Review

Endogenous noradrenaline affects the maturation and function of the respiratory network: Possible implication for SIDS

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Abstract

Breathing is a vital, rhythmic motor act that is required for blood oxygenation and oxygen delivery to the whole body. Therefore, the brainstem network responsible for the elaboration of the respiratory rhythm must function from the very first moments of extrauterine life. In this review, it is shown that the brainstem noradrenergic system plays a pivotal role in both the modulation and the maturation of the respiratory rhythm generator. Compelling evidence are reported demonstrating that genetically induced alterations of the noradrenergic system in mice affect the prenatal maturation and the perinatal function of the respiratory rhythm generator and have drastic consequences on postnatal survival. Sudden Infant Death Syndrome (SIDS), the leader cause of infant death in industrialised countries, may result from cardiorespiratory disorders during sleep. As several cases of SIDS have been observed in infants having noradrenergic deficits, a possible link between prenatal alteration of the noradrenergic system, altered maturation and function of the respiratory network and SIDS is suggested.

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1. Introduction

Respiration is a vital, rhythmic motor act that allows blood oxygenation in the lungs and oxygen delivery to the whole body, including the heart and the brain. Therefore from the very first moments of extrauterine life, the neonatal mammal must be able to produce rhythmic respiratory movements and must be able to adapt it to environmental and behavioral changes. Thus, postnatal survival requires a normal prenatal maturation of the respiratory rhythm generator (RRG), i.e. the brainstem neural network responsible for the production of the central rhythmic drive to the respiratory motoneurons. Postnatal survival also requires a normal prenatal maturation of the different structures that are implicated in the modulation of the RRG, i.e. on the one hand the peripheral receptors that convey messages from the respiratory apparatus (lungs, trachea, carotid bodies, muscles, etc.) and on the other hand the central structures that receive and integrate these peripheral messages to regulate and modulate the RRG activity. Among the latter structures, the central bioaminergic neurons have a crucial role, especially the serotonergic and the noradrenergic ones. As reviewed below, the brainstem noradrenaline (NA) system plays a pivotal role in both the modulation and the maturation of the RRG. This suggests that genetic or epigenetic alterations of the maturation of the NA system during the perinatal period may affect the maturation of the RRG and therefore may have drastic consequences on postnatal survival. Sudden Infant Death Syndrome (SIDS), the leading cause of infant death in industrialised countries, may be due to respiratory disorders (Horne et al., 2004; Ribas-Salgueiro et al., 2004). SIDS victims frequently display brainstem NA deficits (Ozawa et al., 1999, 2003; Cann-Moisan et al., 1999; Obonai et al., 1998; Kopp et al., 1993; Takashima and Becker, 1991; Denoroy et al., 1987; Ozand and Tildon, 1983) and frequently present mutations of genes implicated in specification of NA phenotype (Weese-Mayer et al., 2004). This is fully in agreement with a possible link between prenatal NA deficits, respiratory dysfunction and SIDS.

2. The maturing respiratory network(s)

As first shown by Suzue (1984) in neonatal rats and thereafter by many others in fetal rats, neonatal mice and fetal mice (Di Pasquale et al., 1992; Greer et al., 1992, Hilaire et al., 1997, Viemari et al., 2003), the isolated RRG may be conveniently studied in brainstem–spinal cord preparations (“en bloc” preparations; Fig. 1A), where it continues to produce a rhythmic central drive and to induce rhythmic phrenic bursts for several hours in vitro. In addition, the RRG also continues to function in medullary slice preparations from neonatal and young rodents (Smith et al., 1991; Johnson et al., 1994; Koshiya and Smith, 1999; etc.) as shown by recording the population activity of respiratory neurons from the ventral respiratory group (Fig. 1B).

The use of these “en bloc” and “slice” in vitro preparations has considerably improved our knowledge on the mechanisms through which the RRG produces its rhythmic drive. It has been shown that the neonatal RRG is located in a small region of the ventrolateral medulla, the pre-Botzinger Complex (PBC) where electrolytic lesions disrupt the in vitro rhythmic drive (Onimaru et al., 1988; Viemari et al., 2003). In adult mammals, lesion of the PBC also severely disrupts breathing in vivo (Ramirez et al., 1998; Gray et al., 2001, Solomon, 2002, 2003, Wenninger et al., 2004a,b; McKay et al., 2005). Indeed, the PBC contains respiratory pacemaker neurons that play a crucial role in respiratory rhythmogenesis (Onimaru et al., 1988; Smith et al., 1991; Ramirez et al., 1998; Johnson et al., 1994; Koshiya and Smith, 1999).
In human and rodent fetuses, respiratory movements occur in utero, very early during gestation (for a review, see Achard et al., 2005). Indeed, 3–4 days before birth, the fetal RRG is already able to elaborate a rhythmic drive, although unstable and variable (Di Pasquale et al., 1992; Greer et al., 1992). However, no pacemaker neurons are found in the fetal PBC during this early developmental period (Di Pasquale et al., 1994; Pagliardini et al., 2003). Therefore, several questions arise. Are the fetal and neonatal RRG different networks or the same maturing network? How do this (these) network(s) function (pacemaker vs. network connection properties)? How do pacemaker properties develop within the maturing PBC? To answer these questions, a computational model has been developed that reproduces well the experimental data (Achard et al., 2005). This computational model shows that the “pacemaker vs. network dilemma” can be solved simply: fetal conditional pacemaker neurons only require both a certain amount of noise and synaptic connections from the other neurons to express their bursting properties. Therefore, this means that the prenatal maturation and function of the RRG is at least in part affected by the inputs it receives. As shown below, among plenty of inputs, those from NA neurons may have a pivotal role.

3. The maturing central NA system

As summarized in Fig. 2A, the synthesis of NA from L-tyrosine successively requires two main synthetic enzymes, tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH). The initial and rate limiting step in NA synthesis is the hydroxylation of L-tyrosine in L-Dopa through TH. Then L-Dopa decarboxylation leads to DA, and finally DA hydroxylation leads to NA through DBH.

3.1. Genetic manipulations of NA synthesis

Different mutant mice have been created in which the ability to synthesize NA (TH-deficiency and DBH-deficiency), to remove NA from the synapse (NA transporter deficiency), or to degrade NA are impaired. These mice with genetically induced alterations of the NA system are invaluable tools for the study of NA physiology and for the study of the long-term physiological consequences of altered NA homeostasis (see the excellent review from Carson and Robertson, 2002).

TH-deficiency in mice is lethal, with mortality occurring early during embryonic development, from E11.5 to E15.5, i.e. few days after the normal appearance of the first NA neurons (E9–E10.5). Surgical delivery at E18.5 in mice reveals that 19% of TH-deficient fetuses survive, some take a breath but all die within 1 day. They could be rescued by administration of L-Dopa although they failed to thrive and to survive past 5 weeks (Zhou et al., 1995). The presence of a weak level of DA in TH-deficient mice suggests the existence of an alternative synthesis pathway (Fig. 2A), probably through tyrosinase (Rios et al., 1999). TH transcription is mediated by different kinase pathways in central NA neurons, with possible differences between A1 and A2 neurons (Rusnak and Gainer, 2005).

DBH-deficiency is also lethal in mice (Thomas et al., 1995) but DBH-deficient fetuses could be rescued though administration of L-dihydroxyphenylserine (DOPS) that is
converted directly to NA. In addition, mobilizing NA to vesicles is required for mouse survival and complete removal of the vesicular monoamine transporter is lethal (Carson and Robertson, 2002).

3.2. Specification of NA phenotype during development

During embryonic development, specification of NA phenotype in central neurons depends on BMP protein signaling and on four transcription factors at least, Rux, Mash1, Phox2a and Phox2b, that can act in different orders (Fig. 2B), and can interact with distinct factors in different types of NA cells (Goridis and Rohrer, 2002). Indeed, these transcription factors are linked in transcriptional cascades but their respective positions within the cascade differ from one NA group to another (Tiveron et al., 1996; Hirsch et al., 1998; Lo et al., 1998; Pattyn et al., 1997, 1999, 2000; Qian et al., 2001, 2002). Rux inactivation compromises the formation of most NA neurons but partly preserved A6 neurons (Qian et al., 2001) whereas Phox2a inactivation leads to the agenesis of A6 neurons but does not drastically affect the formation of other NA neurons (Morin et al., 1997). Both Rux- and Phox2a-deficient mice die within 24 h after birth. Phox2b is another genetic factor with an identical homeodomain-binding site than Phox2a that is required for TH and DBH transcriptions and its efficiency is comparable to that of Phox2a (Yang et al., 1998). In the enteric nervous system and the sympathetic ganglia, Phox2b is needed for the expression of the GDNF-receptor subunit Ret, for maintaining Mash1 expression and for the expression of TH and DBH (Pattyn et al., 1999). Indeed, Phox2b regulates the NA phenotype in vertebrates since Phox2b is expressed in all central NA neurons and is required for the differentiation of all NA centers in the brain, including A6 (Pattyn et al., 2000). The molecular mechanisms underlying the regulation of Phox2a and Phox2b gene expression and the relation to NA differentiation are still under debate (Goridis and Rohrer, 2002; Jong Hong et al., 2004).

Besides Mash1, Rux, Phox2a, Phox2b, other genes are implicated in the development of NA neurons, such as Ret and BDNF. Ret gene encodes a transmembrane tyrosine kinase receptor and is expressed in presumptive monoaminergic brainstem neurons at E8–E10 (Pachnis et al., 1993). Ret contributes to the formation of NA neurons (Dauger et al., 2001) and is highly expressed in some brainstem NA areas and in motor nerve nuclei of E18 embryos (Viemari et al., 2005a). Ret-deficient neonates do not survive past 1 or 2 days (Schuchardt et al., 1994; Aizenfisz et al., 2002), and Ret-deficient fetuses at E18 have reduced pontine NA contents and reduced number of A5 and A6 neurons (Viemari et al., 2005a).

BDNF protein is also crucial for survival and plasticity of NA neurons (Copray et al., 1999; Akbarian et al., 2002). The BDNF mRNA is expressed in medullary A1/C1 and A2/C2 neurons (Cho et al., 1999) and BDNF protein increases the number and branching of cultured TH-neurons (Guo et al., 2005). Null mutants for TrkB, the tyrosine kinase receptor for BDNF display a loss of NA neurons whereas TrkB ligands increase their number (Holm et al., 2003).

3.3. Location of maturing brainstem NA neurons

In adult mice, the NA phenotype is expressed by many central neurons forming several anatomical groups among which four main brainstem groups can be distinguished, two groups in the pons (the compact, dense dorsal A6 group or locus coeruleus and the more diffuse, ventral A5 group) and two groups in the medulla (the ventral A1/C1 and the dorsal A2/C2 groups). These NA groups are implicated in numerous neurovegetative functions, such as sleep, mood, thermoregulation, cardiovascular regulations, etc. (see Guyenet, 1991). During the embryonic development, the NA neurons from the A6 group that arise in the dorsal isthmic region of the hindbrain are among the earliest born neurons, at E9–E10.5 in mouse embryos (Lauder and Bloom, 1974). A6 neurons are already well expressed at gestational week 6 in human embryos (Zecevic and Verney, 1995). In neonatal mice, the four brainstem NA groups are easily distinguished within the brainstem although a continuum of scattered NA neurons may be observed in the pons, between the dorsal A6 and ventral A5 groups (Fig. 2C) and NA neurons of the A1/C1 group may extend dorsally, towards the A2/C2 group (Fig. 2C). Counting of TH-expressing neurons in the A1/C1 and A2/C2 areas reveals a 25% larger number of TH-neurons in neonatal than in adult mice (Roux, personal communication). In rats aged from postnatal day 1 to postnatal day 10 (P1 to P10), A6 does not exhibit significant developmental changes but the number of A5 neurons decreases with age (Ito et al., 2002). In addition, measuring the NA content in the whole brain of mice shows 33% lower values in neonates than in adults (Ide et al., 2005). Thus, the maturation of the NA system still continues after birth. Indeed, the postnatal development of TH activity displays two main developmental windows, the first window within a few days after birth (the intra-uterine transition) and the second window between 2 and 3 weeks of age, corresponding to a deep energetic phase related to the maturation of cardiorespiratory processes (Roux et al., 2003). In addition, TH expression during ventilatory acclimatization shows hypoxia-induced plasticity (Dumas et al., 1996).

4. Endogenous NA modulates the neonatal RRG activity

The use of both “en bloc” and “slice” preparations that retain the ability to produce a rhythmic central respiratory drive (Fig. 1) and that contain either the four brainstem NA groups (ponto-medullary “en bloc” preparations) or only the two medullary groups (medullary “en bloc” preparations and medullary slices) has considerably improved our
knowledge on the mechanisms through which endogenous NA may modulate the RRG activity. As illustrated in Fig. 3, the four groups of brainstem NA neurons play different roles in the NA modulation of the RRG activity.

4.1. Pontine A5 group

As reviewed recently (Hilaire et al., 2004), the pontine A5 and A6 groups exert dual, opposite effects on the RRG, with A5 inhibitory and A6 facilitatory modulations. In both rat and mouse neonates, elimination of A5 inputs onto the medullary RRG by either pons resection or A5 electrolytic lesions or pharmacological inhibition of A5 neurons increases the respiratory rhythm. In addition, blockade of medullary $\alpha_2$ adrenoceptors in "en bloc" preparations retaining the A5 areas increased the respiratory rhythm. It has been therefore concluded that the pontine A5 neurons exert a permanent inhibition onto the medullary RRG through medullary $\alpha_2$ adrenoceptors (Hilaire et al., 1989; Errchidi et al., 1990, 1991; Viemari et al., 2003; Hilaire et al., 2004).

In both rat and mouse fetuses, the A5 inhibitory modulation of the RRG already exists prior to birth although it develops rather late during gestation (Di Pasquale et al., 1992; Viemari et al., 2003). The A5 modulatory process persists throughout life since in adult animals, (1) activation of A5 neurons slows down the in vivo respiratory rhythm (Jodkowski et al., 1997; Dawid-Milner et al., 2001), (2) A5 neurons are synaptically connected to the respiratory neurons (Dobbins and Feldman, 1994; Gaytan et al., 2002), (3) A5 neurons display a respiratory-related rhythm and a chemosensitivity (Guyenet et al., 1993), and (4) contribute to respiratory responses to hypoxia and hypercapnia (Coles and Dick, 1996; Soulier et al., 1997; Roux et al., 2000; Peyronnet et al., 2000).

4.2. Pontine A6 group

In neonatal rodents, application of exogenous NA to medullary preparations alters the RRG rhythm, probably through the PBC area (Errchidi et al., 1991; Al-Zubaidy et al., 1996) and the PBC pacemaker neurons (Arata et al., 1998). However, NA effects are complex and depending on experimental conditions (ponto-medullary and medullary preparations) and species (rats or mice), exogenous NA mainly facilitates or mainly inhibits the neonatal RRG, with a mixture of $\alpha_1$ facilitatory and $\alpha_2$ inhibitory effects. Although quantitative differences exist between neonatal rats and mice (Viemari and Hilaire, 2002), the complexity of the NA effects on the respiratory rhythm suggested that, besides the A5 inhibition, other modulatory processes exist.

In "en bloc" preparations retaining the A6 group (but not the A5 group) from neonatal mice, blockade of medullary $\alpha_1$ adrenoceptors decreases the respiratory rhythm and the $\alpha_1$-dependent rhythm depression is abolished after A6 elimination. In addition, blockade of the degradation of the endogenous NA increases the respiratory rhythm and this increase is abolished after A6 elimination. As both effects require A6 groups to be observed, it has been concluded that A6 neurons exert a facilitatory modulation onto the RRG via medullary $\alpha_1$ adrenoceptors. The A6 facilitation of the RRG has also been found in neonatal rat preparations (Hakuno et al., 2004). The A6 facilitation of the RRG is fully supported by in vitro results showing that A6 neurons present respiratory-related activity and participate in the central chemosensitivity (Ito et al., 2004; Ballantyne and Scheid, 2000; Oyamada et al., 1998). In adult mammals, the influence of the A6 group onto the RRG is likely to persist since A6 neurons are connected to the medullary respiratory network (Dobbins and Feldman, 1994) and display respiratory-related firing (Guyenet et al., 1993).

4.3. Medullary A1/C1 and A2/C2 groups

The demonstration that the pontine A5 and A6 neurons exert a dual, opposite control on the medullary RRG (Fig. 3) does not exclude a possible role of medullary A1/C1 and A2/C2 NA neurons in RRG modulation (Zanella et al., in press). In medullary preparations, i.e. in preparations where the pons and the A5 and A6 groups are absent, activation of the NA synthesis through application of the NA precursor L-tyrosine increases the respiratory rhythm whereas blockade of the $\alpha_2$ adrenoceptors through application of the $\alpha_2$ adrenoceptor antagonist yohimbine induces a dose-dependent depression of the respiratory rhythm (Figs. 1C and 3). As the latter effect persists after elimination of the dorsal A2/C2 group, it is concluded that the A1/C1 group exerts a permanent facilitation of the RRG through $\alpha_2$ adrenoceptors. In addition, experiments performed in slice preparations from 2-week-old neonatal mice fully confirm the facilitating role of A1/C1 neurons. As already reported
elimination of the dorsal part of the medulla does not suppress in vitro respiratory rhythm (Hilaire et al., 1990; Infante et al., 2003) but it increases the variability of the respiratory cycle period, suggesting a new, but not yet explained, role of A2/C2 neurons in stabilizing the RRG activity (Fig. 3) (Zanella et al., in press).

The schema of Fig. 3 illustrates the complexity of the NA modulation of the RRG:

- Endogenous NA released from the four NA brainstem groups may facilitate (A1/C1 and A6) and reduce (A5) the RRG activity,
- The pontine A5 and A6 groups exert dual, opposite effects through different subtypes of medullary adrenoceptors, with an A5 break (a2 adrenoceptors) and an A6 accelerator (a1 adrenoceptors),
- The pontine A5 and the medullary A1/C1 groups exert dual, opposite effects, with an A5 break and an A1/C1 accelerator both acting through medullary a2 adrenoceptors; whether the same a2 adrenoceptors constitute the final pathway of A5 and A1/C1 modulatory pathways or whether there exist two functional types of a2 adrenoceptors specifically belonging to the two modulatory pathways remain an open question.
- The medullary A2/C2 group might play a stabilizing role on the RRG through unknown mechanisms and unknown adrenoceptor subtypes.

Indeed the schema of Fig. 3 is too much simple and interactions between the different NA groups as well as interactions between NA neurons and other neurons such as 5HT neurons should be taken into account. However, this simple schema summarizes rather well the observed results.

5. Morphological support of the RRG modulation by NA neurons

To know whether anatomical pathways support the A5–A6 modulations of the medullary RRG at birth, we are performing rabies-virus tracing experiments coupled with immunodetection of TH-neurons (Fig. 2). RV is a neurotropic virus that retrogradely infects, step by step, synaptically connected neurons. We inject rabies virus (RV) in the diaphragm of mice at P1 and sacrifice the neonates at different times after the diaphragmatic RV injection. Phrenic motoneurons are infected first within 24 to 30h post injection, followed by infection of cervical cord interneurons and brainstem bulbospinal neurons (30–36h post injection) and finally propriobulbar respiratory neurons, especially those constituting the classical medullary "respiratory centers" such as the ventral respiratory group (Fig. 4A). In addition, brainstem NA neurons are also infected (36–42h post injection). In the pons, RV-infection and TH immunoreactivity are co-localized in some A5 and A6 neurons (arrows in Fig. 4B, C). This neonatal result is consistent with adult results showing projections from pontine A5 and A6 neurons towards the medullary respiratory network in rats (Dobbins and Feldman, 1994) and mice (Gaytan et al., 2002). Therefore both in neonatal and adult rodents, A5 and A6 neurons are actually connected to the respiratory network and these connections may support the A5 inhibitory and A6 facilitatory modulations reported above. In the medulla, TH and RV immunoreactive neurons were sometimes localized in overlapping areas (Fig. 2) but none of the TH-neurons of the A1/C1 and A2/C2 groups were RV-infected (Fig. 4D). The lack of RV-infection of the medullary A1/C1 and A2/C2 neurons may mean either that these neurons are RV-resistant (Tsiang et al., 1983) or that they do not modulate the RRG through "classical" synaptic relations but through "volume transmission". This latter possibility fits with several reports showing that adrenoceptors are often associated with nonsynaptic processes and that catecholamine released from axon terminals plays an important role in modulating the neural communications without synaptic contacts (Lee et al., 1998a, 1998b; Vizi, 1999, 2000; Liprando et al., 2004).

6. The brainstem NA groups contribute to the maturation of the neonatal RRG

As reported above, the differentiation of NA neurons during CNS development is controlled by several genes such as Rnx, Phox2a, Phox2b and Ret and the deletion of either one of these genes produces mutant mice that do not survive more than P1–P2. The short life span of these mutants might be indicative of fatal respiratory dysfunction at birth and might suggest a possible role of endogenous NA in the prenatal maturation of RRG (Blanchi and Sieweke, 2005). Indeed analysing the in vivo breathing and the in vitro RRG activity in mutant mice with genetically induced NA deficits reveal that the proper development of the NA brainstem groups is required for a normal activity of the neonatal RRG and survival. Schemas in Fig. 5 summarize some of the results obtained in mutant mice with NA deficits.

In Rnx mutants (Fig. 5A), where the formation of most NA neurons is drastically compromised but that of A6 neurons is partly preserved, the respiratory frequency is abnormally high at birth, with respiratory cycle of highly variable duration and frequent respiratory arrests (Shirasawa et al., 2000). This means that the lack of A5, A1/C1 and A2/C2 neurons during the gestation has altered the prenatal maturation of the RRG. Indeed, the loss of A5 break vs. the persistence of A6 accelerator may contribute to their fast respiratory rhythm, the lack of A1/C1 facilitation and A2/C2 stabilizing effects to the respiratory arrests and the variability of cycle duration, respectively.

In Phox2a mutants (Fig. 5B), the formation of most of NA neurons is preserved (A5, A1/C1 and A2/C2 neurons)
but the formation of A6 neurons is drastically altered. The agenesis of the A6 neurons resulted in an altered breathing in fetuses caesarean-delivered by gestational day E18 (Viemari et al., 2004). First plethysmographic recordings of the in vivo breathing frequency of control and mutant E18 fetuses reveal an abnormally low breathing frequency in Phox2a mutants. Second, recording the in vitro rhythm produced by the isolated RRG in medullary preparations (pons excluded) revealed central deficits: the in vitro rhythm was abnormally low in Phox2a mutants (Fig. 5B).
In addition, the response of the isolated RRG to application of exogenous NA was altered in Phox2a mutants, with a transient but huge rhythm facilitation by NA. This suggests that α1 adrenoceptors are expressed by the mutant RRG despite the lack of A6 neurons but that the responsiveness of the α1 adrenoceptors is exacerbated. Indeed, the lack of A6 facilitation onto the maturing RRG has drastic consequence.

In Ret mutants (Fig. 5C), the formation of both A5 and A6 neurons is altered as shown by measurements of NA contents in the pons and by counting of TH-neurons in A5 and A6 areas whereas the formation of medullary NA groups is not significantly altered (Viemari et al., 2005a). In E18 exteriorized fetuses, plethysmographic recordings revealed a tendency to a slower breathing frequency in Ret mutants although not statistically different from control fetuses. In vitro recordings confirmed central respiratory deficits in Ret mutants with a slower respiratory rhythm and an exacerbated response to NA application. Although the NA and respiratory deficits of Ret mutants (partial loss of A5 and A6 neurons) were less marked than those of Phox2a mutants (total loss of A6 neurons), Ret-deficiency has also altered the prenatal maturation of the RRG.

In Mecp2 mutants, on-going experiments performed in our laboratory reveal that deletion of this gene also results in NA and respiratory deficits (Roux et al., 2005; Viemari et al., 2005b). In Mecp2 mutant neonates, breathing frequency, NA contents and number of TH-neurons are normal at birth. However from birth to 1 month of age, NA deficits develop with a reduction of NA contents (Viemari et al., 2005b; Ide et al., 2005) and a loss of A1/C1 and A2/C2 neurons. Meanwhile NA alteration develops in Mecp2 mutants, respiratory deficits appear, with first an increased variability of the cycle duration observed both in vivo and in vitro (A2/C2 alterations?) and second a reduction of breathing frequency with appearance of recurrent, frightening apnoeas (A1/C1 alterations?). The respiratory deficits worsen until death, at about 2 months of age. As the pontine NA contents are not significantly affected in Mecp2 mutants (Viemari et al., 2005b), it is likely that the respiratory deficits originate from the medullary NA deficits. The abnormal variability of cycle duration and the reduction of the breathing frequency are consistent with A2/C2 and A1/C1 deficits, respectively (Zanella et al., 2005).

In BDNF mutants, the expression of a normal respiratory behavior is impaired at birth (Erickson et al., 1996). Both in vivo and in vitro studies revealed a depressed and highly variable respiratory rhythm during the postnatal period in BDNF mutants; indeed BDNF was the first transcription factor identified that is required for normal respiratory rhythm and ventilatory control (Balkowiec and Katz, 1998). As BDNF mRNA is expressed in medullary A1/C1 and A2/C2 neurons (Cho et al., 1999), A1/C1 and A2/C2 alterations might explain the depressed and variable respiratory rhythm of BDNF mutants, respectively. Indeed, BDNF is required for postnatal survival of some dopaminergic primary sensory neurons in vivo and BDNF inactivation may alter peripheral chemoreceptor afferent neurons and the respiratory response to hypoxia (Erickson et al., 2001) as well as the
long-term facilitation of respiration by intermittent hypoxia (Baker-Herman et al., 2004). In piglets, intermittent hypoxic periods markedly increased BDNF mRNA levels in the entire medulla but only transiently affected the BDNF protein level (Peiris et al., 2004).

In Phox2b mutants, expression of all NA neurons and survival is impaired but heterozygous mutant mice for Phox2b survive; they present a normal respiration when awake but frequent sleep apnoeas (Durand et al., 2005). Heterozygous mutations of PHOX2B have been found in a high proportion of patients suffering from central congenital hypoventilation syndrome (CCHS or Ondine’s curse), a rare disorder characterized by an idopathic failure of the autonomic control of breathing (Sasaki et al., 2003; Gaultier et al., 2004). Even if mutations of PHOX2A, RET and BDNF genes have also been reported in some cases of CCHS, it is more likely that mutations of PHOX2B gene are responsible for CCHS. Here again the NA deficits induced by PHOX2B mutations may play a role in the RRG function.

7. Discussion and conclusion

This review shows that endogenous NA contributes to both the prenatal maturation and the neonatal function of the RRG and that maturational NA deficits have deleterious effects on RRG activity. Briefly, it has been reported that:

1. The NA neurons develop early during gestation and NA phenotype specification implicates different genes such as Rnx, Phox2a, Phox2b and Ret.
2. The NA neurons from the different brainstem groups exert complex, specific modulations on the neonatal RRG activity.
3. Some of these NA neurons “belong” to the neonatal respiratory network (i.e. those from the A5 and A6 groups send synaptic connections to the medullary respiratory neurons), while others may act via “volume transmission” processes (those from A1/C1 and A2/C2 groups).
4. Finally, deletion of genes implicated in NA phenotype specification results in lethal respiratory deficits that develop during either the prenatal (Rnx, Phox2a, Phox2b, Ret) or the early postnatal periods (Mecp2).

Even if these results have been obtained in animals, they may be of some relevance with humans. SIDS, the leader cause of infant death in industrialised countries, is highly likely due to neurovegetative disorders and more precisely to cardiorespiratory dysfunction during sleep (Horne et al., 2004; Ribas-Salgueiro et al., 2004). Biochemical and histological postmortem analysis of SIDS victims often reveal alterations of the NA system, with abnormal levels of NA metabolites, abnormal morphology of catecholaminergic neurons, abnormal expression of medullary α2 adrenoceptors and abnormal expression of catecholamine enzymes (Ozawa et al., 1999, 2003; Cann-Moisan et al., 1999; Obonai et al., 1998; Kopp et al., 1993; Takashima and Becker, 1991; Denoroy et al., 1987; Ozand and Tildon, 1983). Moreover, genetic analysis reveal some protein-changing rare mutations in 14 of 92 SIDS cases among the PHOX2a, RET, and RNX genes (Weese-Mayer et al., 2004). Endogenous NA is known to be implicated in sleep, cardiovascular and respiratory regulations. The observations of biochemical, histological and genetic alterations of the NA systems in some SIDS victims strengthen the hypothesis that SIDS, or at least some cases of SIDS, may be at least in part genetically pre-determined. They also suggest that SIDS might originate at least in part from NA deficits that in turn facilitate the occurrence of respiratory deficits. In addition, the rare respiratory disorder CCHS may also originate from NA deficits resulting from Phox2b mutations.

During a normal life span, the rhythmic respiratory motor act occurs about half a milliard of times, whatever the species. From the very first moment of postnatal life until the very last, this rhythmic motor act must be continuously adapted to environmental and behavioral changes in order to secure a proper oxygen delivery to the whole body, including the brain. Although long lasting apnoeas with impaired arousal may be lethal during the neonatal period, possibly leading to SIDS, less drastic but recurrent respiratory deficits also exist, such as central or obstructive sleep apnoeas and CCHS, and may induce a chronic hypoxia. It has been highlighted that stress during the prenatal and neonatal periods can affect the programming of NA and other neurotransmitters and receptors expression, and that this can lead to long-term behavioral effects (Herlenius and Lagercrantz, 2004). Thus besides the genetic factors, the epigenetic factors may also play a role in the maturation of the NA system whose alteration may lead to respiratory dysfunction and chronic hypoxia. The latter may in turn affect the maturation and function of all brain neurons and functional circuits, with deleterious consequences on sleep, memory, behaviors, etc. Indeed, it seems highly likely that normal NA and normal respiratory systems during the perinatal period are required for a normal life to occur.

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