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Breathing dysfunction in Rett syndrome: Understanding epigenetic regulation of the respiratory network

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Abstract

Severely arrhythmic breathing is a hallmark of Rett syndrome (RTT) and profoundly affects quality of life for patients and their families. The last decade has seen the identification of the disease-causing gene, methyl-CpG-binding protein 2 (*Mecp2*) and the development of mouse models that phenocopy many aspects of the human syndrome, including breathing dysfunction. Recent studies have begun to characterize the breathing phenotype of *Mecp2* mutant mice and to define underlying electrophysiological and neurochemical deficits. The picture that is emerging is one of defects in synaptic transmission throughout the brainstem respiratory network associated with abnormal expression in several neurochemical signaling systems, including brain-derived neurotrophic factor (BDNF), biogenic amines and gamma-amino-butyric acid (GABA). Based on such findings, potential therapeutic strategies aimed at improving breathing by targeting deficits in neurochemical signaling are being explored. This review details our current understanding of respiratory dysfunction and underlying mechanisms in RTT with a particular focus on insights gained from mouse models.

Keywords

Rett Syndrome; Methyl-CpG-binding protein 2; Breathing; Synaptic transmission

1. Introduction

Rett syndrome is a severe neurodevelopmental disorder with a prevalence of approximately 1 in 10,000 live female births. Affected children undergo apparently normal postnatal development until 6–18 months of age and then begin a marked neurological decline with a highly variable course that can include an early period of developmental regression followed by phases of symptom stabilization as well as late deterioration (Hagberg et al., 1983; Shahbazian et al., 2002; Vorsanova et al., 2004; Chahrour and Zoghbi, 2007). Initial manifestations of the disease may include loss of acquired speech, head growth deceleration and autistic features such as emotional withdrawal and diminished eye contact. Subsequently, RTT patients develop motor stereotypies, epileptiform seizures, exaggerated responses to stress and autonomic dysfunction. Of particular relevance to this review is that the majority of RTT patients also develop severe breathing abnormalities.

The vast majority of typical RTT cases are attributable to loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2; Amir et al., 1999; Shahbazian and Zoghbi, 2001). MeCP2 belongs to a family of methyl-binding proteins (Klose and Bird, 2006) that regulate gene expression by repressing transcription at methylated promoters. Thus,

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MeCP2 is considered an epigenetic regulator because it is able to influence patterns of gene expression from outside the genome by "reading" methylation patterns in the DNA and repressing target genes.

The *Mecp2* gene is located on the X chromosome and homozygous mutation is invariably lethal. Affected females are heterozygotes and most male infants carrying an *Mecp2* mutation die shortly after birth. Female heterozygotes are somatic mosaics for RTT, i.e., cells in which the mutated allele occurs on the inactive X are phenotypically normal, whereas cells in which the mutated allele occurs on the active X are mutant. Thus, it is thought that disease phenotype depends not only on the specific mutation but also on the skewing of X chromosome inactivation, such that individuals in which inactivation is skewed towards the mutant allele are less severely affected, and *vice versa*. However, recent studies indicate that disease severity cannot always be explained by X inactivation patterns, pointing to a role for other modifiers of *Mecp2* gene function (Takahashi et al., 2008; Xinhua et al., 2008).

There are currently no treatments available for Rett syndrome. Therefore, understanding the molecular pathogenesis of Rett syndrome is important, first and foremost, for developing therapeutic strategies aimed at improving the quality of life for affected individuals. There are likely to be other benefits as well. For example, *Mecp2* mutations, as well as gene duplications, have now been identified in other disorders, including some forms of autism (Moretti and Zoghbi, 2006). More broadly, the study of Rett syndrome is shedding light on basic mechanisms of epigenetic regulation and their importance for nervous system development and function.

2. MeCP2 function and target genes

The MeCP2 protein contains at least 2 functional domains: a methyl-binding domain (MBD) that recognizes methylated CpG dinucleotides with particular flanking sequences (Klose and Bird, 2006), and a transcription repression domain (TRD). Over 200 different *Mecp2* mutations have been found in Rett patients, with many clustered in the MBD and TRD sequences.

The repressor activity of MeCP2 results not only from its intrinsic TRD but also from its ability to recruit complexes comprised of histone deacetylases and other proteins with transcriptional repressor activity (Klose and Bird, 2006). More recently, additional domains have been identified within MeCP2 that bind DNA at non-methylated sites (Nikitina et al., 2007a,b). Thus, it is likely that transcriptional repression at specific gene promoters is not the only function of MeCP2. In particular, increasing evidence supports a role in higher order chromatin structure (Nikitina et al., 2007a) and there are also data to suggest a role in RNA splicing (Young et al., 2005).

Despite extensive characterization of it's repressor activity *in vitro*, relatively few *bona fide* transcriptional targets of MeCP2 have been identified. This is somewhat surprising given that the structure of MeCP2 predicts a widespread role in gene repression. Several laboratories have attempted to identify MeCP2 targets by comparing gene expression profiles in brain samples from wildtype and *Mecp2* null mice using RNA microarrays, the prediction being that many genes would be upregulated in mutant animals in the absence of MeCP2. Another approach has been chromatin immunoprecipitation (ChIP), a technique in which DNA-binding proteins are cross-linked to chromatin in intact cells and then immunoprecipated with anti-MeCP2 antibodies. The immunoprecipitated chromatin fragments are then identified by quantitative real-time polymerase chain reaction with oligonucleotide probes directed against specific DNA sequences. Relatively few validated gene targets have yet emerged from either of these approaches (Colantuoni et al., 2001; Johnston et al., 2001; Traynor et al., 2002; Tudor et al., 2002; Ballestar et al., 2003; Nuber et al., 2005; Delgado et al., 2006; Kriaucionis et al., 2006; Peddada et al., 2006; Deng et al., 2007; Jordan et al., 2007; Smrt et al., 2007). One explanation

may be that patterns of gene regulation by MeCP2 are highly dependent on cellular context, and that differences in expression between wildtype and null cells are diluted or averaged out when analyzing expression profiles in heterogenous tissues such as whole brain. Moreover, ChIP data have recently been used to propose that MeCP2 actually binds to potentially thousands of genes, including both actively transcribed and repressed genes (Yasui et al., 2007). However, recent studies have questioned whether or not such data actually reveal functional binding and have called into serious question the ability of ChIP to distinguish genuine targets of transcription factors from non-specific DNA-binding sites (Li et al., 2008).

3. Mouse models of RTT

Identification of *Mecp2* as the disease-causing gene in human RTT patients stimulated the development of mouse models of RTT in which *Mecp2* is either deleted, mutated or overexpressed, including 1) $Mecp2^{-/y}$ mice with extended exonic deletion of the *Mecp2* gene (Chen et al., 2001; Guy et al., 2001; Pelka et al., 2006), 2) $Mecp2^{308/y}$ mice with truncation of *Mecp2* protein at amino acid 308, a human RTT mutation (Shahbazian et al., 2002), 3) $Mecp2^{Flox/y}$ mice expressing a hypomorphic *Mecp2* allele (Samaco et al., 2008) and 4) $Mecp2^{Tg1}$ mice that overexpress MeCP2 protein (Collins et al., 2004). As discussed in detail below, the loss-of-function mouse models exhibit varying degrees of RTT-like pathophysiology, including disturbances of breathing, and are therefore proving valuable in defining links between *Mecp2* mutations and neurologic dysfunction. As with human RTT patients, homozygous female *Mecp2* mutants are not viable and heterozygous females are phenotypically heterogeneous due to variable patterns of X-chromosome inactivation. Therefore, most laboratories have studied the effects of *Mecp2* loss of function in hemizygous males ($Mecp2^{-/y}$), which are completely null for *Mecp2* and therefore tend to be more phenotypically homogenous than female heterozygotes (see however Bissonnette and Knopp, 2006, 2008; Bissonnette et al., 2007).

4. RTT symptoms can be reversed in mice

A conceptual breakthrough in RTT research came in 2007 with the demonstration that RTT symptoms in mice are at least partially reversible. To approach this issue, Guy et al. (2007) created a mouse line in which the endogenous *Mecp2* gene is silenced by insertion of a *Lox-Stop* cassette linked to a modified estrogen receptor. The *Mecp2* gene is turned off in these animals until the Lox-Stop cassette is excised by Cre-mediated deletion triggered by exposure to tamoxifen. Using these animals, Guy et al. (2007) demonstrated that reactivation of *Mecp2* in severely symptomatic adult “null” mice resulted in significant reversal of their RTT-like phenotype, including marked improvement in lifespan, motor function and grossly observable breathing behavior. Qualitatively similar results were reported by Giacometti et al. (2007) and more recently by Jugloff et al. (2008). These remarkable findings suggest that, in mice, RTT-like symptoms do not result from irreversible changes in brain structure or function and raise the possibility that therapeutic strategies aimed at restoring MeCP2 dependent signaling, even in symptomatic RTT patients, could be clinically beneficial.

5. Morphological changes in the RTT brain

Consistent with the results of genetic reversibility experiments in mice, the brains of RTT patients and *Mecp2* null mice do not exhibit gross morphological abnormalities. Despite profound neurological dysfunction, the major morphological change in the central nervous system of animal models and human RTT patients is a reduction in brain size without detectable cell loss (see however Kitt and Wilcox, 1995). Rather, reduction of brain size in RTT is associated with evidence of hypomorphic neuronal traits, including (1) marked reduction in dendritic branching of layer III and V pyramidal neurons in the frontal, temporal and motor cortices, and of layer II and IV neurons in the subiculum (Armstrong, 2005), (2) shortening of

dendritic spines in the frontal cortex (Belichenko et al., 1994), (3) reduction in neuronal size in the cortex, thalamus, basal ganglia, amygdala, and hippocampus (Kitt and Wilcox, 1995), and (4) dysmorphic olfactory neurons (Ronnett et al., 2003). Similarly, *Mecp2*$^{-/y}$ mice exhibit reduced cortical dendritic arborization (Kishi and Macklis, 2004), delayed neuronal maturation and synaptogenesis in the cerebral cortex (Fukuda et al., 2005) and possible reductions in synapse number in the hippocampus (Chao et al., 2007).

6. Electrophysiological perturbations in RTT

RTT patients exhibit numerous electrophysiological abnormalities suggestive of cortical hyperexcitability, including a higher incidence of epileptiform seizures and appearance of rhythmic slow theta activity (Glaze, 2005). In mouse models, on the other hand, loss of MeCP2 function appears to be associated with an imbalance between excitatory and inhibitory neurotransmission that can favor either excitation or inhibition, depending on the brain region and experimental preparation. For example, spontaneous neuronal activity in adult cortical slices is reduced in *Mecp2*$^{-/y}$ mice due to a decrease in total excitatory synaptic drive and an increase in the total inhibitory drive (Dani et al., 2005). *Mecp2*$^{-/y}$ mice exhibit a reduction in the number of hippocampal excitatory glutamatergic synapses *in vivo* (Chao et al., 2007), as well as decreased frequency of spontaneous excitatory synaptic transmission in cell culture (Nelson et al., 2006). Moreover, in single neuron microcultures, *Mecp2*$^{-/y}$ hippocampal neurons exhibit a reduction in the number of excitatory autaptic synaptic inputs (Chao et al., 2007). In contrast, Zhang et al. (2008) recently demonstrated that hippocampal slices from *Mecp2*$^{-/y}$ mice exhibit a reduction in inhibitory rhythms that apparently renders the slice prone to hyperexcitability. Changes in synaptic plasticity have also been reported, including reduced long term potentiation (LTP) in *Mecp2*$^{-/y}$ cortical slices (Asaka et al., 2006) and in *Mecp2*$^{308/y}$ cortical and hippocampal slices (Moretti et al., 2006), enhanced LTP in MeCP2-overexpressing *Mecp2*^{Tg1} hippocampal slices (Collins et al., 2004) and an increase in the rate of short-term synaptic depression in cultured hippocampal neurons (Nelson et al., 2006).

Loss of MeCP2 function is also associated with an excitatory/inhibitory imbalance in the mouse brainstem, including in cell groups involved in regulation of breathing. Stettner et al. (2007) recently described hyperexcitability of neurons located in the medulla oblongata and pons of *Mecp2*$^{-/y}$ mice, most probably due to increased excitatory drive. In addition, the amplitude and frequency of spontaneous inhibitory synaptic currents is severely depressed in neurons located in the rostral ventrolateral medulla of *Mecp2*$^{-/y}$ mice (Medrihan et al., 2008).

7. Breathing disturbances in RTT patients

Breathing dysfunction in RTT is complex and highly variable, both among affected individuals and within individuals, depending, for example, on the level of behavioral arousal. In general the RTT breathing phenotype is characterized by forced and apneustic breathing, increased occurrence of apneas, and highly unstable breathing patterns including periods of breath-holds, episodes of hyperventilation and heterogeneous breath duration (Weese-Mayer et al., 2006 and references therein). Mean respiratory frequency is increased by approximately 20%. Although originally thought to occur only during wakefulness, some recent studies have observed breathing disturbances during sleep as well (Rohdin et al., 2007). Initial studies aimed at characterizing the pathophysiology of RTT in human patients suggested several possible causes for breathing disturbances, including cortical dysfunction (Elian and Rudolf, 1991; Marcus et al., 1994) and brainstem immaturity (Julu et al., 1997).

8. Breathing disturbances in *Mecp2* null mice

Mecp2$^{-/y}$ male mice develop breathing abnormalities similar to human RTT, however, significant differences in respiratory phenotype exist among different mouse strains. In general,

the two strains studied thus far, i.e., *Mecp2*$^{tm1.1Bird}$ (Guy et al., 2001) and *Mecp2*$^{tm1.1Jae}$ (Chen et al., 2001) both exhibit increased variability in breath duration and episodes of hyperventilation, as determined *in vivo* using whole-body plethysmography (Viemari et al., 2005; Ogier et al., 2007; Zanella et al., 2008) and *in vitro* using electro-physiologic assessment of phrenic and vagal nerve activities from working heart-brainstem preparations (Stettner et al., 2007). Male *Mecp2*$^{tm1.1Bird}$ null mice breathe normally until approximately one month of age, after which they develop erratic breathing characterized by increased variability in the duration of the respiratory cycle, leading to alternating periods of fast and slow breathing frequencies, and by occurrence of apneas (Viemari et al., 2005). Initial breathing disturbances worsen between the first and second months and the mice eventually die from fatal respiratory arrest. Before dying, *Mecp2*$^{tm1.1Bird}$ null mice display a slow and arrhythmic breathing pattern with a highly variable cycle period, decreased mean breathing frequency and an increasing occurrence of long-lasting apneas (Viemari et al., 2005; Roux et al., 2007; Stettner et al., 2007; Zanella et al., 2008). The increased variability of total breath duration has been linked to the duration of post-inspiratory and inspiratory phases (Stettner et al., 2007), and does not depend on altered peripheral chemoreceptor oxygen sensitivity (Bissonnette and Knopp, 2008). Increased respiratory variability and apneas have also been reported in *Mecp2*$^{Flox/y}$ hypomorphs, mice that express approximately 50% lower levels of MeCP2 protein in the brain (Samaco et al., 2008). However, unlike RTT patients, no change in the mean breathing frequency has been reported in *Mecp2*$^{tm1.1Bird}$ or *Mecp2*$^{Flox/y}$ mice.

Male *Mecp2*$^{tm1.1Jae}$ null mice also exhibit severely disordered breathing with alternating periods of high respiratory frequency and normal breathing by 5 weeks of age (Fig. 1; Ogier et al., 2007). Occurrence of apneas shows a high degree of individual variation at this stage. Unlike *Mecp2*$^{tm1.1Bird}$ null mice, mean breathing frequency (and minute ventilation) is significantly increased in 5-week-old *Mecp2*$^{tm1.1Jae}$ null mice compared to wildtype controls by approximately 20%, similar to human RTT patients (Weese-Mayer et al., 2006). Differences in the breathing phenotypes of *Mecp2*$^{tm1.1Jae}$ and *Mecp2*$^{tm1.1Bird}$ null mice could result from subtle differences in the exonic deletion between the two strains. However, a more likely explanation is that the two mouse strains have different genetic backgrounds. For example, the fact that C57BL/6 mice are particularly prone to spontaneous apneas from central origin (Stettner et al., 2008a,2008b) might explain why the apneic phenotype is more pronounced in *Mecp2*$^{tm1.1Bird}$ null mice, engineered on a pure C57BL/6 background, compared to *Mecp2*$^{tm1.1Jae}$ null mice, engineered on a mixed C57BL/6, 129/sv, Balb/c background.

Bissonnette and Knopp (2006) have recently suggested that respiratory dysfunction in *Mecp2* mutant mice is not only of neurogenic origin but also results from MeCP2 deficiency in peripheral tissues. Using plethysmography, they compared the respiratory phenotype of 5-month-old female *Mecp2*$^{tm1.1Bird}$ heterozygotes to age-matched wildtypes and females with neuron-specific mutation of *Mecp2* (*Mecp2*$^{+/nestin\text{-}Cre\text{-}lox}$) and reported that the two mutants display distinct respiratory phenotypes. *Mecp2*$^{tm1.1Bird}$ heterozygotes breathe slowly compared to wild-types, and have an increased mean tidal volume. In contrast, female *Mecp2*$^{+/nestin\text{-}Cre\text{-}lox}$ heterozygotes have a higher mean breathing frequency and a reduced mean tidal volume. In addition, *Mecp2*$^{tm1.1Bird}$ heterozygotes exhibit increased duration of apneas in response to hypoxia-induced hyperventilation compared to *Mecp2*$^{+/nestin\text{-}Cre\text{-}lox}$ heterozygotes. These observations may indicate that peripheral MeCP2 deficiency can influence breathing, however, further studies are needed to rule out possible influences of strain differences between *Mecp2*$^{tm1.1Bird}$ and *Mecp2*$^{+/nestin\text{-}Cre\text{-}lox}$ mice as well.

9. Respiratory network abnormalities in *Mecp2* null mice

9.1. Neurophysiological defects

Several recent electrophysiological studies in *Mecp2* null mice have begun to reveal specific functional alterations in the brainstem respiratory network that likely contribute to the abnormal breathing phenotype observed in intact animals. Using an *in vitro* preparation of acute brainstem slices to record bursting activity from the ventral respiratory group, Viemari et al. (2005) described a significant increase in the coefficient of variation and irregularity score for respiratory cycle length in mutant slices compared to age-matched wildtype controls at 2–3 weeks of age. Although consistent with breathing arrhythmia, as in older *Mecp2* null mice, these changes preceed detectable differences in respiratory function between wildtype and mutant animals by several weeks. Stettner et al. (2007) recently demonstrated that breathing arrhythmia is associated with impaired control of post-inspiratory activity caused by unpredictable fluctuations in the duration and amplitude of the post-inspiratory vagal afferent discharge, leading in turn to a significant increase in the variability of expiratory cycle length. In addition, they observed a loss of desensitization of the Hering–Breuer reflex in response to repeated vagal afferent stimulation in mutant animals compared to wildtype controls. Moreover, they observed hyperexcitablity of respiratory-related neurons in the pontine Kölliker-Fuse nucleus characterized by exaggerated responses to exogenous glutamate. Together, these data indicate disruptions in two different neural systems that play key roles in modulation of the respiratory rhythm in general and expiratory cycle length in particular, i.e., vagal afferent inputs and the Kölliker-Fuse nucleus. Therefore, Stettner et al. (2007) propose that alterations in the control of post-inspiratory activity contribute to the respiratory phenotype in *Mecp2* null mice, and possibly RTT patients, by generating breathing arrhythmias and increasing the incidence of apneas. Moreover, in light of the fact that post-inspiratory motor output plays a key role in regulating laryngeal adductors and, therefore, upper airway patency, these data could help explain upper airway-related problems in RTT, including apneas with laryngeal closure, loss of speech (Budden et al., 1990) and weak coordination of breathing and swallowing (Morton et al., 1997; Isaacs et al., 2003).

Finally, Medrihan et al. (2008) have recently described very early changes in synaptic function in the ventrolateral medulla of *Mecp2* null mice. Specifically, they showed that, as early as one week after birth, the frequency and amplitude of spontaneous inhibitory postsynaptic currents (IPSCs) recorded from the rostal ventrolateral medulla is severely depressed in *Mecp2* null mice compared to wildtype controls. Thus, although overt respiratory dysfunction does not become apparent until several weeks after birth (Viemari et al., 2005; Stettner et al., 2007), increasing evidence indicates that network abnormalities begin to appear much sooner.

9.2. Neurochemical defects in the brainstem respiratory network in Mecp2 null mice

In an attempt to understand the molecular bases of respiratory network dysfunction in RTT, a number of laboratories have examined expression and function of neurotransmitter systems in the *Mecp2* null mouse brainstem. These studies have focused in particular on three signaling systems; brain-derived neurotrophic factor (BDNF), biogenic amines (notably norepinephrine and serotonin) and the inhibitory transmitter gamma-amino butyric acid (GABA), all of which play pivotal roles in the development and/or function of the brainstem respiratory network.

9.2.1. BDNF—BDNF plays critical roles in survival, maturation and plasticity of neurons throughout the neuraxis and is essential for development of the respiratory controller and normal breathing behavior (for review see Katz, 2005). In the perinatal period, BDNF is required for survival of peripheral cranial sensory afferents in the nodose and petrosal ganglia, including baroreceptor and chemoafferent neurons, as well as neurons in the pontine A5 noradrenergic cell group (Guo et al., 2005; Huang et al., 2005), an important source of

modulatory input to medullary respiratory neurons. Moreover, BDNF is required for perinatal development of central respiratory output (Balkowiec and Katz, 1998) and modulates synaptic function in newborn and adult animals at multiple sites within the respiratory network, including the nucleus tractus solitarius (Balkowiec et al., 2000), preBötzinger complex (Thoby-Brisson et al., 2003), Kölliker-Fuse nucleus (Kron et al., 2007a,b) and spinal phrenic motoneurons (Baker-Herman et al., 2004). The synaptic modulatory role of BDNF derives from the fact that many neurons express BDNF throughout life and release it in an activity-dependent manner (Balkowiec and Katz, 2000, 2002). BDNF null mice die within a few weeks of birth, most likely from breathing complications that include severely depressed minute ventilation, highly variable respiratory cycle length and increased occurrence of apneas (Erickson et al., 1996).

A possible link between BDNF and RTT was suggested by evidence that the BDNF gene is a transcriptional target of MeCP2 (Chen et al., 2003; Martinowich et al., 2003). Subsequently, studies in this and other laboratories demonstrated that *Mecp2* null mice exhibit marked progressive deficits in brain content of BDNF after birth (Wang et al., 2006; Chang et al., 2006). The finding that BDNF gene expression is reduced in *Mecp2* null mutants conflicts with the notion that MeCP2 is a transcriptional repressor of BDNF, and mechanisms of BDNF regulation by MeCP2 remain a subject of controversy.

The earliest and most profound declines in BDNF mRNA and protein in *Mecp2* null mice occur in the nodose ganglion and brainstem, including nTS, with mutants exhibiting 40–50% wildtype levels by 5 weeks after birth (Wang et al., 2006). These declines are not due to loss of BDNF expressing neurons, as cell survival is unchanged in mutant mice compared to controls (Wang et al., 2006). The lack of an effect on cell number reflects the fact that BDNF deficits in *Mecp2* null mice occur several weeks after birth, at a time when neurons are no longer dependent on BDNF for survival.

Deficits in BDNF protein are a likely factor contributing to synaptic dysfunction in *Mecp2* null mice. Indeed, we have previously shown that BDNF can potently modulate activity of second order relay neurons in nTS by inhibiting postsynaptic glutamatergic AMPA receptors (Balkowiec et al., 2000). We hypothesize that decreased BDNF expression by nodose ganglion cells in *Mecp2* null mice, leading to reduced synaptic inhibition at primary afferent synapses in nTS, contributes to the loss of reflex desensitization to repeated vagal stimulation in mutant animals (Stettner et al., 2007). Further work is required to test this hypothesis, as well as the possibility that dysregulation of BDNF expression contributes to synaptic dysfunction in the Kölliker-Fuse nucleus (Kron et al., 2007a,b), preBötzinger complex (Thoby-Brisson et al., 2003) and phrenic motoneurons (Baker-Herman et al., 2004).

9.2.2. Biogenic amines—Norepinephrine, dopamine and serotonin levels in the *Mecp2* null brain as a whole exhibit significant gradual decline after birth compared to wildtype animals (Ide et al., 2005). This is particularly true in the brainstem, where a postnatal deficit in norepinephrine is associated with a decrease in the number of neurons in the A1/C1 and A2/C2 cell groups expressing the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH; Viemari et al., 2005; Roux et al., 2007) and decreased TH content in the pontine A6 cell group of *Mecp2*$^{-/y}$ mice (Ogier et al., 2006). Based on these findings, pre-clinical and clinical studies are currently in progress to examine the efficacy of increasing noradrenergic signaling for improving respiratory function in RTT (see below).

9.2.3. GABAergic signaling—As noted above, Medrihan et al. (2008) recently demonstrated reduced inhibitory synaptic inputs to rostral ventrolateral medullary neurons in *Mecp2* null mice. They further showed that reduced inhibitory synaptic transmission was due to a specific decrease in IPSCs mediated by GABA, with no change in glycinergic signaling.

Furthermore, loss of GABAergic transmission was associated with decreased expression of both pre- and post-synaptic markers of GABA function, including presynaptic levels of GABA and the vesicular inhibitory amino acid transporter, as well as reduced levels of the $GABA_A$ receptor α2 subunit mRNA and protein.

9.3. Neurochemical alterations in RTT patients

Numerous studies in RTT patients have attempted to identify neurochemical markers of disease (Wenk, 1997; Blue et al., 1999a,b; Lipani et al., 2000; Armstrong, 2001; Calamandrei et al., 2001; Saito et al., 2001; Riikonen, 2003; Guideri et al., 2004a,b; Paterson et al., 2005). However, many of the findings that have been reported thus far have not been consistent across studies. In addition, some analyses included very small numbers of samples across a broad spectrum of ages, sometimes without adequate normal controls. Nonetheless, some of the human data are consistent with findings of neurochemical alterations in *Mecp2* null mice discussed above.

The possibility that monoaminergic transmission is decreased in RTT patients has been a subject of debate for more than 20 years. Initial studies reported that levels of norepinephrine, serotonin and their respective metabolites are severely decreased in postmortem brain tissues from patients with RTT (Riederer et al., 1985; Brucke et al., 1987; Lekman et al., 1989), with a tendency for more severe phenotypes in older patients (Lekman et al., 1989). These results were corroborated by analysis of cerebrospinal fluid samples from RTT patients (Zoghbi et al., 1985, 1989). However, other investigators failed to demonstrate deficits in serotonin and norepinephrine metabolites in cerebrospinal fluid of RTT patients (Perry et al., 1988; Lekman et al., 1990). More recently, a reduction in the level of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, was observed in the locus coeruleus region in the brain of one patient with RTT, suggesting hypoactivity of brain norepinephrinergic transmission (Saito et al., 2001). However, this observation has not yet been corroborated and more such studies are needed. As discussed above, *Mecp2* null mice exhibit significant decreases in brainstem levels of norepinephrine and serotonin and these changes, in norepinephrine in particular, are thought to play an important role respiratory dysfunction (Viemari et al., 2005). Thus, it is critical, particularly given current efforts to develop RTT therapies that target noradrenergic signaling (see below), that the presence or absence of abnormalities in catecholamine metabolism in RTT patients be resolved.

Glutamate is reported to be elevated in the cerebrospinal fluid (Hamberger et al., 1992; Lappalainen and Riikonen, 1996) and postmortem brain samples (Pan et al., 1999) from RTT patients. Substance P, on the other hand, is decreased in the cerebrospinal fluid (Matsuishi et al., 1997), and in the dorsal horn and intermediolateral column of the spinal cord, spinal trigeminal tract, solitary tract and nucleus, parvocellular and pontine reticular nuclei, the parabrachial complex and locus coeruleus, and, to a lesser extent in the substantia nigra, central gray of the midbrain, frontal cortex, caudate, putamen, globus pallidus and thalamus (Deguchi et al., 2000; Saito et al., 2001). Given the pivotal roles played by glutamate and substance P in regulation of respiratory function (Hilaire and Duron, 1999; Ramirez and Viemari, 2005), these findings, while still quite general, may be relevant to respiratory disturbances in RTT.

10. Potential therapeutic targets for treatment of breathing dysfunction in RTT: preclinical studies in mice

The mortality rate among RTT patients is significantly increased (up to 13 times) compared to the general population, and up to 26% of RTT patients may die suddenly due to cadiorespiratory failure (Kerr et al., 1997; Kerr and Burford, 2001). However, there are currently no treatments available for respiratory dysfunction in RTT. Therefore, a number of laboratories are actively

working on preclinical development of potential therapies based on insights gained from mouse models of RTT.

10.1. BDNF

Several lines of evidence suggest that strategies aimed at enhancing BDNF signaling could be of therapeutic benefit in RTT. For example, genetic overexpression of BDNF in *Mecp2*$^{tm1\text{-}1Jae}$ null mice results in improved somatomotor function and increased lifespan (Chang et al., 2006). More recently, our laboratory has begun exploring the possibility that pharmacologic elevation of endogenous BDNF expression with ampakine drugs could improve respiratory function in *Mecp2*$^{tm1\text{-}1Jae}$ null mice. Ampakines are benzamide derivatives that acutely facilitate the activity of glutamatergic AMPA receptors (Arai and Kessler, 2007). However, repeated treatment with ampakines increases expression of BDNF mRNA and protein in the forebrain of mice and rats for several days (Lauterborn et al., 2000, 2003; Rex et al., 2006) and augments BDNF dependent synaptic plasticity (Ingvar et al., 1997; Porrino et al., 2005; Rex et al., 2006; Wezenberg et al., 2007). These effects far outlast the very short half-life of ampakine drugs, which is on the order of a few hours, as well as the acute effects on AMPA receptor currents. Based on this long-term action on BDNF expression, ampakine drugs are thought to have potential therapeutic value for treatment of diseases characterized by impaired BDNF function. Recently, our laboratory demonstrated that treatment of *Mecp2*$^{tm1\text{-}1Jae}$ null mice with one ampakine, CX546, for 3 days restores normal respiratory frequency and minute ventilation (Fig. 2A; Ogier et al., 2007). These behavioral effects were accompanied by a significant increase in expression of BDNF protein in vagal afferent neurons in the nodose ganglion (Fig. 2B). Further work is needed to establish whether or not respiratory improvement following ampakine treatment is a direct result of enhanced BDNF expression. Nonetheless, these data are consistent with the hypothesis that decreased BDNF levels contribute to the respiratory phenotype of *Mecp2* null mice and that BDNF may be a druggable target for improving respiratory function in RTT.

10.2. Noradrenergic signaling

Based on the finding that brainstem levels of norepinephrine are decreased in *Mecp2* null mice, and that exogenous norepinephrine can normalize rhythmic respiratory bursts in *Mecp2* null brainstem slices, Viemari et al. (2005) proposed that deficits in brainstem noradrenergic signaling play a key role in the development of respiratory dysfunction in these animals. Based on these observations, Roux et al. (2007) and Zanella et al. (2008) explored the possibility that increasing noradrenergic signaling *in vivo* with desipramine, a tricyclic antidepressant that inhibits the reuptake of norepinephrine, could improve respiratory function in *Mecp2*$^{tm1.1Bird}$ null mice. Either repeated intraperitoneal injection (Roux et al., 2007) or oral administration (Zanella et al., 2008) of desipramine significantly reduced the incidence of apneas and extended lifespan in mutant mice. Further work is required to understand the mechanism of these effects, particularly given that desipramine affects multiple signaling mechanisms in addition to norepinephrine reuptake, including, for example, expression of BDNF (Nibuya et al., 1995). It would be interesting if the efficacy of desipramine in improving respiratory function in *Mecp2* null mice is related both to its action as an inhibitor of norepinephrine uptake and by increasing levels of BDNF. Currently, a Phase II clinical trial of desipramine in Rett patients is underway in France (Villard et al., 2007).

11. Conclusions

Studies on the pathogenesis of breathing dysfunction in RTT are still at an early stage. It is clear from mouse models that loss of MeCP2 function profoundly affects the functional integrity of the brainstem respiratory network. However, whether or not these changes result from direct or indirect effects of loss of MeCP2 function is unknown. For example, no direct

link has yet been made between dysregulation of a *bona fide* MeCP2 target and a specific deficit in respiratory behavior. Understanding the molecular pathogenesis of respiratory dysfunction is complicated by the fact that MeCP2 likely regulates multiple target genes, each of which may influence numerous downstream signaling pathways and diverse neuronal subtypes. Thus, it is not surprising that the respiratory phenotype of RTT patients and *Mecp2* null mice is complex, and that numerous neurochemical and electrophysiological abnormalities have been described thus far. In general, the picture that is emerging is that genetic loss of MeCP2 has significant effects on synaptic transmission in several brain regions important for respiratory control, including the ventrolateral medulla, the Kölliker-Fuse nucleus in the pons, and the nucleus tractus solitarius. Mechanisms that appear to underlie these transmission defects include reduced levels of biogenic amines, including norepinephrine and serotonin, reduced GABAergic signaling and marked deficits in BDNF expression. However, much more work is needed to fully understand the complex chain of events leading from loss of MeCP2-mediated gene regulation to respiratory dysfunction in RTT. For example, the observation that breathing disturbances in RTT are strongly influenced by the level of behavioral arousal (Kerr, 1992; Marcus et al., 1994; Woodyatt and Murdoch, 1996; Weese-Mayer et al., 2006) suggests that interactions between the brainstem and forebrain (and *vice versa*) that mediate state-dependent regulation of respiration merit further investigation in mouse models of the disease. The fact that at least some RTT-like symptoms in *Mecp2* null mice are reversible (Guy et al., 2007) raises hope that efforts to apply our growing understanding of neurochemical and synaptic defects in mouse models of RTT to preclinical trials will lead to the development of new therapies for RTT patients.

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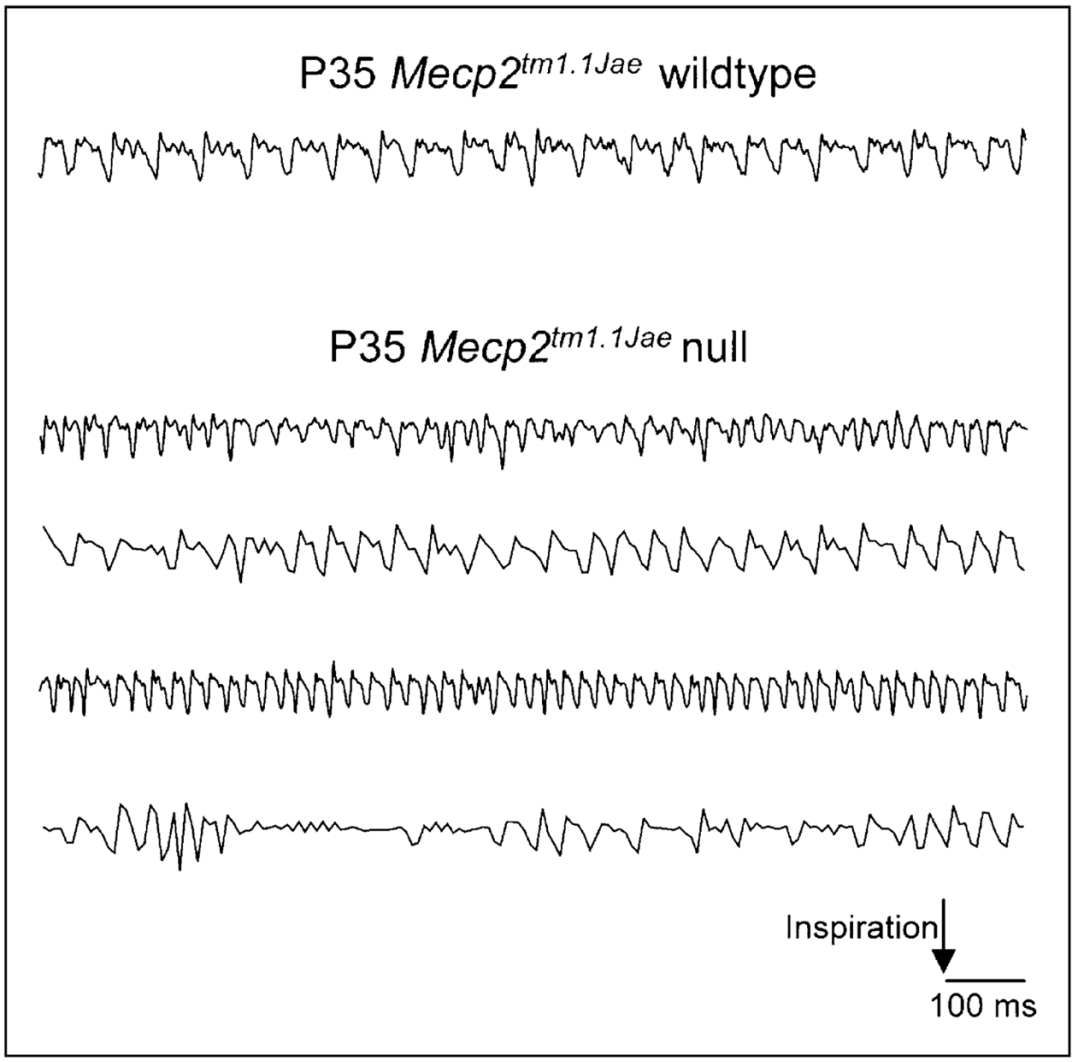

Fig. 1.

Mecp2$^{tm1.1Jae}$ null mice exhibit a Rett-like respiratory phenotype at 5 weeks of age (P35). Representative plethysmographic recordings of quiet breathing from wildtype and *Mecp2*$^{tm1.1Jae}$ null mice. In contrast to the wildtype pattern of regular breathing, the null phenotype is extremely heterogeneous and includes significant periods of high breathing frequency, highly variable breath amplitudes and respiratory pauses. Modified from Ogier et al. (2007).

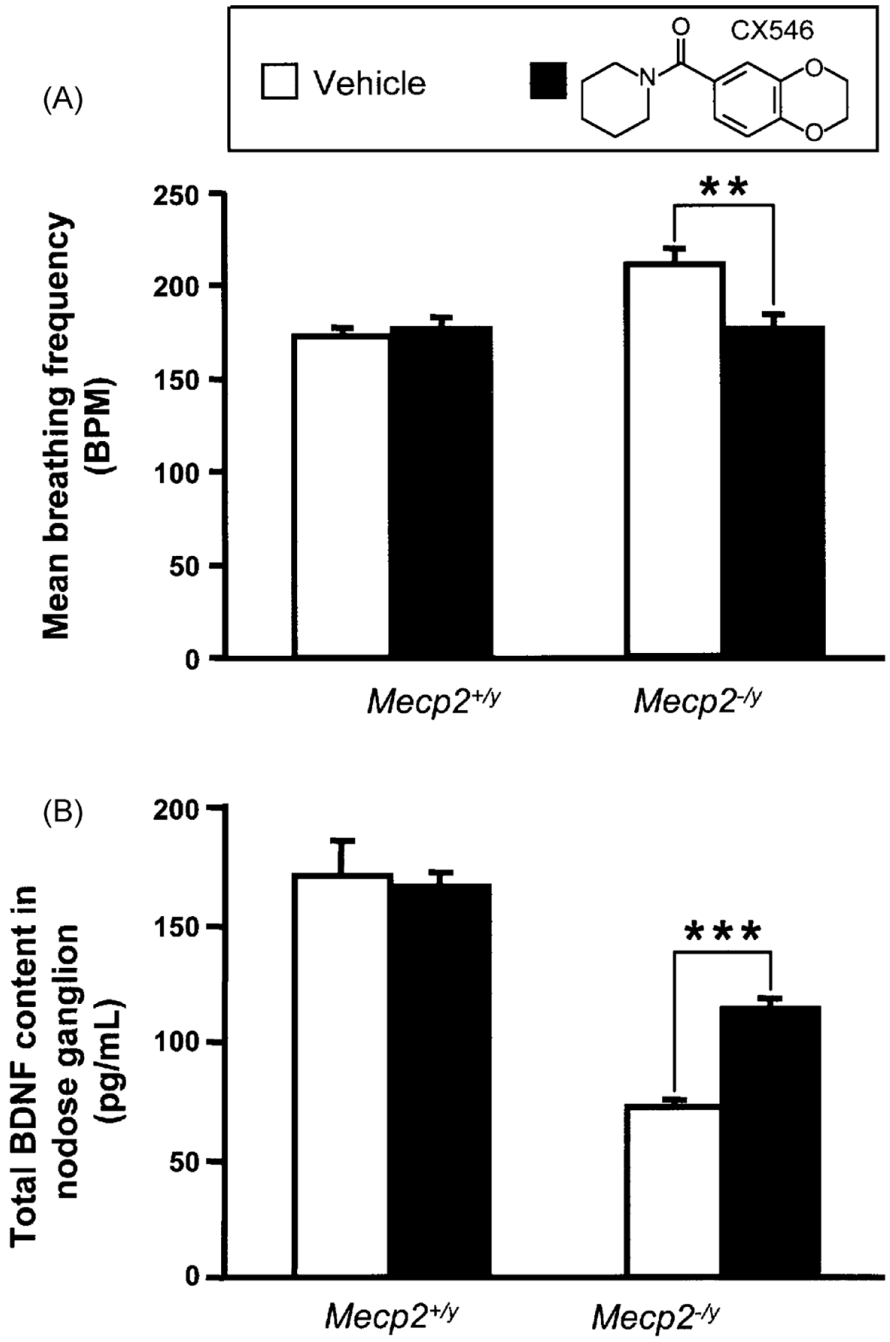

Fig. 2.

Chronic treatment with CX546 restores normal breathing frequency and increases BDNF levels in the nodose ganglia in P35 *Mecp2*$^{tm1.1Jae}$ null mice. (A) Mean respiratory frequency is higher in vehicle-treated *Mecp2* null (−/*y*) mice compared to wildtype controls (+/*y*). Following 3-day treatment with CX546 (40 mg/kg, i.p., b.i.d.) respiratory frequency in mutant mice returns to wildtype levels; $^{**}p < 0.01$, ANOVA I with post hoc Tukey test. (B) Brain-derived neurotrophic factor (BDNF) content is markedly decreased in the nodose ganglia of vehicle-treated *Mecp2* null mice compared to wildtype controls (Wang et al., 2006). Treatment of *Mecp2* null mice with CX546 results in a significant 42% increase in BDNF levels whereas drug treatment

has no effect on nodose BDNF content in wildtype animals; $^{***}p < 0.001$, ANOVA I with post hoc Tukey test. Modified from Ogier et al. (2007).