University, Montreal, Quebec, Canada) and with the Canadian Council on Animal Care.

Briefly, male Sprague-Dawley rats, born in-house, were assigned to one of two groups: A recurrent hypoxia group (n = 7) was exposed to 12% oxygen balance nitrogen for 7 h daily (0900–1600 h, during sleep phase in rat) from postnatal day 17 through 33 (for a total of 17 days), a period representing “childhood” in rats. In unsedated, unanesthetized, and freely moving rats, such a level of hypoxia decreases arterial partial pressure of oxygen from approximately 97 mmHg to 47 mmHg.7,8 The rats were placed in a sealed acrylic chamber (41-cm length by 22-cm width by 41-cm height), and were provided with food and drinking water. The slatted floor of the chamber was raised over carbon dioxide-absorbent material (soda lime) placed over a liquid absorbent pad (Fisher Scientific, Montreal, Quebec, Canada). The box was continuously flushed with the gas mixture at a
flow rate of approximately 10 l/min. Levels of oxygen and carbon dioxide inside the chamber were continuously monitored (MP100; Biopac Systems, La Jolla, CA) to verify the target oxygen level and to ensure that carbon dioxide levels did not exceed 0.6% throughout. At the end of each daily hypoxic exposure, the rats were returned to their cages and remained in normoxia until they were brought back into the hypoxia chamber on the next day. After the 17 consecutive days of such hypoxia, the rats were kept in normoxia in their colony room. A control group (n = 6), comprising Sprague-Dawley rats matched in age, feeding, and housing to the hypoxic group, was never exposed to recurrent hypoxia and remained in room air throughout the study period.

Each rat from the recurrent hypoxia and control groups was studied once, during daylight hours, between 49 and 53 postnatal days. The rat was placed in a cylindrical whole body plethysmograph, 8 cm in diameter (PLY3213; Buxco Electronics, Sharon, CT), flushed with room air at 2 l/min. Baseline respiration was recorded electronically for 30 min.6 The rat was then injected subcutaneously with a single dose of 120 μg/kg fentanyl. This is the ED$_{90}$ fentanyl dose for respiratory depression in the adult rat, i.e., a dose that reduces minute ventilation by 50%, as previously determined in rats raised in normoxia.5,6 After the fentanyl injection, each rat was immediately returned to the plethysmograph. Measurement of respiratory indices started 8 min thereafter and lasted 26 min. The respiratory indices measured were minute ventilation, respiratory frequency, tidal volume, and tidal volume divided by inspiratory time, representing respiratory drive.5,6

Data Analysis and Statistics

Airflow and respiratory timing were derived from air pressure fluctuations generated by the breathing of the rats. These pressure fluctuations were measured as a pressure difference between the animal chamber and a reference chamber in the whole body plethysmograph, using Epstein’s equations for estimation of tidal volume in a flow-through system.9 Minute ventilation, respiratory frequency, tidal volume, and respiratory drive were calculated automatically for each breath (Buxco Electronics) using additional measurements of chamber humidity as well as chamber and rectal temperatures (Cole Parmer YSL-55; Vernon Hills, IL).5,6 For each rat, the respiratory indices were averaged over 2-min intervals. Minute ventilation, tidal volume, and respiratory drive were expressed per 100 g body weight. Data were sorted by study group, i.e., hypoxia-exposed versus control group, and averaged. After a confirmation of variance homogeneity (Cochran C statistic), respiratory variables of the hypoxia-exposed and control groups were analyzed by a two-way analysis of variance (time by group), with Greenhouse-Geisser correction for repeated measures over time. Additional analyses included post hoc pairwise comparisons between groups at specific time points by contrasts, and comparisons of respiratory values during fentanyl effect to values at baseline using the Dunnett test. Statistical analysis was performed with Statistica (version 6; Statsoft, Tulsa, OK). Significance was defined at $P \leq 0.05$.

Results

Overall, respiratory indices at baseline did not differ between the hypoxia-exposed and control rats, whereas previous exposure to recurrent hypoxia heightened the respiratory depression in response to an acute fentanyl administration in the rats.

After fentanyl administration, minute ventilation decreased in both the hypoxia-exposed and control groups throughout the entire period of measurement (main effect of time, $P = 0.0004$). For each rat group, a significant difference in minute ventilation was seen between baseline and each measurement during fentanyl effect (fig. 1A). Minute ventilation was lower in the hypoxia-exposed group than in the control group throughout the 26-min measurement period after fentanyl injection (main effect of group, $P = 0.0077$). There was no interaction between groups and time of measurement after fentanyl administration.

Respiratory frequency also decreased in both rat groups throughout the observation period after fentanyl injection, thus displaying a main effect of time ($P = 0.0037$), and also showed a significant difference between baseline and most time points after fentanyl administration (fig. 1B). The temporal pattern of the decrease in respiratory frequency was similar to that of minute ventilation. Although the hypoxia-exposed rats exhibited lower mean values of respiratory frequency than did controls, the $P$ value for the group main effect was 0.0627. There was no interaction between groups and time during the period of observation.

Tidal volume also decreased in both rat groups after fentanyl administration, resulting in a main effect of time ($P = 0.0009$). Unlike minute ventilation and respiratory frequency, however, the tidal volume response displayed an interaction between rat groups and time ($P = 0.0351$), in which the decrease in tidal volume of the hypoxia-exposed rats was greater than that of the control rats only during the earlier portion of the observation period after fentanyl injection (fig. 1C). The greater reduction in tidal volume in the hypoxia-exposed group was further shown by a significant difference from baseline at 9 of 10 time points as compared with a significant difference at only 5 of 10 time points in the control group.

The response of respiratory drive after fentanyl administration was similar to that of minute ventilation. The decrease in respiratory drive occurred in both rat groups (main effect of time, $P < 0.0001$). For each rat group,
significant difference in respiratory drive was seen between baseline and each measurement during fentanyl effect (fig. 1D). The magnitude of this decrease was greater in the hypoxia-exposed than in the control rats throughout the period of observation (main effect of group, \(P = 0.0013\)). There was no interaction between any rat group and time.

Discussion

The findings reported here show that a history of recurrent hypoxia heightens the respiratory sensitivity to subsequent opiates in rats. These findings complement the enhanced analgesic sensitivity to opiates found in children with a history of severe hypoxemia preoperatively.\(^1\) The difference in experimental design between the two studies was that, in the clinical study, various opiate doses were given to reach a uniform level of a physiologic function, analgesia, whereas in the animal study, a uniform opiate dose was used to differentiate the sensitivity of a physiologic function, respiration.

The increased respiratory sensitivity to fentanyl found in rats after exposure to recurrent hypoxia may explain the respiratory complications observed in children with severe obstructive sleep apnea\(^3,10\) who may have received too great an opiate dose relative to their previous level of hypoxemia. In the clinical study in this issue of *Anesthesiology*,\(^1\) no postsurgical, opiate-induced hypoventilation was observed, as evidenced by a lack of increase in transcutaneous carbon dioxide tension or decrease in respiratory frequency. Based on the results from the current animal study, this absence of hypoventilation may be explained by the reduced analgesic opiate dosing given to those children who had severe previous oxygen desaturation.

Although the processes underlying an increased sensitivity to opiates after recurrent hypoxemia during development are unknown, we speculate that such hypoxemia produces a repeated release of endogenous opioid ligands at central pain and respiratory regions,\(^11\) which, in turn, activate \(\mu\)-opioid receptive pathways and sensitize them to subsequent opioid agonist administration. Presuming that endogenous opioid ligands use the same pathways as exogenous opiates, this hypothesized sequence is supported by a recent report\(^12\) that showed an increased sensitivity to a morphine injection subsequent to consecutive daily injections of morphine in adolescent rats. There are, however, important differences between the two studies: In that study, the sensitizing agonist was an exogenous opiate, administered as a single injection on 3 consecutive days, and the physiologic function assessed was locomotion.\(^12\) By contrast, in our study, the sensitizing agonist was a hypoxia-induced and repeatedly released endogenous opioid ligand, and the physiologic function assessed was respiration. Nevertheless, it is probable that the sequence of events leading to an increased sensitization at \(\mu\)-opioid receptors is similar in both cases. As to possible molecular mechanisms underlying the heightened \(\mu\)-opioid sensitization, many signal transduction pathways common to a variety of G-protein receptors\(^13\) might be invoked.

In summary, the current study shows that a history of recurrent hypoxia in developing rats is associated with an increased respiratory sensitivity to subsequent admin-
istration of fentanyl. The findings from this study corroborate the heightened analgesic sensitivity to opiates observed in children after recurrent hypoxemia due to obstructive sleep apnea.\textsuperscript{1} If mechanisms underlying this heightened sensitivity apply to humans, opiate dosing in children with a history of recurrent hypoxemia should be reduced to avoid respiratory depression.

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References

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