**Phox2b controls the development of peripheral chemoreceptors and afferent visceral pathways**

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**Summary**

We report that the afferent relays of visceral (cardiovascular, digestive and respiratory) reflexes, differentiate under the control of the paired-like homeobox gene Phox2b: the neural crest-derived carotid body, a chemosensor organ, degenerates in homozygous mutants, as do the three epibranchial placode-derived visceral sensory ganglia (geniculate, petrosal and nodose), while their central target, the nucleus of the solitary tract, which integrates all visceral information, never forms. These data establish Phox2b as an unusual ‘circuit-specific’ transcription factor devoted to the formation of autonomic reflex pathways. We also show that Phox2b heterozygous mutants have an altered response to hypoxia and hypercapnia at birth and a decreased tyrosine hydroxylase expression in the petrosal chemosensory neurons, thus providing mechanistic insight into congenital central hypoventilation syndrome, which is associated with heterozygous mutations in **PHOX2B**.

Key words: Transcription factor, Autonomic nervous system, Sensory neurons

**Introduction**

A key neuroanatomical substratum for the maintenance of bodily homeostasis is the set of reflex circuits of the autonomic [better termed ‘visceral’ (Blessing, 1997)] nervous system. These circuits modulate the activity of internal organs via a two-neuron efferent pathway that includes visceral motoneurons of the hindbrain and spinal cord, and their targets (the sympathetic, parasympathetic and enteric ganglionic neurons). The afferent pathway of visceral reflexes (Fig. 1A) conveys to the hindbrain baroreception, chemoreception (including taste) and osmoreception from blood vessels, airways and the digestive tract, and consequently modulates the discharge of visceral motoneurons as well as that of central respiratory rhythm generators. Primary visceral afferents make up the three epibranchial-derived cranial ganglia: the geniculate, petrosal and nodose. Among them, the chemosensory neurons of the petrosal ganglion are in fact postsynaptic to bona fide chemosensors, in particular the glomus cells of the carotid body (Katz et al., 1987), responsible for sensing hypoxia, hypercapnia, low pH (Gonzalez et al., 1994) and, as recently established, hypoglycemia (Pardal and Lopez-Barneo, 2002). Primary visceral sensory neurons project onto second order visceral sensory neurons in the hindbrain, which form the nucleus of the solitary tract (nTS), the first central relay for visceral information (Blessing, 1997). Dorsal to the nTS and also projecting on it, the area postrema (AP), a circumventricular organ whose neurons are in direct contact with the bloodstream and cerebrospinal fluid, serves as a chemoreceptive center responsive to a variety of toxins and responsible for chemically induced vomiting and conditional taste aversion (Borison, 1989).

All the neuronal classes listed above (with the exception of spinal visceral motoneurons, i.e. sympathetic pre-ganglionic neurons) are, from the earliest phase of their differentiation (and irrespective of their developmental origin or eventual phenotype), marked by the expression of the same transcription factor: the paired-like homeobox gene Phox2b (and of its paralogue Phox2a) (Brosenitsch and Katz, 2002; Pattyn et al., 1997; Tiveron et al., 1996). Conversely, expression of Phox2 genes is largely restricted to these neurons, and is therefore a simple, non combinatorial predictor of their eventual integration in autonomic reflex loops. We previously showed that Phox2b is actually required for the differentiation of the two neuron efferent visceral reflex pathway (with the exception of sympathetic pre-ganglionic neurons, see above): in Phox2b-null mutants, parasympathetic ganglia never form, enteric neuron precursors never migrate past the gastroesophageal junction, and sympathetic ganglionic cells fail to undergo their pan-neural as well as type-specific differentiation and eventually degenerate (Pattyn et al., 1999). Moreover, hindbrain visceral motoneurons (i.e. pre-ganglionic enteric and parasym pathetic neurons), normally born in the neuroepithelial pMNv domain of rhombomeres 2 to 7, are never generated (Pattyn et al., 2000b).

We show that, quite intriguingly, this Phox2b-dependency extends to the three-relay visceral sensory pathway comprising...
the carotid body, cranial ganglia and the nTS. In the context of our previous work, these new data reveal the developmental requirement for Phox2b throughout the nervous system to be unusually coherent and to correlate neither with neural phenotype, nor spatial coordinates, but connectivity.

Recently, heterozygous mutations in PHOX2B have been found to correlate with congenital central hypoventilation syndrome (CCHS) or Ondine’s curse, a complex dysautonomic syndrome (Amiel et al., 2003). The dependency of visceral afferent pathways on Phox2b that we report here sheds light on the etiopathology of this disease. In addition, we show that heterozygous mutants display respiratory anomalies which partially model the impaired autonomic control of breathing, pathognomonic for CCHS.

Materials and methods

Mouse breeding, genotyping, and rescue

The generation and genotyping of Phox2b mutant mice have been reported previously (Pattyn et al., 1999). Homozygous Phox2b mutants, which normally die at midgestation, were rescued beyond E10.5 with noradrenergic agonists as described (Pattyn et al., 2000a).

Histology, immunodetection and quantitative analysis

In situ hybridization using lacZ, peripherin, Rnx (Tlx3 – Mouse Genome Informatics), Tbx20 and Th antisense riboprobes, immunohistochemistry using Phox2a, Phox2b or Lmx1b antisera, and combined in situ hybridization with immunohistochemistry were performed as previously described (Tiveron et al., 1996). Double-immunofluorescence experiments using Phox2b and Lmx1b antibodies were analyzed on a Leica microscope. Pictures were superimposed in Photoshop.

Quantitative analysis of cranial sensory ganglia in Phox2b mutants was carried out by calculating the surface of the ganglia on serial transverse sections stained with peripherin using the QFluoro program (Leica).

Whole-body plethysmography

Breathing variables were measured non-invasively in unanaesthetized, unrestrained pups using whole-body flow barometric plethysmography (Dauger et al., 2001). Frequency and amplitude of respiratory movements, and their product, ventilation, were calculated from the plethysmographic signal in air (baseline ventilation), and during hypoxia and hypercapnia. The respiratory tests were run and analyzed before genotyping 2 days after birth (P2) on 38 Phox2b+/− (weight, 1.52±0.15 g; mouth temperature, 33.7±0.75°C) and 44 Phox2b+/+ pups (1.57±0.18 g and 33.5±0.58°C); at P6 on 18 Phox2b+/− (2.83±0.23 g and 32.1±0.35°C) and 13 Phox2b+/+ pups (2.89±0.15 g, and 32.5±0.38°C); and at P10 on 18 Phox2b+/− (4.96±0.28 g and 32.69±0.25°C) and 21 Phox2b+/+ pups (5.95±0.27 g and 33.5±0.12°C).

Results

Requirement of Phox2b for the formation of the nucleus of the solitary tract

We first examined the involvement of Phox2b in the development of the nTS. The nTS is an elongated nucleus which spans the rostrocaudal extent (Fig. 1A). At the obex of the IVth ventricle, it is capped by the AP, a wedge of neural tissue dorsal to the lumen of the tube. We previously showed that, at birth, most if not all neurons in the nTS and AP express Phox2b (Pattyn et al., 1997). At E10.5, the dorsal stripe of Phox2b expression that extends from r4 to the caudal hindbrain (Pattyn et al., 1997) is, by its dorsoventral position and rostrocaudal extent, a likely candidate as the source of nTS precursors (Qian et al., 2001). To document the development of the nTS, we focused on levels caudal to r6. At E10.5, Phox2b+ cells could be divided in a ventral and a dorsal population. The ventral population, where Phox2b expression started in the ventricular zone (i.e. in proliferative progenitors) corresponded to the visceral and branchial motor (vm/bm) neuronal precursors as evidenced by their post-mitotic coexpression of Phox2b and Isl1 (Fig. 1B,C) (Pattyn et al., 1997). The dorsal population (where Phox2b expression started in the mantle zone, i.e. postmitotically) coexpressed Rnx and Lmx1b (Fig. 1B,D,E) (Qian et al., 2002). At E11.5, the ventral bm/vm neurons had migrated dorsally to form the anlage of the dorsal motor nucleus of the vagus nerve (dmnX) and nucleus ambiguous (nA), while the dorsal population of Lmx1b+/Phox2b+ cells had considerably expanded, suggesting an ongoing dorsal production of ventral-bound migratory cells (Fig. 1F,J). By E12.5, many of these cells had accumulated close to the incipient dmnX (Fig. 1G,I), while others were still dorsal, presumably still being born, in agreement with birth dating experiments (Taber Pierce, 1973). By E13.5, a dramatic spatial reconfiguration, which might involve active cell migration, passive displacement or both, had given to the Phox2b+/Lmx1b+ population the recognizable, almost mature shape of the nTS (Fig. 1H,J). We compared this developmental sequence in heterozygous and homozygous mutants using lacZ as a marker. In Phox2b-null mutants the dorsal population of lacZ+/Lmx1b+/Rnx+ cells was preserved until E11.5, showing that the nTS precursors are born in Phox2b mutants, express Rnx and Lmx1b (not shown) and start migrating ventrally (Fig. 1J-M). At E12.5, however, the pattern of mutant nTS precursors was massively altered: dorsally, lacZ+ cells were present, but no lacZ expression was detectable more ventrally, where the majority of nTS precursors had accumulated by this stage in the heterozygotes (Fig. 1N,O). No cell death was detected by TUNEL analysis (not shown) suggesting that nTS precursors undergo a fate switch. At E13.5, lacZ expression in the mutants was restricted to scattered cells in the lateral medulla and no nTS was detectable (Fig. 1P,Q). At E18.5, on a dorsal whomelent view and on cross-sections of the medulla, a conspicuous loss of tissue affected the region where the nTS and AP were found in the wild type (Fig. 1R-U). Altogether, these data show that the nTS and AP never form in Phox2b-null mutants.

Degeneration of epibranchial placode-derived ganglia and of the carotid body in the absence of Phox2b

Projections to the nTS come from the geniculate, petrosal and nodose ganglia – the distal ganglia of, respectively, cranial nerves VII, IX and X. These ganglia develop from epibranchial placodes. Neuronal precursors first express Phox2a (Tiveron et al., 1996; Valarché et al., 1993) and, in mouse, Ngn2 (Fode et al., 1998) [Ngn1 in chick (Begbie et al., 2002)], then delaminate and start expressing Phox2b as they accumulate close to their site of aggregation (Begbie et al., 2002; Fode et al., 1998; Pattyn et al., 1997). We have previously shown that these ganglia form but become atrophic by midgestation in both, Phox2a−/− and Phox2bjacZjacZlacZ embryos (Morin et al., 1997; Pattyn et al., 1999). We now show that, in Phox2bjacZlacZ...
embryos, they lose expression of Phox2a and lacZ (i.e. of the Phox2b locus) as early as E13.5 (Fig. 2A-F) and continue to degenerate until virtual disappearance by E16.5 (Fig. 2G-I). The atrophy of the petrosal-nodose complex, visualized by peripherin expression, was estimated at 93% by surface area measurements (Fig. 2I) and, on most sections, the ganglion appeared fragmented (not shown). The atrophy was thus more complete than in Phox2a mutants where it was estimated at 77% with the ganglion retaining histological cohesion at birth (Morin et al., 1997). This denervation is unlikely to be secondary to the absence of the nTS, as ganglionic neurons of Rnx mutants survive up to birth even in the absence of their normal central target (Qian et al., 2001). Therefore, Phox2b expression is absolutely required for the differentiation and survival of most, and possibly all, epibranchial placode-derived ganglionic cells.

Finally, we examined the development of the carotid body. Its chemosensors, the glomus cells, which are derived from the neural crest (Le Douarin et al., 1972), express a variety of neuropeptide and neurotransmitter synthetic enzymes,
including tyrosine hydroxylase (TH) (Gonzalez et al., 1994) as well as the transcription factors Phox2a and Phox2b (Brosenitsch and Katz, 2002). In heterozygous mutants, at E13.5, when the carotid body was first detectable, a loose aggregate of cells expressing lacZ, Phox2a and Tbx20 (Kraus et al., 2001) could be seen at the prospective site of carotid body formation, i.e. at the divergence of the internal and external carotids (Fig. 2J,L,N). In homozygous mutants, a smaller aggregate was also detected and expressed lacZ, but neither Phox2a nor Tbx20 (Fig. 2K,M,O). At E16.5 the carotid body has switched on Th (R) in the heterozygote, is no longer detectable in the homozygote (Q,S). ec, external carotid artery; ic, internal carotid artery.

A haploinsufficient respiratory phenotype in Phox2b mutants
From a physiopathological standpoint, our finding that Phox2b controls the ontogeny of afferent visceral pathways is directly relevant to the recent report that heterozygous mutations in human PHOX2B are frequently associated with CCHS syndrome (Amiel et al., 2003). The defining dysfunction of CCHS is in the autonomic control of breathing resulting in hypoventilation, most severe during the non-rapid eye movements phase of sleep (Gozal, 1998). The proposed mechanism of CCHS is an impairment of central integration of chemoreceptor input (Spengler et al., 2001). Furthermore, post-mortem examinations have revealed abnormal carotid bodies in two individuals (Cutz et al., 1997). Thus, the carotid body, the petrosal ganglion that innervates it and the nTS which integrates chemoreceptive inputs are all candidates for the main site(s) of dysfunction. The dependency of all three structures on Phox2b in mice provides the straightforward basis for a cell-autonomous mechanism of PHOX2B involvement in CCHS. It is unclear, however, to what extent the PHOX2B mutations implicated in CCHS [polyalanine expansions and C-terminal frame shifts (Amiel et al., 2003)] are functionally equivalent to the mouse mutation, which is presumably a null (Pattyn et al., 1999). To investigate whether some aspects of CCHS are modeled by heterozygous mutant mice, we studied the respiratory phenotype of freely moving Phox2blacZ/+ and Phox2b+/+ pups. We first tested pups at 48 hours after birth to minimize the possible confounding effects of postnatal development and recovery processes. [Such recovery of respiratory impairments have been reported, for example, in heterozygous Mash1+/– (Dauger et al., 1999).] We examined the ventilatory changes caused by hypoxia (5% O2), which are mainly mediated by afferences from the carotid body glomus cells to the nTS via the petrosal ganglion. In newborns, the initial increase in ventilation caused by hypoxia is followed by a strong ventilatory depression (Bissonnette, 2000). We found that hypoxia resulted in a similar increase in ventilation in Phox2blacZ/+ and Phox2b+/+ pups, but the total duration of post-

Fig. 2. Involution of the petrosal-nodose ganglionic complex and carotid body between E13.5 and E16.5 in Phox2b lacZ/lacZ mutants. (A-F) At E13.5 the petrosal-nodose complex is already markedly atrophic, as assessed by peripherin in situ hybridization (A,B, quantified in I) and has lost expression of lacZ (C,D) and Phox2a (E,F), which was initially expressed in the mutant placodal precursors at E10.5 (Pattyn et al., 1999). (G,H) In situ hybridization with peripherin showing that, at E16.5, the involution is almost complete. (I) Quantification of the ganglionic atrophy at E13.5 and E16.5. Measurements of control ganglia were considered as 100±5.5% at E13.5 (n=6) and 100±3.7% at E16.5 (n=4). The relative measurements of mutant ganglia are 25.8±5.3% at E13.5 (n=4) and 74±2% at E16.5 (n=4). (J-O) The anlage of the carotid body is first detected at E13.5 in a heterozygous mutant (arrowhead in J) by immunohistochemistry against Phox2a (L) or in situ hybridization for lacZ (J) or Tbx20 (N). In a homozygous mutant, lacZ expression is already affected at this stage (arrowhead in K) and neither Phox2a (M) nor Tbx20 (O) expression occurs. (P-S) At E16.5 the carotid body, which still expresses lacZ (P) and has switched on Th (R) in the heterozygote, is no longer detectable in the homozygote (Q,S). ec, external carotid artery; ic, internal carotid artery.
Phox2b and afferent visceral pathways

Development and disease

Hypoxic apneas was strikingly longer in Phox2b lacZ/+ than in Phox2b+/+ pups (Fig. 3A,B). We also examined the ventilatory increase caused by hypercapnia (8% CO2), which is mediated by CO2/H+-sensitive cells widely distributed within the brainstem and also by carotid body glomus cells (Nattie, 2001), which significantly contribute to this increase [40% in dogs (Rodman et al., 2001)]. We found that the ventilatory response to hypercapnia was markedly lower in Phox2b lacZ/+ than in Phox2b+/+ pups (Fig. 3C,D). It is notable that, in CCHS, a blunted response to hypercapnia is also the most prominent ventilatory defect (Spengler et al., 2001). We then examined the respiratory phenotype of Phox2b lacZ/+ and Phox2b+/+ pups at P6 and P10. We focused on the ventilatory response to hypercapnia, which presented the closest similarity between the CCHS and the Phox2b lacZ/+ respiratory phenotypes. These responses were not distinguishable between Phox2b+/+ and Phox2b lacZ/+ pups at P10 (Fig. 3E), whereas 6-day-old pups presented an intermediate phenotype between 2-day- and 10-day-old pups (P<0.024, not shown).

We then looked for neuronal differentiation defects in heterozygous Phox2b mutants that could underlie this transient respiratory phenotype. We focused on Th, which is regulated by hypoxia and expressed in both glomus cells (Gonzalez et al., 1994; Wang et al., 1998) and the petrosal neurons that innervate them (Brosenitsch and Katz, 2002; Katz et al., 1987). Whereas Th expression was intact in glomus cells of late gestational (E16.5) heterozygous mutant embryos (Fig. 4A,B), it was decreased by 45% in the petrosal ganglion (P<0.01) (Fig. 4C,D,E). Whether this decrease is due to the disappearance of Th+ neurons or their loss of Th expression is not known, but it shows a dose-related effect of Phox2b on the differentiation of at least one class of neurons known to be involved in respiratory control. At P10, when the respiratory phenotype was no longer detectable, a small but not statistically significant (P>0.1) difference in TH+ cell counts was found in the petrosal ganglion between wild type and heterozygous mutants (Fig. 4E).

Discussion

Phox2b as a neural circuit-specific transcription factor

We have previously shown that the two-neuron efferent pathway of parasympathetic and enteric reflexes is Phox2b dependent (Pattyn et al., 2000b; Pattyn et al., 1999). We now show that Phox2b is also required for the differentiation of peripheral afferent visceral pathways (carotid body and
visceral sensory ganglia) and their central projection site, the nTS and associated AP. The afferent and efferent pathways thus defined constitute, via synapses of nTS interneurons onto visceral motoneurons, four- or five-neuron circuits that account for a variety of autonomic reflexes [examples can be found elsewhere (Blessing, 1997; Marshall, 1994)]. The dependence of the sympathetic reflex circuits is less complete, as it excludes spinal visceral motoneurons. However it does include noradrenergic sympathetic premotor neurons (Blessing, 1997; Pattyn et al., 2000a).

Conversely, the role of Phox2b seems largely restricted to the ontogeny of these pathways. The major class of Phox2b-dependent neurons, which seem not to fit in, are bm neurons (Pattyn et al., 2000b). However, from a phylogenetic perspective, this exception is only apparent. Although in amniotes, bm neurons have evolved voluntary functions in the control of head and jaw muscles, their original major function retained in fish and amphibia is the control of breathing mostly through the innervation of gill muscles. Phox2b thus appears to be dedicated to the differentiation of a set of neuronal classes whose sole, yet salient, point in common is to interconnect to form the reflex circuits of the visceral nervous system, most notably in its parasympathetic and enteric divisions. Indeed, the Phox2b-dependence of all those neurons is not paralleled by any common feature (other than their belonging to visceral reflex circuits), be it neurotransmitter phenotype, morphology, position or developmental origin (which ranges from dorsal and ventral neural tube to neural crest and neurogenic placodes).

After our suggestion of a hodological correlate for Phox2 gene expression (Tiveron et al., 1996), Rax (Qian et al., 2001) and Math1 (Bermingham et al., 2001) have been proposed to play roles in specifying visceral and proprioceptive circuits, respectively. However, among the components of visceral reflex circuits, only the nTS and medullary noradrenergic centres have been shown to depend on Rax, which is also required for proper formation of somatic sensory neurons and their connections (Qian et al., 2002). Math1 is necessary for the development of several of the synaptic sensory neurons and their eventual synaptic connection to other Phox2b-dependent neurons. Remarkably, the closest structural relative of Phox2b, Drg11, is expressed in both, primary and secondary somatic sensory neurons (Saito et al., 1995) and required for projection of the former to the latter (Chen et al., 2001). However, in the case of Phox2b, such a role cannot be tested in simple knockouts, as abrogation of Phox2b function entails an early differentiation block (Pattyn et al., 2000a; Pattyn et al., 2000b; Pattyn et al., 1999).

How could expression of a same transcription factor by two (or more) neuronal classes determine their interconnection? As proposed by Lin et al. (Lin et al., 1998), the same transcription factor could, in two synaptic partners, regulate the expression of homophilic molecules, such as cadherins, which are thought to be required for synaptogenesis. This scenario could underlie some cases of matching expression of the Ets-class transcription factors PEA3 and ER81 by connected proprioceptive sensory and motor neurons of the spinal cord observed in chick (Lin et al., 1998) [but not in mouse (Arber et al., 2000)]. However, unlike PEA3 and ER81, Phox2b is expressed very early, before any neurite outgrowth. Therefore, no aspect of the axonal navigation of visceral neurons can be instructive for Phox2b expression – unlike PEA3 expression by spinal motor and sensory neurons (Haase et al., 2002; Patel et al., 2003) – and, conversely, axonal navigation itself should be under the control of Phox2b if Phox2b is to specify connectivity. One hypothesis is that Phox2b controls the expression in both presynaptic and postsynaptic partners of the

![Fig. 4. Decrease in Th expression in the petrosal ganglion of Phox2b+/+/-- E16.5 embryos and normalization at P10. (A, B) In situ hybridization for Th expression shows that the heterozygous mutant glomus expresses normal levels of Th. (C, D) The subpopulation of petrosal ganglionic neurons, positioned ventrally, which expresses Th seen in the wild type in C is sparser in heterozygous mutants (D). The contour of the ganglion is outlined in red. (E) Quantification of Th-positive cells in the petrosal ganglion of E16.5 wild-type (n=6 ganglia) and heterozygous mutant (n=6 ganglia) embryos and P10 wild-type (n=4 ganglia) and heterozygous mutant (n=4 ganglia) pups. Values are means±s.e.m. (**P<0.01).](image-url)
same receptor for a chemotactic signal that steers the coordinated migration and appropriate positioning of their processes or cell bodies. In this respect, it is remarkable that the establishment of connectivity in visceral reflex circuits is often accomplished by such coordinated movements. For example, the nTS neurons and the dmX neurons [the dendrites of which will eventually invade the nTS (Shapiro and Miselis, 1985)] are born at opposite ventral and dorsal poles of the rhombencephalon and migrate towards each other to form the compact and extensively connected ‘dorsal vagal complex’ (this study). Another example is provided by the di-synaptic extrinsic motor innervation of the enteric nervous system, which is preceded by the roughly simultaneous rostrocaudal invasion of the gut mesenchyme by enteric neuronal precursors (Taraviras and Pachnis, 1999) and vagal axons (Baetge and Gershon, 1989). Interestingly, in this case, Phox2b is required for the navigation of at least one partner, because, in enteric neurons, it controls the expression of Ret (Pattyn et al., 1999), a co-receptor for GDNF that is required for their migration (Natarajan et al., 2002). As Ret is also regulated by Phox2b in at least two other visceral neuronal types [sympathetic ganglia and cranial sensory ganglia (Pattyn et al., 1999)] and as GDNF-family ligands (GFLs) are also involved in the migration and/or axonal guidance of other visceral neurons, namely sympathetic and parasympathetic ganglionic neurons (Enomoto et al., 2001; Enomoto et al., 2000; Hashino et al., 2001; Honma et al., 2002), the Ret/GFL signalling system is an appealing candidate for a mechanistic underpinning of our model.

**Phox2b heterozygous neonates as models of congenital central hypoventilation syndrome**

Recently, the pleiotropic role of Phox2b in the ontogeny of the visceral nervous system was given a physiopathological dimension as human PHOX2B was found to be mutated in a majority of cases of a complex genetic dysautonomia: congenital central hypoventilation syndrome or Ondine’s Curse (Amiel et al., 2003). It has already been noted by these authors that most of the incompletely penetrant symptoms of CCHS [such as multiple neuroblastomas, Hirschsprung disease, paralysis of the pupils, cardiac rhythm disturbances or dysphagia (Croaker et al., 1998; Gozal, 1998)] involve Phox2b-dependent neuronal classes (Pattyn et al., 1999), in these cases, autonomic ganglionic neurons. The present study provides mechanistic insight into the main, defining symptom of CCHS (impaired autonomic control of breathing) by showing that three neuronal types involved in sensing hypoxia and hypercapnia are strictly dependent on Phox2b for their differentiation: the carotid body, the petrosal chemoreceptors that innervate it and the nTS on which they project (Finley and Katz, 1992). Moreover, our study demonstrates that partial loss of function of Phox2b (by heterozygosity) leads to dysfunction of the respiratory system, which partly model the respiratory phenotype of CCHS and to dysgenesis of petrosal chemoreceptors, which may underlie this neonatal respiratory phenotype. Our data do not preclude additional haploinsufficient defects in CO2/H+ sensitive cells located in the carotid body, the nTS or the locus coeruleus (Nattie, 2001), which all depend on Phox2b (this study) (Pattyn et al., 2000a).

The recovery of the ventilatory response to hypercapnia in elder mutant pups confirms the considerable postnatal plasticity of respiratory control (Feldman et al., 2003). It was paralleled by a normalization of the number of Th+ neurons in the petrosal ganglion – most probably owing to the de novo expression of Th in Phox2+ neurons (Brosenitsch and Katz, 2002) – consistent with a causative role of these cells in the transient respiratory anomalies of heterozygous mutants. However, it is possible that Phox2b-dependent respiratory inputs are superceded by CO2/H+-sensitive sites that do not express Phox2b and are therefore spared by its mutation [e.g. the midline raphé, the hypothalamus and the fastigial nucleus of the cerebellum (Feldman et al., 2003)]. Determining whether this recovery, not observed in individuals with CCHS, reflects a difference in the control of breathing between humans and mice, or in the severity of the Phox2b mutation will await future studies. The polyalanine extension and C-terminal frame shift mutation found in CCHS (Amiel et al., 2003) could lead to hypomorphic or dominant negative alleles or even cellular toxicity in the case of polyalanine extensions, which may not be modeled by the mouse mutation. However, as frame-shift mutations cause a clinically indistinguishable syndrome, a dose-related effect, resulting from haploinsufficiency or dominant-negative action, seems most likely.

It should be noted that Phox2b expression persists at postnatal stages in several neural structures, such as the carotid body (Brosenitsch and Katz, 2002) or the nTS (Pattyn et al., 1997). Therefore, it is conceivable that, apart from developmental defects such as the one we report in petrosal chemoreceptors, the respiratory disorder of CCHS could also reflect the disruption of post-developmental roles of Phox2b.

Finally, this study raises the possibility that Phox2b could be involved in cases of sudden infant death syndrome, in which anomalies in the catecholamine content of carotid bodies have been found (Perrin et al., 1984). More generally, given the richness and variability of the clinical picture of CCHS, it is tempting to speculate that mutations in this gene could underlie yet other congenital autonomic dysfunctions or dysplasia.

We thank Q.-F. Ma for the gift of the Rnx probe, T. Jessell for the Isl1 and Lmx1b antibodies, M.-M. Fortier for the peripherin probe, J. Ericson for the Tbx20 probe, J. Amiel and S. Lyonnet for communication of results prior to publication, and D. Katz for sharing his expertise on the carotid body. This work was supported by grants from the EC (QLG2-CT-2001-01467) and AFM (to C.G.).

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