Bradycardia in serotonin-deficient Pet-1⁻/⁻ mice: influence of respiratory dysfunction and hyperthermia over the first 2 postnatal weeks

Kevin J. Cummings, Aihua Li, Evan S. Deneris, and Eugene E. Nattie

Dartmouth Medical School, Lebanon, New Hampshire; and Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio

Submitted 11 February 2010; accepted in final form 5 March 2010

Bradycardia in serotonin-deficient Pet-1⁻/⁻ mice: influence of respiratory dysfunction and hyperthermia over the first 2 postnatal weeks. Am J Physiol Regul Integr Comp Physiol 298: R1333–R1342, 2010. First published March 10, 2010; doi:10.1152/ajpregu.00110.2010.—Neonatal rodents deficient in medullary serotonin neurons have respiratory instability and enhanced spontaneous bradycardias. This study asks if, in Pet-1⁻/⁻ mice over development: 1) the respiratory instability leads to hypoxia; 2) greater bradycardia is related to the degree of hypoxia or concomitant hypopnea; and 3) hyperthermia exacerbates bradycardias. Pet-1⁺/⁺, Pet-1⁻/⁻, and Pet-1⁻/⁻ mice [postnatal days (P) 4–5, P11–12, P14–15] were held at normal body temperature (Tb) and were then made 2°C hypo- and hyperthermic. Using a pneumotach-mask system with ECG, we measured heart rate, metabolic rate (V0₂), and ventilation.

We also calculated indexes for apnea-induced hypoxia (total hypoxia: apnea incidence × O₂ consumed during apnea = μL·g⁻¹·min⁻¹) and bradycardia (total bradycardia: bradycardia incidence × magnitude = beats·min⁻¹). Resting heart rate was significantly lower in all Pet-1⁻/⁻ animals, irrespective of Tb. At P4–5, Pet-1⁻/⁻ animals had approximately four- to eightfold greater total bradycardia (P < 0.001), owing to an approximately two- to threefold increase in bradycardia magnitude and a near doubling in bradycardia incidence. Pet-1⁻/⁻ animals had a significantly reduced V0₂ at all Tb, thus there was no genotype effect on total hypoxia. At P11–12, total bradycardia was nearly threefold greater in hyperthermic Pet-1⁻/⁻ animals compared with controls (P < 0.01). In both genotypes, bradycardia magnitude was positively related to the degree of hypopnea (P = 0.02), but there was no genotype effect on degree of hypopnea or total hypoxia. At P14–15, genotype had no effect on total bradycardia, but Pet-1⁻/⁻ animals had up to seven times more total hypoxia (P < 0.001), owing to longer and more frequent apneas and a normalized V0₂. We infer from these data that 1) Pet-1⁻/⁻ neonates are probably not hypoxic from respiratory dysfunction until P14–15; 2) neither apnea-related hypoxia nor greater hypopnea contribute to the enhanced bradycardias of Pet-1⁻/⁻ neonates from approximately P4 to approximately P12; and 3) an enhancement of a temperature-sensitive reflex may contribute to the greater bradycardia in hyperthermic Pet-1⁻/⁻ animals at approximately P12.

Cummings KJ, Li A, Deneris ES, Nattie EE. Bradycardia in serotonin-deficient Pet-1⁻/⁻ mice: influence of respiratory dysfunction and hyperthermia over the first 2 postnatal weeks. J Physiol 298: R1333–R1342, 2010. First published March 10, 2010; doi:10.1152/ajpregu.00110.2010.—Neonatal rodents deficient in medullary serotonin neurons have respiratory instability and enhanced spontaneous bradycardias. This study asks if, in Pet-1⁻/⁻ mice over development: 1) the respiratory instability leads to hypoxia; 2) greater bradycardia is related to the degree of hypoxia or concomitant hypopnea; and 3) hyperthermia exacerbates bradycardias. Pet-1⁺/⁺, Pet-1⁻/⁻, and Pet-1⁻/⁻ mice [postnatal days (P) 4–5, P11–12, P14–15] were held at normal body temperature (Tb) and were then made 2°C hypo- and hyperthermic. Using a pneumotach-mask system with ECG, we measured heart rate, metabolic rate (V0₂), and ventilation.

We also calculated indexes for apnea-induced hypoxia (total hypoxia: apnea incidence × O₂ consumed during apnea = μL·g⁻¹·min⁻¹) and bradycardia (total bradycardia: bradycardia incidence × magnitude = beats·min⁻¹). Resting heart rate was significantly lower in all Pet-1⁻/⁻ animals, irrespective of Tb. At P4–5, Pet-1⁻/⁻ animals had approximately four- to eightfold greater total bradycardia (P < 0.001), owing to an approximately two- to threefold increase in bradycardia magnitude and a near doubling in bradycardia incidence. Pet-1⁻/⁻ animals had a significantly reduced V0₂ at all Tb, thus there was no genotype effect on total hypoxia. At P11–12, total bradycardia was nearly threefold greater in hyperthermic Pet-1⁻/⁻ animals compared with controls (P < 0.01). In both genotypes, bradycardia magnitude was positively related to the degree of hypopnea (P = 0.02), but there was no genotype effect on degree of hypopnea or total hypoxia. At P14–15, genotype had no effect on total bradycardia, but Pet-1⁻/⁻ animals had up to seven times more total hypoxia (P < 0.001), owing to longer and more frequent apneas and a normalized V0₂. We infer from these data that 1) Pet-1⁻/⁻ neonates are probably not hypoxic from respiratory dysfunction until P14–15; 2) neither apnea-related hypoxia nor greater hypopnea contribute to the enhanced bradycardias of Pet-1⁻/⁻ neonates from approximately P4 to approximately P12; and 3) an enhancement of a temperature-sensitive reflex may contribute to the greater bradycardia in hyperthermic Pet-1⁻/⁻ animals at approximately P12.

heart rate; apnea; body temperature; breathing; sudden infant death syndrome

Address for reprint requests and other correspondence: E. E. Nattie Jr., Dartmouth Medical School, 1 Medical Center Dr., Lebanon, NH 03766 (e-mail: Eugene.e.nattie.jr@dartmouth.edu).

MATERIALS AND METHODS

Animals. Data were obtained from 51 littermates obtained from 8 different litters that originated from 8 female Pet-1⁺/⁺ females paired with 4 Pet-1⁻/⁻ males. Animals were studied at three postnatal ages:...
P4–5 (Pet−/−, 8; littermates, 10); P11–12 (Pet−/−, 7; littermates, 10), and P14–15 (Pet−/−, 9; littermates, 7). Dams were provided food and water ad libitum and were housed with a 12:12-h light-dark cycle at a Ta of 21–23°C. There is no difference in medullary 5-HT between recordings began. A 10-min recording of V˙E, V˙O2, cycle at a Ta of 21–23°C. There is no difference in medullary 5-HT

food and water ad libitum and were housed with a 12:12-h light-dark

34°C), with 10-min recordings at each new steady-state Tb. The

adjusting Ta, at either 35°C/min and 0.5°C (P11–12 and P14–15, 31–32°C

in younger animals and 33–34°C in older animals (i.e., slightly

hypothermic).

Effects of genotyping. Pups were ear-notched at each respective age. Genotyping on isolated DNA was performed according to a previous study (15) using primers: 5’-CGC ACT TGG GGG GTC ATT ATC AC-3’

and 3’-CCG TGG ATG TGG AAT GTG TGC-3’, and 5’-GCC TGA TGT

TCA AGG AAG ACC TCG G-3’. PCR was performed using an initial

5-min denaturing step at 95°C, followed by 35 cycles of 94°C for 1

min, 62°C for 30 s, and 72°C for 50 s. PCR products generated were

a wild-type allele and knockout allele of 209 and 361 base pairs,

respectively.

Experimental setup. Experiments were performed using a setup that allows for precise control of Ta, and the accurate determination of tidal volume (Vt) in neonatal animals, where the Ta–Tb difference is very small (8). Briefly, the animal chamber (volume 40 ml) was constructed from a water-jacketed glass cylinder. Tb (and thus Ta) was altered by changing the temperature of the water perfusing the glass chamber. Ta and Tb changed 1°C/min and 0.2°C/min, respectively. Ve in all animals was measured with a mask and pneumotach. The head chamber was made by fitting a section of vinyl over the end of syringe tube (volume 3 ml), held in place with another rubber gasket that fit into the anterior end of the chamber. The snout of the animal (fur removed) was placed into a small hole in the vinyl and sealed with polyether material (Impregum F Polyether Impression material, 3M, St. Paul, MN).

A downstream pump (AEI Technologies, Naperville, IL) connected to the outlet port of the mask pulled air through the pneumotach and mask at a flow of either 50 ml/min (P4–5), 110 ml/min (P7–8, P11–12), or 160 ml/min (P14–15). Air was pulled through a small, vertical column of Drierite (Hammond Drierite, Xenia, OH) before being analyzed for the fractional concentrations of CO2 and O2 by gas analyzers (AEI Technologies). Tb (rectal) and Ta were continually monitored with fine thermocouples (Omega Engineering, Stamford, CT). The Tb thermocouple was mounted above the animal, 0.5 cm displaced from the inner surface of the chamber. Both thermocouples and ECG leads exteriorized by way of a hole in a rubber gasket (Terumo Medical) in the posterior end of the chamber.

Inspiratory and expiratory airflows were detected by connecting both side-arms of the pneumotach to a differential pressure transducer (Validyne Engineering, Northridge, CA). Integration of the flow trace provided respiratory volume, calibrated by injecting and withdrawing known volumes of air (0.025, 0.05 ml) at the end of each experiment. The pneumotach responded in a linear fashion to these volumes.

Experimental protocol. Experimentation was performed while blinded to genotype. Pups were removed from the litter and immediately weighed. To minimize heat loss, animals were held in-hand while instrumentation was performed; we limited the duration animals were exposed to room air temperature to 5 min. Animals were instrumented with ECG leads (contained in a small vest made from sensor bandage). A rectal thermocouple was then inserted 1 cm and lightly glued to the base of the tail. At this point, Tb was 31–32°C in younger animals and 33–34°C in older animals (i.e., slightly hyperthermic).

P4–5, P11–12, and P14–15 mice were placed within the preheated glass chamber. Over a period of time, normothermia Tb was established, by adjusting Tb, at either 35 ± 0.5°C (P4–5) or 36 ± 0.5°C (P11–12 and P14–15) before recordings began. A 10-min recording of Ve, V˙O2, and HR was then made at normothermia, after which Tb was made either 2°C hyperthermic (P4–5: ~37°C, P11–12 and P14–15: ~38°C) or hypothermic (P4–5: ~33°C, P11–12 and P14–15: ~34°C), with 10-min recordings at each new steady-state Tb. The

order of the hyper- and hypothermic step was randomized and was followed immediately by the other temperature.

Data analysis. Data are expressed as means ± SE. Ventilatory parameters reported are frequency (f; min−1), Vt (ml/kg), and V˙E = f × Vt (ml·min−1·kg−1). V˙O2 was calculated for all animals during room-air breathing, at all Tb, using the difference in the fractional concentrations of each gas entering and leaving the mask, multiplied by the flow rate (expressed as ml·min−1·kg−1). Ve and V˙O2 were determined across all breaths during 5 min of breathing at each steady-state Tb. Breathing was analyzed automatically, within LabChart 6 (ADI Instruments), using peak detection to determine breath amplitude in conjunction with a slope trigger to detect the beginning of each breath and the breath duration.

Resting HR is reported in beats/min. Spontaneous bradycardias were counted and quantified if an acute drop in HR of at least 30 beats/min occurred. Rather than simply reporting the minimum HR achieved during the bradycardia, we expressed the magnitude of bradycardias as the integrated area above the HR curve (Fig. 1). This value is reported as “beats lost” [drop in HR (Hz) × duration (s)]. In this way, both the duration and fall in HR are taken into consideration for each event. In addition, we calculated a total bradycardia (beats lost/min) that represents the product of bradycardia magnitude (beats lost) and incidence (min). To determine apnea-related hypoxia, we assessed incidence of all apneas greater than two times the average respiratory period (apneas/min), regardless of when they occurred in the respiratory cycle. Importantly, respiratory disruptions where there was detectable inspiratory flow were not counted as apneas. As it is prohibitively difficult to accurately measure oxygen saturation in mice weighing $<5 g, we report an estimate for hypoxia occurring during the apnea (O2% = μO2/g) that represents the product of the apnea duration (s) and the V˙O2 before the apnea (μO2·g−1·s−1). The key assumption we are making is that V˙O2 does not change in the period of the apnea from hypoxia or the reduced breathing (see discussion). Finally, as a measure of the total exposure to apnea-related hypoxia, we calculated a second index: total hypoxia (μO2·g−1·min−1), representing the product of the apnea incidence (apneas/min) and O2% (μO2·g−1·apnea−1).

Bradycardias in neonatal mice and rats frequently occur simultane-ously with a drop in Ve that is not an apnea (i.e., hypopnea where respiratory flow continues) (Fig. 1). For each animal, we assessed the relationship of bradycardia magnitude to maximum change in Ve during the bradycardia (measured breath to breath). We did this by subtracting the minimum Ve occurring during the bradycardia from the average Ve in the 10 breaths preceding the bradycardia (Fig. 1). Regression analysis was performed on the average bradycardia and average hypoxia for every animal of each genotype.

Statistical analysis. Effects of genotype and Tb on respiratory and metabolic variables were assessed using a two-factor, repeated-measures ANOVA within each age group. Tukey’s post hoc tests were performed when significant effects were found. Effects were considered significant at P < 0.05.

RESULTS

Effects of Pet-1 genotype on V˙O2 and breathing. Given the metabolic status of newborn mice deficient in medullary 5-HT neurons had not previously been established, we were inter-ested in measuring V˙O2 in Pet−/− neonates throughout the first 2 postnatal weeks. At P4–5, the V˙O2 of Pet−/− animals is reduced ~25% relative to littermates, irrespective of Tb (Fig. 2A, left; P < 0.001). This relative hypometabolism is met with a proportional decrease in Ve (Fig. 2B, left; P < 0.001) such that there is no effect of Pet-1 deficiency on the ventilatory equivalent (Ve/V˙O2) (not shown). The relative decrease in Ve in Pet−/− animals is due to reduced f (Fig. 2C; P < 0.001) with no change in Vt (Fig. 2D). Warming is associated with a decrease in Vt (P < 0.001) and an
increase in $f_b$ ($P < 0.001$), resulting in an increase in $V_E$ ($P = 0.001$). The effect of $T_b$ does not depend on genotype.

P11–12 and P14–15 Pet-1<sup>−/−</sup> neonates have the same $V_{O_2}$ as littermates. The breathing of P11–12 Pet-1<sup>−/−</sup> animals is the same as that for littermates (Fig. 2, A–D; middle). By P14–15, however, Pet-1<sup>−/−</sup> animals again breathe more slowly (Fig. 2C; $P < 0.001$), but with an elevated $V_E$ (Fig. 2D; $P < 0.001$) that normalizes $V_E$ (Fig. 2B, right). Thus, at ages beyond P4–5, Pet-1 deficiency has no effect on $V_{O_2}$ or overall $V_E$.

Effects of Pet-1 genotype on the potential for apnea-related hypoxia. Given the developmental changes in $V_{O_2}$, we reexamined apneas in Pet-1<sup>−/−</sup> animals throughout the first 2 postnatal weeks, with particular attention to the potential for apnea-related hypoxia. As accurately measuring $O_2$ saturation was not possible in these small animals, we developed an index for the hypoxia developing during the apnea ($O_{2}^{tot}$), as well as for overall hypoxia (total hypoxia) that takes into account both $O_{2}^{tot}$ and the apnea incidence.

The incidence and duration of apneas in Pet-1<sup>−/−</sup> animals compared with littermates depends on postnatal age, but not $T_b$. P4–5 Pet-1<sup>−/−</sup> neonates have no more apneas compared with littermates (Fig. 3A, left), but their apneas are, on average, 50–60% longer in duration (Fig. 3B, left; $P < 0.001$). Owing to a reduced $V_{O_2}$ at this age, however, the apnea prolongation in Pet-1<sup>−/−</sup> animals does not translate into greater $O_{2}^{tot}$ or total hypoxia compared with littermates (Fig. 3, C and D, respectively, left). The effect of Pet-1 deficiency on apnea duration resolves at P11–12 (Fig. 3B, middle), but reappears at P14–15 ($P < 0.001$), when apneas also become more frequent ($P = 0.01$) (Fig. 3, A and B, right, respectively). In addition, owing to the combined influence of apnea duration and a normal $V_{O_2}$, P14–15 Pet-1<sup>−/−</sup> animals experience, on average, an 50% greater $O_{2}^{tot}$ (Fig. 3C, right; $P = 0.003$) that, along with increased apnea incidence, results in a hypoxia index that can be up to seven times greater than that of controls (Fig. 3D, right; $P < 0.001$).

Effects of Pet-1 genotype on resting HR and body size. At all $T_b$, the HR of Pet-1<sup>−/−</sup> animals is significantly lower than that of littermates [Fig. 4A; $P < 0.001$ (P4–5); $P = 0.002$ (P11–12); $P = 0.008$ (P14–15)]. In animals younger than P14–15, HR increases as $T_b$ increases [Fig. 4A; $P < 0.001$ (P4–5); $P = 0.047$ (P11–12); $P = 0.17$ (P14–15)]. Pet-1<sup>−/−</sup> animals are significantly smaller than littermates at all developmental time points (Fig. 4B; $P < 0.001$). In animals younger than P14–15, there is a significant relationship between HR and size (the relationship between size and HR at P4–5: $R^2 = 0.52$, $P < 0.001$; at P11–12: $R^2 = 0.37$, $P < 0.01$; at P14–15: $R^2 = 0.03$, $P = 0.54$). On the other hand, there is no relationship between $V_{O_2}$ and HR in Pet-1<sup>−/−</sup> neonates (relationship between $V_{O_2}$ and HR at P4–5: $R^2 = 0.36$, $P < 0.01$; at P14–15: $R^2 = 0.27$, $P = 0.04$).

Effects of Pet-1 genotype and $T_b$ on the occurrence and magnitude of spontaneous bradycardias. Pet-1<sup>−/−</sup> neonates can have qualitatively larger spontaneous bradycardias compared with wild-type littermates (Fig. 5). However, the specific influence of Pet-1 genotype on the incidence, magnitude, and temperature-sensitivity of spontaneous bradycardias depends on postnatal age. At P4–5, Pet-1<sup>−/−</sup> animals experience more bradycardias, irrespective of $T_b$ (Fig. 6A, left; $P = 0.02$). The most striking effect of genotype at this age, however, is on bradycardia magnitude, with Pet-1<sup>−/−</sup> animals experiencing events that are approximately two to three times larger than those of littermates (Fig. 6B, left; $P < 0.001$). As a result, total bradycardia can be approximately four to eight times greater in Pet-1<sup>−/−</sup> animals compared with littermates (Fig. 6C, left; $P < 0.001$).

By P11–12, Pet-1<sup>−/−</sup> animals are no longer having more frequent bradycardias than littermates (Fig. 6A, middle). However, Pet-1<sup>−/−</sup> animals continue to have larger bradycardias than littermates (Fig. 6B, middle; $P = 0.03$), most evident at warmer $T_b$ ($T_b$ effect: $P = 0.02$). Thus, although the overall effect of genotype at P11–12 is not as prominent as at P4–5, total bradycardia remains significantly elevated in Pet-1<sup>−/−</sup> animals.
animals (e.g., nearly 3 times greater than controls at hyperthermic Tb; Fig. 6 C, middle; P < 0.007).

By P14–15, Pet-1−/− animals experience bradycardias at the same frequency and of the same magnitude as littermates (Fig. 6, A and B, respectively, right). As a result, unlike at younger ages, there is no significant genotype effect on total bradycardia (Fig. 6 C, right).

Relationship of spontaneous bradycardia magnitude to associated hypopnea. Genotype has no effect on the magnitude of the hypopnea that occurs concomitantly with bradycardia. At P4–5, the average maximum fall in V̇E for wild-type animals is 6.8 ± 0.9, 5.3 ± 0.8, and 4.5 ± 0.5 µL/g at hypo-, normo-, and hyperthermic Tb, respectively, while Pet-1−/− animals experience a fall in V̇E of 5.2 ± 0.7, 5.3 ± 1.0, and 4.6 ± 0.5 µL/g, respectively. At P11–12, wild-type animals experience a fall in V̇E of 4.2 ± 0.8, 3.8 ± 0.8, and 4.6 ± 1.1 µL/g at hypo-, normo-, and hyperthermic Tb, respectively, while the V̇E of Pet-1−/− animals fell 4.6 ± 1.6, 3.6 ± 1.2, and 5.5 ± 2.3 µL/g, respectively.

We analyzed the relationship between the magnitude of bradycardias and the fall in V̇E (Fig. 1). At P4–5, no significant relationship exists, irrespective of Tb \[ R^2 < 0.00, P = 0.99 \] (hypo); \[ R^2 < 0.001, P = 0.93 \] (norm); \[ R^2 = 0.08, P = 0.3 \] (hyper); not shown]. At P11–12, there exists a positive overall relationship between the degree of hypopnea and bradycardia magnitude, but only when animals are hyperthermic [Fig. 7, bottom (hyper); \[ R^2 = 0.35; P = 0.02 \]].

DISCUSSION

Previous data from our laboratory and others have demonstrated respiratory instability and a propensity for large, spontaneous bradycardias associated with these hypopneas in neonatal rodents deficient in medullary 5-HT neurons (7, 11). Our results extend these findings, suggesting that a prenatal loss of medullary 5-HT neurons not only enhances spontaneous bradycardias until P12, but also that 1) Pet-1−/− animals display respiratory instability at P4–5 and P14–15, but are only susceptible to hypoxia at P14–15, owing to a relative suppression of V̇O₂ at P4–5; 2) contrary to our original hypothesis, neither greater hypoxia nor greater concomitant hypopnea explain the augmentation of bradycardias in Pet-1−/− neonates; and 3) at approximately P12, hyperthermia augments the bradycardias
of both Pet-1−/− and littermates, with the largest bradycardias associated with the greatest concomitant fall in V\(\dot{E}\).

Pet-1 deficiency and potential for respiratory-related hypoxia over development. Similar to other rodent models with medullary 5-HT disruption (including rat pups with pharmacological lesions, tryptophan hydroxylase−2−/− mice, and adult lmx-1b−/− mice) Pet-1−/− neonates have a lower f\(B\) and are prone to apnea (2, 7, 16, 17). Importantly, these new data suggest that P4–5 Pet-1−/− animals do not hypoventilate (and are thus not hypoxic), as their reduced f\(B\) and V\(\dot{E}\) are matched appropriately to a reduced V\(\dot{O}_2\). At P14–15, the f\(B\) of Pet-1−/− animals again lags behind that of

Fig. 3. Effect of Pet-1 genotype and T\(b\) on apnea and potential for apnea-induced hypoxia throughout the first 2 postnatal weeks. Apnea incidence (A), duration (B), O\(\dot{2}^\text{tot}\) (an estimate of apnea-related hypoxia; C), and total hypoxia index (apnea incidence × O\(\dot{2}^\text{tot}\); D) in P4–5, P11–12, and P14–15 Pet-1−/− animals (open bars; \(n = 8, 7, \text{and } 9\), respectively) and wild-type and heterozygous littermates (solid bars; \(n = 10, 10, \text{and } 7\), respectively) during hypothermia (C), normothermia (N), and hyperthermia (H) are shown. Total hypoxia is not influenced by T\(b\) and is not significantly elevated in Pet-1−/− animals until P14–15. *Genotype effect: \(P < 0.01\). Values are means ± SE.
littermates, suggesting the reemergence of a 5-HT-dependent defect in fB. A compensatory increase in VT, possibly as a result of adaptation of respiratory motoneurons, normalizes overall V̇E. Why the fB of Pet-1/−/− animals temporarily recovers at approximately P12 is a matter of speculation. Variability in 5-HT receptor expression may partially explain this effect. There is a transient reduction in 5-HT2 receptor expression at P12 in brain stem nuclei involved in respiratory control (22). It is possible nuclei participating in the respiratory rhythm become less reliant on medullary 5-HT during this developmental window.

P14–15 Pet-1/−/− animals also experience longer, more frequent apneas compared with controls that, unlike in younger animals, result in a considerably higher total hypoxia. This persistent respiratory instability and potential hypoxia toward the end of the second postnatal week (and not in the first week) are novel findings and do not support our original hypothesis that the most hypoxia would occur along with the greatest respiratory instability. Previously, animals were only studied up until P9.5, and, with no measure of V̇O₂, it was unknown whether neonates deficient in medullary 5-HT neurons were susceptible to respiratory-related hypoxia, or if this susceptibility changed with development. Our findings also underline the importance of obtaining accurate measurements of both VT and V̇O₂ in small neonatal animals, where airway resistance is high and is coupled with a small Tₜ₃–Tₜ₄ difference.

HR control in Pet-1/−/− mice: potential mechanisms. While novel, our data do not support our original hypothesis that the augmented bradycardia in animals deficient in medullary 5-HT neurons is related to greater hypopnea or apnea-related hypoxia. What mechanisms could account for their lower resting HR and increased transient bradycardias? Similar to other rodent models of brain stem 5-HT deficiency (e.g., tryptophan hydroxylase−2/−, Lmnx-1b/−/−) (2, 16), Pet-1/−/− mice were significantly lighter than littermates. However, a smaller size does not contribute to the cardiac phenotypes of Pet-1/−/− mice because,
over development, cardiac phenotypes resolve, while body mass becomes more disparate. A relatively reduced $\overline{V_\text{O}_2}$ may be a contributing factor to the reduction in resting HR, but it is difficult to ascertain how this might contribute to the enhanced transient bradycardias. A more plausible explanation is that a reduction in medullary 5-HT neurons leads to autonomic dysfunction through a physiological effect within relevant nuclei. Not only do medullary 5-HT neurons impinge on premotor, presympathetic neurons in the ventrolateral medulla, but a significant proportion of premotor presympathetic neurons are themselves serotonergic (19). Changes in vagal tone are also possible, as it is well established that medullary 5-HT neurons project to preganglionic vagal neurons, expressing a wide variety of 5-HT receptors (18, 34, 37). 5-HT also participates in the development of neuronal networks (5, 29). Thus an alternative possibility is that the cardiac phenotypes are owing to a developmental abnormality within premotor sympathetic, or preganglionic, parasympathetic nuclei, either directly from a lack of 5-HT, or through another downstream factor. Although 5-HT neurons begin synthesizing 5-HT almost immediately after their formation, full innervation of 5-HT targets continues until the third postnatal week in rodents (21). Hence, sympathetic, parasympathetic, and other nuclei involved in cardiorespiratory control may not be under the influence of medullary 5-HT at the same time, which could, in part, explain the variability in both cardiac and respiratory phenotypes over early postnatal life. Further research exploring autonomic dysfunction in Pet-1$^{-/-}$ mice and other models of medullary 5-HT deficiency is warranted.

At approximately P12, bradycardia magnitude is greater in Pet-1$^{-/-}$ animals, augmented by hyperthermic conditions.
Given that the degree of hypopnea is not different between genotypes, a plausible explanation for the enhanced bradycardia in Pet-1⁻/⁻ mice is an enhancement of a temperature-dependent respiratory reflex. One possibility is the carotid chemoreflex, which is not only enhanced by mild warming (8, 12), but may also be a key contributor in early life to the bradycardia associated with apnea (14, 32). Interestingly, stimulation of the caudal raphe has been shown to mitigate the bradycardic response elicited by carotid body activation (38, 39). Alternatively, it may be that reflexes related to pulmonary stretch are enhanced, either centrally or peripherally, in Pet-1⁻/⁻ animals. It has been previously demonstrated that the prevailing lung volume and hence activity of pulmonary stretch receptors can significantly influence the magnitude of chemoreflex-induced bradycardia magnitude, with greater bradycardia occurring as lung volume is reduced (3).

Methodological considerations. A main finding of our study is that neonates deficient in 5-HT neurons are not susceptible to apnea-related hypoxia until P14–15. We acknowledge, however, that, for technical reasons, we were unable to measure O₂ saturation directly. Previous data in human infants do suggest that apneas of only a few seconds (e.g., a few breaths; comparable in relative terms to apneas experienced by our P14–15 Pet-1⁻/⁻ animals) can result in significant hypoxemia (<80% O₂ saturation) (32). Of course, V̇O₂ could be reduced during the apnea in response to hypoxia or to the reduced breathing. Alternatively, V̇O₂ could increase as part of a stress response. In terms of processes that could limit hypoxemia, we feel our assumption is valid. First, it has been previously demonstrated across species that hypoxia-induced hypometabolism is an active process requiring a number of physiological adaptations, including a resetting of the Tₜ set-point and a downregulation of muscle activity, among others (25). These processes are more likely to occur in the order of minutes (not seconds) and are thus unlikely to occur during the short apneas we describe. The reduced breathing itself could lower energy expenditure and thus limit the fall in blood gases during the apnea. However, the cost of breathing is <1% of total V̇O₂ in normoxia, so this is unlikely to have a big effect on blood gases (26). In addition, the dynamics and magnitude of oxygen desaturation during apnea are complex and are influenced by a number of additional factors, such as lung volume, hemoglobin affinity, and cardiac output (35). Because Pet-1⁻/⁻ animals are smaller and have a lower resting HR, our hypoxia index may actually underestimate the degree of hypoxia they experience (35). However, as we have no measure of V̇O₂ during apnea, our data should be interpreted with the above caveats in mind.

Conducting experiments on Pet-1⁻/⁻ mice rather than postnatally lesioned rat pups allowed us to circumvent technical issues related to body size, allowing us to examine the control of HR and breathing in animals beyond P12. Rather than reporting the minimum HR achieved during the course of the spontaneous event [as our laboratory did in rat pups (7)], we have reported the integrated HR, i.e., the area under the HR curve, which we feel is a more physiologically relevant indicator of changes in cardiac output, resulting from bradycardia magnitude and duration. Our results in mice not only support the contention that medullary 5-HT neurons are important for the maintenance of HR in early postnatal life, but also provide the nidus for future experiments utilizing novel transgenic techniques that will further delineate the molecular mechanisms underlying the effect of medullary 5-HT on the control of HR.
BRADYCARDIA AND HYPOXIA IN NEONATAL Pet-1−/− MICE

Perspectives and Significance

While our data suggest that respiratory dysfunction and associated hypoxia do not explain the greater bradycardia in neonates with reduced medullary 5-HT, hypoxia alone has clear implications for neonatal mortality. SIDS is a syndrome associated with medullary 5-HT deficiency (10) and is thought to occur after one or more hypoxic events, based on evidence of brain stem gliosis, right ventricular enlargement, extramedullary hematopoiesis, and enlarged adrenal chromaffin cell mass, all of which are hallmarks of chronic hypoxemia (20, 27, 28).

There is direct evidence of bradycardia in cardiorespiratory tracings from SIDS cases (24, 31). In a background of medullary 5-HT deficiency, an augmentation of spontaneous, transient bradycardias superimposed on a background of reduced resting HR could result in inadequate perfusion of the brain and other vital organs, thereby increasing SIDS risk. This assumes there is no compensatory increase in stroke volume or adjustments to the organs, thereby increasing SIDS risk. This assumes there is no compensatory increase in stroke volume or adjustments to the organs, thereby increasing SIDS risk. This assumes there is no compensatory increase in stroke volume or adjustments to the organs, thereby increasing SIDS risk. This assumes there is no compensatory increase in stroke volume or adjustments to the organs, thereby increasing SIDS risk. This assumes there is no compensatory increase in stroke volume or adjustments to the organs, thereby increasing SIDS risk. This assumes there is no compensatory increase in stroke volume or adjustments to the organs, thereby increasing SIDS risk. This assumes there is no compensatory increase in stroke volume or adjustments to the organs, thereby increasing SIDS risk.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


