HIGHLIGHTED TOPIC | Neural Control of Perinatal Respiration

Neural control of breathing: insights from genetic mouse models

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Gaultier C, Gallego J. Neural control of breathing: insights from genetic mouse models. J Appl Physiol 104: 1522–1530, 2008; doi:10.1152/japplphysiol.01266.2007——Recent studies described the in vivo ventilatory phenotype of mutant newborn mice with targeted deletions of genes involved in the organization and development of the respiratory-neuron network. Whole body flow barometric plethysmography is the noninvasive method of choice for studying unrestrained newborn mice. Breathing-pattern abnormalities with apneas occur in mutant newborn mice that lack genes involved in the development and modulation of rhythmogenesis. Studies of deficits in ventilatory responses to hypercapnia and/or hypoxia helped to identify genes involved in chemosensitivity to oxygen and carbon dioxide. Combined studies in mutant newborn mice and in humans have shed light on the pathogenesis of genetically determined respiratory-control abnormalities such as congenital central hypoventilation syndrome, Rett syndrome, and Prader-Willi syndrome. The development of mouse models has opened up the field of research into new treatments for respiratory-control disorders in humans.

Newborn mice; whole body plethysmography; congenital central hypoventilation syndrome; Rett syndrome; Prader-Willi syndrome

MUTANT MOUSE MODELS constitute powerful tools for understanding the organization and development of respiratory control. In vivo models incorporate the numerous factors that affect respiratory control and allow direct preclinical assessments of pharmacological treatments for respiratory control disorders. Analyzing the respiratory phenotype of mutant mice at birth is of crucial importance for two main reasons. First, null mutants for many genes of interest die within a few hours after birth, which leaves very little time to investigate respiratory function. Second, plasticity and learning effects may deeply alter respiratory control, thereby masking the effects of a gene mutation. Furthermore, studies in mutant newborn mice combined with studies in humans have provided valuable information on the pathogenesis of genetically determined respiratory-control disorders such as congenital central hypoventilation syndrome (CCHS), Prader-Willi syndrome (PWS), and Rett syndrome (RTT).

This review starts with a brief description of noninvasive methods for in vivo determination of the ventilatory phenotype in newborn mice, with emphasis on similarities in breathing between newborn mice and human infants. Then we discuss the ventilatory phenotypes of mutant newborn mice with targeted deletions of genes involved in the development of rhythmogenesis and/or chemosensitivity to oxygen and carbon dioxide. Finally, tentative models of CCHS, PWS, and RTT are described.

NONINVASIVE VENTILATORY PHENOTYPING IN NEWBORN MICE

Breathing is usually quantified based on respiratory rate or total breathing cycle time (T Tot), inspiratory and expiratory times, tidal volume (V T), and ventilation (V̇ ). V T and V̇ are divided by body weight to adjust for inter- and intraindividual differences in growth. Breathing variables are measured under both baseline conditions, i.e., while the animals are breathing air and during exposure to chemical stimuli such as hypercapnia, hypoxia, or hyperoxia, to test for chemosensitivity. Peripheral chemoreceptor function can be assessed by administering pure oxygen to newborn mice to achieve physiological chemodenervation (66), taking into account the fact that repeated hyperoxia results in increasing levels of respiratory depression (48).

The number and duration of apneas and periodic breathing episodes are indicators of respiratory instability, a general characteristic of newborn mammals. The resolution of respiratory instability during development varies considerably across individuals, and instability may even persist in adulthood. In particular, the C57BL/6 strain spontaneously exhibits central apneas and periodic breathing (39, 77), a specific feature to be considered when using C57BL/6 as the genetic background, which is often the case. Thus a genetic deficiency may aggravate pre-existing respiratory instability in specific strains. Mutant mice should be compared with wild-type littermates whenever possible.

The small birth weight (~1–2 g) and small V T (3–4 μl/g) of newborn mice preclude the use of measurement devices suitable for adult mice (e.g., pneumotachometers, thermistors,
respiratory inductance spirometers, and magnetometers). Two methods are suitable for newborn mice, namely, head-out plethysmography [e.g., (13), and whole body plethysmography (e.g., 51)].

In head-out plethysmography, the pup is placed in a chamber and its head is slipped outside the chamber through an opening with an airtight seal around the neck. The amount of air that moves in and out of the chamber as a result of breathing is roughly proportional to the changes in chest volume. This method provides a relatively direct evaluation of breathing, although the animal must be tightly restrained to ensure that no air leaks around the neck. The effects of the neck collar on upper airway resistance in these tiny animals are difficult to control.

Restraint can be avoided by using whole body flow barometric plethysmography, which consists in placing the animal in a chamber and measuring the pressure changes in the chamber. Pressure increases during inspiration and decreases during expiration. Theoretical considerations indicate that the pressure increase during inspiration is caused by addition of water vapor to the inspired gas and by warming of the inspired gas from the temperature in the chamber to that in the alveoli. Whole body plethysmography has been validated against pneumotachography in adult mice (58) but not in newborn mice. While not free from interpretation difficulties (27a, 53a), whole body plethysmography is the only method that provides semi-quantitative measurements of VT and V̇ while allowing valid measurements of T_{Tot} and apneas in freely moving animals. However, this method does not discriminate between central and obstructive apneas.

Continuous measurements can be obtained by using a bias flow through the plethysmograph to prevent carbon dioxide accumulation and ambient temperature drifts over time (67). Automatic methods that rely on spectral analysis for apnea detection facilitate phenotype determination in large numbers of mutant animals (51). As a rule, respiratory signals must be processed automatically to achieve reasonably high-throughput physiological screening of newborn mutant pups and to improve the identification of genetic factors involved in early respiratory-control disorders (32).

SIMILARITIES IN BREATHING BETWEEN NEWBORN MICE AND HUMAN INFANTS

Newborn mice resemble preterm human infants regarding many neurodevelopmental characteristics. Structural brain maturity in mice aged 4, 7, and 10 days roughly corresponds to that in human infants of 26, 36, and 40 weeks’ gestational age, respectively (38). The ventilatory response to hypoxia is virtually nonexistent during the first 6–12 postnatal hours in mice (70) and during the first few days in humans, whereas the ventilatory response to hypercapnia is present at birth in mice (70) and humans. The ventilatory response to hypoxia is biphasic, with an initial increase in ventilation followed by a decrease, which may fall below the prehypoxic level during hypoxia (hypoxic ventilatory decline) or on return to normoxia [post-hypoxic ventilatory decline (20, 70, 72)]. The magnitude of the hypoxic or post-hypoxic decline closely depends on the strength of the hypoxic stimulus, ambient temperature, and mouse strain, leading to some variability in previously reported data (6).

The response to hypoxia is similar in newborn mice and human infants in terms of arousal from sleep, defensive movements, and alerting cries. Newborn pups learn to produce alerting ultrasonic calls in anticipation of hypoxic stimuli (10). The arousal response to hypoxia is present at birth, when the ventilatory response to hypoxia is virtually nonexistent; moreover, later after birth, arousal occurs after the ventilation peak, i.e., during the hypoxic ventilatory decline (20). Repeated hypoxic episodes depress the arousal response, probably via habituation, but not the ventilatory response (27).

MUTANT NEWBORN MICE WITH ABNORMAL RHYTHMOGENESIS

Inactivation of genes involved in hindbrain segmentation during early embryogenesis, such as Krox20 (43), leads to severe breathing instability at birth with numerous long apneas and death of most of the mutants [see Invited Review by Thoby-Brisson and Greer (78a)].

Little was known about the genes that specify the identity of rhythm-generating neurons in the pre-Bötzing complex generator (pre-BötC) until a recent study in null mutant newborn mice lacking the transcription factor MafB (the basic leucin zipper transcription factor of the Maf family) showed that MafB was a marker for a subpopulation of pre-BötC neurons (9). MafB plays an important role in transcriptional regulation and cellular differentiation and is essential for hindbrain patterning. Null mutant MafB newborn mice die from central arrest of breathing activity soon after birth (9).

The role for neurotrophic factors in the development of rhythmo genesis has been investigated. The neurotrophin brain-derived neurotrophic factor (BDNF) is important in establishing connections between hindbrain pre-motor reticular neurons and motoneurons at early stages of development. BDNF is active until adulthood, particularly on the serotonergic spinal plasticity of breathing caused by intermittent hypoxia. Null mutant newborn mice lacking BDNF had a low respiratory rate and numerous apneas (29). Discharge frequency recorded in brainstem-spinal cord preparations from BDNF mutant newborn mice was more severely decreased in homozygous than in heterozygous animals (3). Thus BDNF appears critical to respiratory rhythmo genesis in neonatal mice, its effect being mediated by the BDNF receptor tyrosine kinase B in pre-BötC neurons. Exposure of neurons in the neonatal pre-BötC to exogenous BDNF specifically modified the membrane properties of rhythmically active neurons (78). Also, BDNF acutely modulated glutamatergic transmission in the nucleus of the solitary tract, the primary relay for peripheral afferent input to the brainstem respiratory rhythm-generating network (4).

The effects of respiratory-drive modulation by neurotransmitters and/or their receptors have been assessed in mutant newborn mice. To investigate the role for NMDA receptors in functional respiratory rhythm-generating neurons, newborn mice lacking the gene for NMDA receptor R1 subunit were used (31). Respiratory depression was noted at birth (63). Impaired glutaminergic transmission results in altered breathing at birth. Thus mice lacking brain/kidney phosphatase-activated glutaminase type 1 (GLS1) exhibited hypventilation due to decreased VT and died shortly after birth (50). The critical role for glutamatergic signaling in the development of respiratory rhythm-generating networks received further sup-
port from a study in mice having a genetic deficit in VGLUT2, a vesicular glutamate transporter. The pre-Botzinger rhythm generator failed to become active in the mutant embryos, and the mutant neonates died immediately after birth because of respiratory failure (83).

A role for GABA in the development of respiratory rhythm generation was looked for in null mutant newborn mice lacking the GABA-synthesizing enzyme 67-kDa glutamic acid decarboxylase (GAD67) (47). Irregular breathing and periodic gasp-like respirations were noted. Whole cell recordings demonstrated decreased firing of inspiratory neurons in the ventral medulla of GAD67-/- mice. These data suggest that GABAergic transmission may be nonessential for respiratory rhythm generation but may contribute to maintain a regular respiratory rhythm and normal inspiratory pattern in neonatal mice (47).

The maturation of neonatal rhythm generation appears to require an appropriate balance between excitatory input from the A6 nucleus and inhibitory input from the A5 nucleus (41). Loss of the noradrenergic A5 nucleus due to gene inactivation leads to abnormally fast breathing, as observed in null mutant newborn mice deficient for Mash1 (22) or Rnx (75), which died within a few hours after birth, and in null mutant mouse embryos lacking the glial cell line-derived neurotrophic factor (42). Also, genetic deficiency in glial cell line-derived neurotrophic factor (GDNF) is associated with a reduced number of tyrosine-hydroxylase neurons in the A5 nucleus and with an increased frequency of respiratory output in vitro (42). The homeobox gene Rnx (for respiratory neuron homeobox, also known as TLX3 or Hox11L2) is a homeobox gene that plays an essential role in the development of somatic and visceral neurons. Phox2a gene deficiency in mice results in loss of A6 nucleus neurons, causing respiratory rhythm abnormalities in vivo and in vitro and death of null mutants in the neonatal period (80, 86). The Ret gene contributes to the prenatal maturation of the noradrenergic A5 and A6 nuclei (81). Ret inactivation has no obvious effects on the in vivo fetal breathing pattern. However, Ret inactivation causes a decrease in the respiratory rate of in vitro fetal preparations. Null mutant newborn mice lacking Ret die soon after birth, whereas heterozygous mice have a normal breathing pattern at birth and develop normally.

Serotonin has long been identified as a main determinant of rhythmogenesis and apneas in newborn mammals (40). Excessive exposure to serotonin can be achieved by inactivating the gene for monoamine oxidase-A, the main serotonin-degrading enzyme. Monoamine-oxidase gene deficiency resulted in respiratory abnormalities at birth with unstable respiratory rhythm, defective serotonin modulation, and abnormal phrenic motoneuron activity (11). Although there is little doubt that serotonin is crucial to rhythmogenesis in newborn mice, the specific receptor subtypes involved in this effect have not been determined. Activation of serotonin-2A receptors is required for respiratory rhythm generation in vitro (62). However, null mutant newborn mice lacking the gene for serotonin-1A receptors had a normal breathing pattern, suggesting in vivo compensatory mechanisms (64).

The role for substance P, which modulates respiratory rhythm by acting through neurokinin-1 receptors (NK1), has been examined in mutant newborn mice. Findings in mutant newborn mice lacking the NK1 receptor gene showed that this receptor was not indispensable for producing a resting respiratory rhythm at birth (65). Moreover, in a study of medullary slice preparations from 4- to 12-day-old mice lacking the preprotachykinin gene, which codes for the substance P precursor, the pre-Bötzinger generated normal breathing activity under normoxic conditions, suggesting adaptation or compensation for long-term substance P deficiency. In another study, excess acetylcholine led to increases in VT and V˙ within the first few postnatal days in mutant newborn mice lacking the acetylcholinesterase gene (12).

Abnormally unstable breathing occurs in a number of other mutant newborn mouse models. Null-mutant newborn mice lacking the transcription factor Nurr1 (nuclear receptor related 1) exhibited increased breathing instability with apneas and died soon after birth (56). Nurr-1 is an orphan nuclear receptor that is critical for cell growth and apoptosis and for inducing the dopaminergic phenotype of nigrostriatal neurons. Studies have investigated the role of the constituents of the pituitary adenylate cyclase-activating intestine peptide (PACAP) superfamily, which are potent stimulators of cAMP production and subsequent protein kinase-A activation. PACAP is central to the early development of the central nervous system. Null mutant mice lacking PACAP exhibited hypoventilation during the neonatal period and were prone to sudden death preceded by prolonged apneas between 1 and 3 wk after birth (18). Furthermore, hypothermia worsened the respiratory depression. Recent experiments showed that the protein NALCN (sodium leak channel, nonselective), a member of the sodium/calcium channel family, formed the background sodium leak conductance and was required for normal rhythmogenesis at birth (49). Homozygous NALCN gene knockout mice exhibited severe respiratory rhythm disruption (as shown by brain stem-spinal cord recordings) with increased periods of apnea. All animals died of respiratory arrest within 24 h after birth. Finally, findings in mutant newborn mice supported a causal link between genetic abnormalities and the respiratory rhythm abnormalities found in human disorders such as CCHS, PWS, and RTT (see additional comments in this review).

**MUTANT NEWBORN MICE WITH ABNORMAL CHEMOSENSITIVITY**

**Chemosensitivity to oxygen.** Studies of chemosensitivity to oxygen in mutant newborn mice have shed light on the biological significance of neurotransmitters and neurotrophic factors acting on the arterial chemoreflex. Table 2 shows abnormal responses to sustained hypoxia or hyperoxia in the mutant newborn mouse models investigated so far.

A role for the endothelin 1 (Edn1) pathway in chemosensitivity to oxygen has been established in mutant newborn mice. Null mutant newborn mice lacking Edn1 (a potent vasoconstrictor peptide) and the endothelin receptor a (Ednra) had blunted ventilatory responses to hypoxia (15, 45). Furthermore, heterozygous mutant newborn mice lacking endothelin-converting-enzyme 1 (Ece1) allele exhibited abnormal hypercapnic ventilatory responses to hypoxia (71). Responses to hypoxia were blunted in null mutant newborn mice lacking Nurr1, a gene involved in dopamine transmission and expressed in the carotid bodies and nucleus of the solitary tract (56). Furthermore, studies have shown that PACAP is involved in chemosensitivity to oxygen. Null mutant newborn mice lacking the
gene for PACAP had blunted ventilatory responses to hypoxia (18). In vitro data indicated that PACAP stimulated the carotid bodies (17).

Information on the genes involved in the hypoxic ventilatory decline has been obtained by studies in mutant newborn mice. The decline was augmented in newborn mice with heterozygous disruption of the Ret (1) or Phox2b (23) gene (see below). Conversely, the decline was attenuated by disruption of other genes. Thus the hypoxic ventilatory decline was smaller in null mutant mice lacking the β2-subunit of the nicotinic acetylcholine receptors (21), a model replicating many of the abnormalities caused by perinatal nicotine exposure (16). Preliminary data suggest that loss of the serotonin transporter protein in 5HTT null mutant newborn mice may result in attenuation of the hypoxic ventilatory decline (30).

The neurotrophic factor BDNF ensures the survival of neurons involved in the arterial chemoreflex (28). Null mutant newborn mice lacking BDNF have arterial chemoreflex deficiencies, as shown by the absence of ventilatory responses to hyperoxia (29).

Chemosensitivities to carbon dioxide. Studies looking at respiratory phenotypes in newborn mice with targeted gene deletions have started to establish links between the expression of specific genes and the development of carbon-dioxide sensitivity (see Table 2). Null mutant newborn mice lacking the Edn1 and Ednra genes had blunted ventilatory responses to hypercapnia (45, 46). Ret (“rearranged during transfection”) is a transmembrane tyrosine kinase signaling receptor for members of the glial cell line-derived neurotrophic factor (GDNF) family of ligands. The Ret signaling pathway, which involves sequential expression of the Mash1, Phox, Ret, and TH genes (see below), is responsible for the development of all noradrenergic derivatives. Blunting of ventilatory responses to hypercapnia occurred in null mutant newborn mice lacking both Ret and TH genes but not in those lacking a single allele (1, 13). The basic helix-loop-helix transcription factor Mash-1 is expressed in embryonic neuronal precursors of the peripheral and central nervous systems and is essential for the development of most of the peripheral autonomic neurons. Deficient ventilatory responses to hypercapnia were observed in heterozygous Mash-1 (mammalian achaete-scute homologue-1) mutant newborn mice (24) and in mice with heterozygous mutation of the Phox2b gene, as described below.

Null mutant newborn mice with PACAP deficiency had a blunted ventilatory response to hypercapnia, suggesting that the PACAP signaling pathway may also contribute to the mechanisms underlying chemosensitivity to carbon dioxide (18). The role for potassium (K+) channels in the mechanisms underlying chemosensitivity to carbon dioxide was investigated using gene inactivation. Mutant mice lacking the gene for the K+ channel Kir2.2 were studied between postnatal days 9 and 18 (59). They exhibited decreased ventilatory responses to hypercapnia on days 14–15, suggesting a transient role for Kir2.2 in central chemosensitivity during postnatal development. Finally, null GLS1 mutant newborn mice had severe alterations in chemosensitivity to carbon dioxide (50), possibly related to dysfunction of glutaminergic neurons in central chemosensory areas (85).

MOUSE MODELS OF CONGENITAL CENTRAL HYPOVENTILATION SYNDROME

Congenital central hypoventilation syndrome (CCHS) is a rare condition in which abnormal autonomic control of breathing results in alveolar hypoventilation that is most marked during slow-wave sleep (33). Heterozygous mutations of PHOX2B, consisting mainly in polyalanine expansions, were recently identified in patients with CCHS (2). The paired-like homebox gene Phox2b is specifically expressed and required in neurons that go on to form the visceral reflex circuits that control the digestive, cardiovascular, and respiratory systems. Studies of the respiratory phenotype of neonatal mice with one invalidated Phox2b allele (Phox2b+/−) established the role for Phox2b in the development of respiratory control and suggested pathophysiological mechanisms for CCHS. Homozygous Phox2b knockout mice (Phox2b−/−) died in utero around embryonic day 14, whereas Phox2b+/− pups survived and were fertile without special care. The normal development of Phox2b−/− mice contrasted with the extremely severe CCHS phenotype. This difference in severity may be due to the fact that the alanine expansions generally found in the PHOX2B genes of CCHS patients exert a toxic gain-of-function or dominant-negative effect, whereas the Phox2b mutation in the mouse model abolishes Phox2b allele function. To investigate this possibility, a second mouse model was developed using a knock-in approach to introduce the most common mutation found in patients with CCHS, namely, addition of seven alanine residues.

Heterozygous Phox2b knockout newborn mice. Sleep apnea time was increased about sixfold in Phox2b+/− mutant pups and ventilation during active sleep was decreased by ~20%, compared to wild-type pups on postnatal day 5 (26), suggesting a decrease in the tonic drive to breathe provided by chemosensitive sites acting predominantly during sleep (19, 53; Table 1). In rats, the carbon dioxide-sensitive neurons of the caudal medullary raphe were active only during sleep (19) and those of the rostral nucleus of the solitary tract were more effective during sleep than during wakefulness (53).

Phox2b−/− mice examined 2 days after birth exhibited blunting of the ventilatory response to 8% carbon dioxide, a feature reminiscent of CCHS (23). The ventilatory increase during hypercapnia was ~40% smaller in Phox2b+/− than Phox2b−/− pups, due to depression of the breath duration (TV) response (Table 2). Carbon dioxide-sensitive sites [locus ceruleus and area postrema (19, 74)] and afferent pathways from the carotid bodies (which contribute to carbon-dioxide sensitivity) also depend on Phox2b for their development (23). However, no differences in ventilatory responses to carbon dioxide were detected between Phox2b+/− and Phox2b−/− pups aged 10 days (Table 2). Thus the postnatal impairment of the hypercapnic ventilatory response seen in Phox2b−/− pups was short lived. The mechanisms of this rapid functional recovery are unknown. Such mechanisms obviously do not operate in patients with CCHS, whose symptoms are typically severe and lifelong.

In 2-day-old Phox2b+/− pups, a biphasic pattern of V changes occurs in response to hypoxia [5% oxygen (23)]. The immediate hypopneic response to hypoxia (i.e., the ascending limb of the biphasic ventilatory response) is normal in these mutant pups. In CCHS patients, sensitivity to hypoxia varies.
across patients (61). Patients who can ventilate adequately during wakefulness have normal peripheral chemosensitivity to oxygen (35), suggesting that defects in peripheral chemosensitivity may exist only in the most severe cases. In contrast to the normal hyperpneic response to hypoxia, the hypoxic ventilatory decline is markedly increased in Phox2b<sup>+/−</sup> pups, mainly as a result of abnormal T<sub>tot</sub> control (Table 2). During the decline, total apnea duration is considerably longer in Phox2b<sup>+/−</sup> than in Phox2b<sup>+/+</sup> pups. Posthypoxic ventilatory depression has not been looked for in CCHS patients. Arousal responses to hypoxia are not significantly different between Phox2b<sup>+/−</sup> and Phox2b<sup>+/+</sup> pups.

The ventilatory decrease caused by hypoxia is larger in newborn Phox2b<sup>+/−</sup> mutant mice than in their wild-type littermates and is magnified by longer apnea durations (66). Furthermore, compared to wild-type pups, mutant pups have a longer-lasting ventilatory decline that persists beyond the return to normoxia. These results suggest stronger tonic activity of oxygen-sensitive peripheral chemoreceptors in mutant pups. This augmented peripheral tonic input may be ascribable to low arterial PO2 levels, which, unfortunately, cannot be measured in newborn mice using currently available techniques. The impairment in the ventilatory response to carbon dioxide in 2-day-old Phox2b<sup>+/−</sup> pups, as assessed comparatively to wild-type pups, was larger at 35°C ambient temperature than at 29°C (67). Interestingly, this effect of higher ambient temperature was not related to differences in body temperature changes measured subcutaneously in the interscapular region, which were closely similar in the two groups. The hypothalamus, which plays a key role in thermoregulation and directly controls respiratory brain stem structures, does not express Phox2b (23). However, hypothalamic abnormalities secondary to structural and neurological abnormalities in Phox2b-expressing brain regions may account for the influence of ambient temperature on ventilatory control in mutant pups.

### Table 1. Breathing pattern abnormalities under normoxic conditions in mutant newborn mice investigated in vivo

<table>
<thead>
<tr>
<th>Newborn Mice</th>
<th>Reference</th>
<th>Age at Study</th>
<th>Respiratory Frequency</th>
<th>Respiratory Frequency Variability</th>
<th>Apneas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krox20&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>43</td>
<td>Day 1</td>
<td>Decreased*</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>MafB&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>9</td>
<td>Birth</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>GAD67&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>29</td>
<td>Day 2</td>
<td>Decreased*</td>
<td>Increased*</td>
<td>+</td>
</tr>
<tr>
<td>Mash1&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>75</td>
<td>Day 1</td>
<td>Increased*</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Nurr1&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>56</td>
<td>First day</td>
<td>Decreased*</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>PACAP&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>18</td>
<td>Day 4</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Phox2b&lt;sup&gt;−+&lt;/sup&gt;</td>
<td>26</td>
<td>Day 5</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Phox2b27Ala&lt;sup&gt;+++&lt;/sup&gt;</td>
<td>25</td>
<td>First day</td>
<td>Decreased*</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

+, Abnormal number and/or duration of apneas compared with wild-type littermates. Data for PACAP<sup>−−</sup> apneas apply to hypothermic conditions. *Significantly decreased compared with wild-type littermates. †Significantly decreased in newborn males.

CCHS is frequently associated with thermoregulatory disorders, such as spurious profuse sweating, decreased basal body temperature with cool extremities, and absence of fever during infections (79). Parents of children with CCHS frequently report exacerbated breathing discomfort at high ambient temperatures (44), suggesting that warmer ambient temperatures may worsen the breathing-control disorders in CCHS. The impairment in the ventilatory response to carbon dioxide in 2-day-old Phox2b<sup>+/−</sup> pups, as assessed comparatively to wild-type pups, was larger at 35°C ambient temperature than at 29°C (67). Interestingly, this effect of higher ambient temperature was not related to differences in body temperature changes measured subcutaneously in the interscapular region, which were closely similar in the two groups. The hypothalamus, which plays a key role in thermoregulation and directly controls respiratory brain stem structures, does not express Phox2b (23). However, hypothalamic abnormalities secondary to structural and neurological abnormalities in Phox2b-expressing brain regions may account for the influence of ambient temperature on ventilatory control in mutant pups.

### Table 2. Ventilatory responses to hypercapnia, hypoxia, and hyperoxia in mutant newborn mice investigated in vivo

<table>
<thead>
<tr>
<th>Newborn Mice</th>
<th>Reference</th>
<th>Age at Study</th>
<th>Hypercapnic VR</th>
<th>Hypercapnic HVR</th>
<th>Hypoxic VR</th>
<th>Hypoxic HVD</th>
<th>Hyperoxic VR</th>
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<tbody>
<tr>
<td>Bdhf&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>29</td>
<td>First 4 days</td>
<td>Present</td>
<td>Blunted</td>
<td>Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edn1&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>45</td>
<td>First day</td>
<td>Blunted</td>
<td>Blunted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eedra&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>15</td>
<td>First day</td>
<td>Blunted</td>
<td>Blunted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ece&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>71</td>
<td>A few hours</td>
<td>NS</td>
<td>Decreased*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nurr1&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>56</td>
<td>First day</td>
<td>NS</td>
<td>Blunted</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PACAP&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>18</td>
<td>Day 4</td>
<td>Blunted</td>
<td>Blunted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mash1&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>24</td>
<td>12 h</td>
<td>Decreased*</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>13</td>
<td>A few hrs</td>
<td>Decreased*</td>
<td>NS</td>
<td>Augmented</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>1</td>
<td>12 h</td>
<td>NS</td>
<td>Augmented</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Phox2b&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>23</td>
<td>Day 2</td>
<td>Decreased</td>
<td>NS</td>
<td>Augmented</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Day 5</td>
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<td></td>
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<tr>
<td>23</td>
<td>Day 10</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phox2b27Ala&lt;sup&gt;+++&lt;/sup&gt;</td>
<td>25</td>
<td>First day</td>
<td>Abolished</td>
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</tr>
<tr>
<td>β-2n AchR&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>21</td>
<td>48 h</td>
<td>NS</td>
<td>Augmented</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>30</td>
<td>Days 1-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kir2.2&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>59</td>
<td>Days 14-15</td>
<td>Decreased*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLS1&lt;sup&gt;−−&lt;/sup&gt;</td>
<td>50</td>
<td>First day</td>
<td>Decreased</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

VR, ventilatory response; HVR, hyperpneic ventilatory response; HVD, hypoxic ventilatory decline; β-2nACh, β-2-subunit of the nicotinic acetylcholine receptors; NS, not significantly different from wild-type littermates. *Significantly decreased compared with wild-type littermates; †significantly decreased in males.
few postnatal hours with extremely severe respiratory disorders that replicate those seen in severe CCHS (25). The pups exhibit a defective response to hypercapnia, which is a key symptom of CCHS. The respiratory phenotype of heterozygous P{hox2b<sup>27Mel</sup>+/} mutant pups immediately after birth varied widely, with some mutants breathing only by intermittent gasps, others exhibiting highly unstable breathing interrupted by apneas (Table 1), and yet others breathing rhythmically albeit at a slower rate than wild-type pups. This phenotypic variability is reminiscent of the large individual differences in respiratory phenotype in newborns with CCHS. On average, total apnea duration was six times longer in mutant than in wild-type pups. All mutant pups died within 24 h after birth. The ventilatory response to 8% carbon dioxide was abolished in mutant pups (Table 2). This model characterized by a defective response to hypercapnia, which is a cardinal symptom of CCHS, should prove useful for designing treatments for CCHS.

**MOUSE MODEL OF PWS**

Major criteria for PWS include hypotonia and poor weight gain in infancy, rapid onset of excessive weight gain between 1 and 6 yr of age leading eventually to morbid obesity, dysmorphic facial features, hypogonadism, and developmental delay. Many of the patients exhibit sleep-disordered breathing with apneas, which may occur in the neonatal period, and episodes of hypoventilation (55). These patients have reduced ventilatory responses to hypoxia with absence of peripheral chemoreceptor responses (34). Severe blunting of carbon-dioxide chemosensitivity is an occasional finding that may be related to the morbid obesity seen in PWS (34). The genetic abnormality underlying PWS consists in lack of expression of several genes including the <i>necdin</i> gene on the paternally derived chromosome 15 (54). <i>Necdin</i> (neurally differentiated embryonal carcinoma-derived protein) is a “growth-suppressor” gene that may facilitate cell-cycle exit and maintenance of the neuronal postmitotic state.

The role for necdin deficiency in the respiratory control abnormalities seen in PWS has been investigated (36). <i>Necdin</i> null mutant newborn mice had respiratory instability at birth with apneas and died soon after birth. Studies of brain stemspinal cord preparations showed irregular diaphragmatic motor discharges recorded by electromyography, and studies of medullary slice preparations documented irregular rhythmic discharges. Furthermore, prolonged periods of suppressed rhythmogenesis were identified by whole cell path-clamp recordings, suggesting that developing respiratory-rhythm-generating neurons may be particularly sensitive to loss of necdin activity (60, 68). Detailed neuroanatomic investigations of <i>necdin</i>-deficient mutant mice detected developmental abnormalities with defects in the formation and morphology of the axonal tracts within the medulla. Immunolabeling for substance P, 5-HT, and noradrenergic fibers showed evidence of abnormal development. In addition, the tyrosine hydroxylase-positive fibers were enlarged and dystrophic. Thus deficiencies in the modulatory drive for pre-BötzC function may contribute to respiratory dysfunction in necdin-deficient mutant mice. Finally, preliminary data indicate a decreased response of isolated respiratory networks to hypoxia in <i>necdin</i> null mutant newborn mice compared with wild-type preparations (88).

Therefore, necdin deficiency may also be involved in the altered chemosensitivity to oxygen seen in patients with PWS.

**MOUSE MODELS OF RTT**

RTT is a neurodevelopmental disease characterized by severe neurological symptoms and respiratory-control disorders, including irregular breathing pattern, episodes of hyperventilation, and life-threatening apneas. The breathing abnormalities occur during wakefulness. Symptoms develop starting at 6–18 mo of age, concomitantly with deficiencies in bioaerobic systems. The breathing disorders improve with advancing age. RTT results from mosaicism for a mutation in the X-linked gene encoding the methyl-CpG-binding protein 2 (<i>MeCP2</i>) in neurons (84). <i>MeCP2</i> is a nuclear protein that binds specifically to methylated DNA and functions as a general transcription repressor.

Several mouse models of RTT have been developed (reviewed in Ref. 5), in particular using Cre-<i>loxP</i> recombination technology (14, 37). Null males (<i>+/−</i>) and females (<i>−/−</i>) were normal until 3–8 wk of age, when they showed neurological symptoms with a stiff uncoordinated gait and irregular breathing. Heterozygous females were normal until 3–4 mo of age, when some of them started to exhibit inertia, ataxia, and clasping. By 10 mo of age, 70% of heterozygous females had symptoms including breathing irregularities (37). <i>MeCP2</i>-null mice died between 6 and 12 wk of postnatal age (14). Female mice heterozygous for a null mutation in <i>MeCP2</i> (<i>MeCP2</i>+/−) and those with selective deletion of the protein in neurons (<i>MeCP2</i>+/nestin-Cre <i>lox</i>) displayed different respiratory phenotypes (8). In particular, marked respiratory depression following hypoxic hyperventilation was seen in <i>MeCP2</i>+/− mice but not in MeCP2+/nestin-Cre lox mice (8). MeCP2 deficiency in mature neurons was sufficient to cause neuronal dysfunction responsible for symptoms resembling those of RTT. In vivo investigation of respiratory control in <i>MeCP2</i>-null males (<i>MeCP2</i>−/−) by whole body plethysmography showed that juvenile MeCP2-deficient mice had normal breathing patterns with periods of stable cycles, although brief apneas lasting 1–2 s were observed occasionally in one of eight mice aged 10–14 days and three of five mice aged 21 days (82). In contrast, adult mice showed impaired breathing patterns, with considerable interindividual variability. Episodes of periodic breathing, a common symptom of RTT, occurred more often and lasted longer in heterozygous MeCP2-deficient female mice than in wild-type mice (7). Furthermore, hypoxia exacerbated the periodic breathing, indicating that increased peripheral chemosensitivity was not the cause of periodic breathing (7). Studies of heart-brain stem preparations showed highly variable postinspiratory activity in <i>MeCP2</i>-null males that correlated with breathing arrhythmias and apneas. The modulation of postinspiratory activity by pontine structures and by peripheral inputs from pulmonary stretch receptors was impaired in mutant mice. Similar mechanisms may account for respiratory-related abnormalities in patients with RTT (76).

<sup>GABA</sup> deficits in <i>MeCP2</i> mutant mice, which are highly relevant to respiratory control disorders, were recently reported. Although null males (<i>−/−</i>) and females (<i>−/−</i>) were apparently normal until 3–8 wk of age, marked depression of GABAergic synaptic transmission was observed in null males (<i>−/−</i>) as early as postnatal day 7 (52).
Mouse models of RTT have proven useful for developing drugs that may have therapeutic potential (73, 87). Noradrenergic stimulation by desipramine in MeCP2-deficient mice improved the breathing pattern for several weeks, increased the survival time, and increased the number of tyrosine hydroxylase-expressing neurons in the A1C1 and A2C2 cell groups (73). Chronic treatment of MeCP2 null mice with amphetamine, a drug that facilitates activation of glutamatergic AMPA receptors and elevates BDNF levels, caused marked functional improvements. The respiratory rate and minute volume returned to normal levels in treated mice 18–24 h after the last amphetamine injection (57). The effectiveness of amphetamine for treating respiratory control disorders may be different in newborn and older mice. In particular, studies in brain stem-spinal cord preparations in newborn rats showed that amphetamine increased breathing frequency when given within 3 days after birth (69). The duration of this effect has not been investigated. MeCP2 null mice exhibit progressive deficits in BDNF expression (57). Interestingly, the respiratory phenotypes of adult mice lacking BDNF and MeCP2-null mice share several similarities. Both models display depressed and irregular breathing, although only BDNF mutant mice exhibit reduced chemosensory drive.

CONCLUSION

Studies of the ventilatory phenotype of newborn mouse models for genetically determined breathing disorders have helped to understand how genetic abnormalities may disrupt one or more components of the neural control of breathing. In vivo studies of the ventilatory phenotype should be combined with in vitro studies of brain stem-spinal cord preparations, with working-heart brain stem preparations, and with cultured brain slices to obtain comprehensive information about the impact of gene disruptions on isolated neurons, isolated brain stems, and live animals.

Furthermore, technological improvements should allow the development of integrative approaches that encompass not only respiration, but also all vital functions such as heart rate, temperature, and behavioral states (26, 67).

Research into the ventilatory phenotype of mutant newborn mouse models is an important component of current international efforts to determine the function of genes and their role in human diseases using the mouse-to-humans approach and vice versa. Mutant newborn mouse models have improved our understanding of the genetic mechanisms underlying the pathogenesis of respiratory-control disorders in humans, such as CCHS. In the opposite direction, data from patients with genetically determined respiratory disorders led to investigations of mutant mouse models, which shed light on the pathogenic mechanisms underlying respiratory-control abnormalities in human diseases such as PWS and RTT. Also, mouse models have proven useful for identifying the pathogenic mechanisms involved in respiratory-control disorders whose genetic basis has not yet been elucidated, such as apnea of prematurity and sudden infant death syndrome. Genetic mouse models are making a strong contribution to efforts aimed at entering a new phase in the field of human respiratory-control disorders, namely, the development of innovative treatment strategies.

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