Heritable differences in respiratory drive and breathing pattern in mice during anaesthesia and emergence

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Background. Postanaesthetic hypoxia and ischaemia can lead to postoperative morbidity and mortality. We studied the effect of isoflurane anaesthesia in two inbred mouse strains known for phenotypic differences in breathing pattern and respiratory drive during carbon dioxide challenge and their first-generation offspring (F1).

Methods. Using whole body plethysmography, we assessed respiratory rate (RR) and pressure amplitude (Amp) in male B6 (high responder to hypercapnia), C3 (low responder), and F1 mice at rest, during anaesthesia with isoflurane, and during recovery from anaesthesia. At each time point, the magnitude and pattern of breathing were determined during hypercapnic challenge (FICO2 = 0.08). Data (mean (SD)) were analysed by generalized ANOVA with post hoc Bonferroni’s correction (P<0.05).

Results. During isoflurane anaesthesia, strain differences between B6 and C3 mice in RR were obscured while differences in Amp persisted. In contrast to baseline RR responses to carbon dioxide were significantly reduced at 0.5 MAC (increase in RR: 175 (33) bpm, 147 (44) bpm, 127 (33) bpm, for B6, C3, and F1 strains respectively) and completely blocked at 1.5 MAC (change in RR: -3 (10) bpm, -2 (1) bpm, -4 (5) bpm, for B6, C3, and F1 strains, respectively). During recovery, B6 mice showed a significant increase in RR (77 (33) bpm; P<0.0001) as well as in Amp. This was not observed in either C3 (±22 (31) bpm) or F1 mice (23 (51) bpm).

Conclusion. Isoflurane anaesthesia abolished the strain differences in respiratory drive between B6, C3, and F1 mice. However, during recovery from anaesthesia, significant strain variation in respiratory drive reappeared and was more pronounced compared with pre-anaesthetic levels. These results suggested, that genetic differences may have minimal contribution to decreased respiratory drive during anaesthesia, but may be a major risk factor for postoperative hypoventilation and the associated morbidity and mortality.

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Reduced respiratory drive can increase morbidity and mortality in patients diagnosed with central or obstructive sleep apnoea. The risks for these patients are aggravated when sedatives, anaesthetics, or centrally active analgesic drugs are administered.¹²

The determination of respiratory drive and the response to hypercapnia or hypoxia in humans is limited by wide inter-individual differences in human populations.³⁴ Moreover, results observed under similar environmental conditions in awake, unmedicated individuals suggests genetic determinants contribute to the differences in respiratory drive.⁵–¹⁰

The identification of genetic determinants of respiratory drive and their contribution to the risk of hypoxia and...
ischaemia in the immediate postoperative period, and during recovery from anaesthetics remains to be determined. Previous studies have shown that even concentrations of volatile anaesthetics as low as 0.1 MAC can significantly reduce the response to hypercapnia and hypoxia.

We have studied this gene–environment interaction more closely using an inbred mouse model. Mice within an inbred strain are genetically identical but differ between strains based on genotype variation, which influences physiological outcomes. C57BL/6J (B6) and C3H/HeJ (C3) mouse strains differ in their baseline breathing pattern and their response to hypercapnia. Furthermore, the first generation offspring (F1) of B6 and C3 progenitors have been shown to resemble one or the other parental strain suggesting that breathing traits are heritable.

Therefore, we tested the effect of isoflurane on respiratory rate (RR), tidal volume, and the response to hypercapnia in B6 compared with C3 mice and their F1 progeny.

Methods

Animals

Our study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Ten C57BL/6J (B6), 11 C3H/HeJ (C3), and 10 B6C3F1/J (F1; i.e., female B6 crossed with male C3) male mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and housed in the animal facilities at Johns Hopkins University for 5–7 weeks before the first experiments. Water and chow (Agway Pro-Lab RMH 1000) were provided ad libitum. All experiments were performed at an age of 14 weeks (±10 days). Mean weight was 29.9 (3.4) g for B6, 31.9 (2.7) g for C3, and 33.7 (2.8) g for F1 mice.

Measurements

Whole body plethysmograph

Ventilatory function was assessed by whole body plethysmography under unrestrained conditions. Each animal was permitted to acclimatize in a cylindrical chamber (400 cm³) for at least 30 min before ventilation measurements were obtained. The chamber was placed in an insulated box to control environmental conditions (i.e., quiet and dark surroundings) and prevent heat loss. Chamber temperature was maintained within the thermoneutral zone for mice (26–28°C) and was recorded with each measurement using a type T thermocouple device. Hyperoxic air (oxygen 100%) was humidified (90% relative humidity) and directed through the chamber at a flow rate of ~300 ml min⁻¹.

At constant chamber volume, changes in pressure because of inspiration and expiration were measured using a differential pressure transducer (model 8510B-2, Endevco, San Juan Capistrano, CA, USA) and recorded on a strip chart recorder (Grass Polygraph model 7D, Grass Instrument Co., Quincy, MA, USA). The expired air at the outlet of the chamber was analysed for oxygen, carbon dioxide, nitrogen, and isoflurane using a CAPNOMAC ULTRA ULT-V-27–06 (DATEX, Division of Instrumental Cooperation, Helsinki, Finland).

Protocol

When the animal was quiescent the chamber was sealed for 60 s to permit measurements of RR and the pressure amplitude (Amp; in mm) resulting from each tidal breath as a correlate of tidal volume. While the chamber was sealed metabolically, induced changes in carbon dioxide and oxygen were <1% of the target level.

Subsequently, gas with an inspired fraction of carbon dioxide 8% balanced with oxygen (Puritan-Bennett Medical Gases, Overland Park, KS, USA) was administered to the chamber for 6 min. At the end of this carbon dioxide challenge, measurements of RR and Amp were repeated as described above.

After these baseline measurements, isoflurane (Abbott Laboratories, North Chicago, IL, USA) was added to inspired oxygen, via an agent-specific vaporizer integrated in the gas flow system. On different days B6, C3, or F1 mice were studied in random order.

Isoflurane was administered in increasing concentrations of 0.5, 1.0, and 1.5 MAC specific for C3 (1.5%) and B6 (1.30%) mice. MAC for F1 was taken as the average of B6 and C3 mice (1.40%). Isoflurane was administered for 30 min at each concentration to achieve a steady state of anaesthesia. At steady state, measurements of RR and Amp were repeated and a carbon dioxide challenge was performed as described above.

Directly after the carbon dioxide challenge at the highest concentration of anaesthetic agent administered, the agent was turned off and the mice were observed during recovery from anaesthesia for the next 30 min. During the recovery period, measurements were performed at 5, 10, 20, and 30 min. At the end of the recovery the mice were challenged again with inspired carbon dioxide 8% for another 6 min.

Data analysis

Data are presented as mean (SD). The following a priori null hypotheses were tested: first, there is no difference between B6 and C3 mice and their F1 progeny in their breathing pattern during isoflurane anaesthesia and in the recovery from anaesthesia; second, there is no strain difference between B6, C3, and F1 mice in response to hypercapnia under the influence of isoflurane; and third, there is no strain difference in the response to hypercapnia between B6, C3, and F1 mice during recovery from anaesthesia. Comparisons of RR and amplitude were separately analysed among strains controlling for anaesthetic concentration and carbon dioxide challenge using generalized ANOVA with Bonferroni’s correction for multiple pair-wise comparisons. Significant differences were considered to be P<0.05.
Results

Breathing pattern under the influence of isoflurane

At baseline, B6 mice showed a significantly greater RR compared to C3 mice ($P=0.0008$) accompanied by significantly smaller tidal volumes ($P=0.0016$; Fig. 1). The F1 offspring showed a RR similar to the B6 ($P<0.0001$, compared with C3 mice) and a tidal volume similar to the C3 progenitor ($P=0.0119$, compared with the B6 mice).

Increasing concentrations of isoflurane led to decreased RR in each mouse strain to ~17% of baseline (Fig. 1). The RR response of each strain returned to baseline during the recovery period (Fig. 1). Furthermore, increasing concentrations of isoflurane anesthesia initially blunted, and then abolished, the strain difference in RR response at baseline. At 0.5 MAC, the only remaining significant difference in RR was between C3 and F1 mice ($P=0.008$). While at higher concentrations of isoflurane, and during the recovery of anesthesia, there was no significant difference in RR between the three mouse strains.

Increasing concentrations of isoflurane had mixed effects on Amp. At the highest concentration studied, there was a significant increase in Amp in all strains (Fig. 2). In addition, at 0.5 or 1.0 MAC Amp was significantly smaller for B6 mice as compared with F1 ($P=0.008$) or C3 mice ($P=0.013$, Fig. 2). At 1.5 MAC, there was no significant difference among the three strains.

During recovery from anesthesia, the strain difference in Amp between B6 and C3 was restored ($P=0.0001$) and was still maintained at 30 min of recovery ($P=0.0002$, Fig. 1) when the mice were considered completely recovered and subsequently returned to their cages.

Response to hypercapnia

Before isoflurane anesthesia, B6 mice responded with a significantly greater carbon dioxide-induced increase in RR compared with either C3 ($P=0.0093$) or F1 mice ($P=0.0041$). During isoflurane anesthesia, strain differences in RR in response to hypercapnia were abolished. However, there was no significant difference in the carbon dioxide-induced increase in Amp among the three strains (Fig. 2).

At 0.5 MAC, a significant difference in carbon dioxide-induced breathing occurred between B6 and F1 mice for RR and Amp ($P=0.0001$ and $P=0.0036$, respectively; Fig. 2). At higher concentrations of isoflurane (1.0 and 1.5 MAC) the RR increase during hypercapnia was reduced (1.0 MAC) and abolished (1.5 MAC) in all three strains (Fig. 2). In addition, the carbon dioxide-induced Amp responses decreased significantly compared with pre-anaesthetic Amp responses for all mouse strains at 1.5 MAC abolishing the strain differences in Amp responses to hypercapnia.

At the end of recovery (30 min), while compared to their response at 1.5 MAC, the carbon dioxide-induced, augmented breathing was restored for both RR and Amp in B6 mice (Fig. 2). However, compared with the baseline responses, the response in RR to hypercapnic challenge remained depressed. Furthermore, the carbon dioxide-induced responses of B6 mice were significantly greater than the responses of both C3 and F1 mice for RR ($P<0.0001$ and $P=0.011$, respectively) and for Amp ($P=0.0001$ and $P=0.0016$, respectively). In C3 and F1 mice, there were no significant increases in either RR or Amp for the hypercapnic challenge during recovery from anesthesia.

Exhaled isoflurane concentrations during recovery did not show any significant differences between the three mouse strains.

Discussion

In the current genetic model of breathing control, strain differences in breathing pattern and hypercapnic responses were completely abolished with clinically relevant concentrations of the inhalation anaesthetic isoflurane. However, during emergence from anesthesia, these genetically determined differences in respiratory drive were accentu-
ated. The C3 parental strain, characterized by a blunted response to hypercapnia, continued to show a profound depression of their response to hypercapnia after isoflurane anaesthesia. This phenotype was inherited by their F1 progeny.

Inbred mouse strains are genetically identical and homozygous at every locus. After extensive studies of breathing patterns and respiratory drive in different inbred mouse strains, two strains had been identified as having two widely different phenotypes. The B6 strain, was characterized by a rapid and shallow baseline breathing pattern and a strong response to hypercapnia, while the C3 strain has a slow and deep baseline breathing pattern with a weak response to hypercapnia. Studies of first and second generations of B6 and C3 progenitors have suggested a relatively simple genetic model incorporating a few numbers of genes controlling each trait.

To explore the mechanisms controlling respiratory drive and the relevance of genetic variations under the influence of central nervous system active agents, the current work examined the effects of a volatile anaesthetic agent on baseline breathing pattern and respiratory drive. We found in strains with significantly different baseline breathing patterns and carbon dioxide responses while awake, that administration of clinically relevant isoflurane concentrations dramatically affected each strain. The differences in RR among the two mouse strains and the F1 progeny were eliminated at concentrations up to 1.5 MAC and during emergence. However, strain differences in tidal volume were essentially unaffected.

We consider the responses to hypercapnia as a model for postanaesthetic hypoventilation. At 0.5 MAC of isoflurane, the response to carbon dioxide for both parental strains and the F1 progeny was reduced. At 1.0 MAC, the lack of increase in RR was partially compensated for by an increase in tidal volume. At 1.5 MAC the response to carbon dioxide was entirely suppressed. At all concentrations of isoflurane anaesthesia, strain differences between B6 and C3 mice in breathing responses to hypercapnic challenges were abolished.

In contrast, the effect of the low concentrations of isoflurane during recovery showed significant differences between the two strains and the F1 progeny. More than 30 min after isoflurane had been discontinued, the response to hypercapnia during recovery remained suppressed in C3 and F1 mice, representing little to no change from the hypercapnic response at 1.5 MAC, while in B6 mice the carbon dioxide-induced RR and tidal volume responses increased significantly. However, B6 responses did not return to the pre-anaesthetic levels even at the end of the recovery period.

It is noteworthy, that the responses in B6 mice were in accordance with results obtained in human volunteers inhaling 0.1 MAC isoflurane in a silent room with dimmed light. Van Elsen and colleagues demonstrated that controlling environmental conditions is a fundamental criterion in the study design. The response to hypoxia and hypercapnia varied significantly with the state of arousal and were uncompromised when patients were kept awake with music or videos comparable to a noisy recovery room.

While the clinical relevance of an inherited predisposition to reduced respiratory drive may seem of only scientific interest in otherwise healthy individuals, it can be critically important during recovery from anaesthesia. Even at very low concentrations, volatile anaesthetic agents in combination with commonly administered potent analgesic medi-
cation can accentuate hypoventilation and further lead to ischaemic events. Our results demonstrate that genetic factors influence the blunted hypercapnic responses in both C3 and F1 mice suggesting a dominant inheritance pattern. Although the breathing pattern of F1 mice at baseline resembled the pattern of B6 mice, their blunted carbon dioxide breathing phenotype at baseline was predictive of a blunted response to hypercarbia during recovery from anaesthesia.

Establishing genotypic markers for blunted respiratory drive would facilitate identifying patients at risk of postoperative hypoventilation. In the clinic, one could presumably adjust anaesthesia and postoperative care to decrease the risk of hypoventilation and thus prevent hypoxia and ischaemic events. There are groups of patients with obvious pathological breathing patterns and blunted respiratory drive, including patients with Pickwickian syndrome or other central apnoea syndromes. In these groups of patients, the increased risk for postoperative complications is well known and a matter of clinical concern. Greater clinical impact on patient safety is anticipated when patients with disturbances of respiratory drive that only became evident under the influence of anaesthetics, sedatives, or analgesic drugs could be identified a priori.1221

In conclusion, during isoflurane anaesthesia, strain differences in breathing pattern and responses to hypercapnic challenge were abolished. However, during recovery from anaesthesia, a critical time for perioperative events, the carbon dioxide-induced response was restored only in B6 mice. In C3 and F1 mice characterized by blunted carbon dioxide-induced responses, the response to hypercapnia remained depressed during recovery from anaesthesia. These results support a genetic basis for the increased risk of postanaesthetic hypoventilation and demonstrate the genetic predisposition to be heritable.

References

2 Thalhofer S, Dorow P. Central sleep apnea. Respiration 1997; 64: 2–9
18 Haldane JBS, Waddington CH. Inbreeding and linkage. Genetics 1931; 16: 357–74
20 Temp JA, Henson LC, Ward DS. Effects of a subanesthetic minimum alveolar concentration of isoflurane on two tests of the hypoxic ventilatory response. Anesthesiology 1994; 80: 739–50