Treatment with desipramine improves breathing and survival in a mouse model for Rett syndrome

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Abstract
Rett syndrome (RS) is a severe X-linked neurological disorder in which most patients have mutations in the methyl-CpG binding protein2 (MECP2) gene. No effective treatment exists. We previously showed that the MeCP2-deficient mice, a mouse model of RS, have highly variable respiratory rhythm and frequent apneas due to reduced norepinephrine (NE) content, and a drastic decrease of tyrosine hydroxylase (TH)-expressing neurons in the medulla. We showed here that treating these mice with desipramine (DMI), which specifically inhibits NE reuptake, significantly improved their respiratory rhythm during several weeks. In addition, the treatment significantly extended their lifespan. At the cellular level, we showed that the reduced number of TH-expressing neurons before treatment in the mutant animals was not due to apoptosis. Conversely, we found that DMI treatment increased the number of TH-expressing neurons in the mutant brainstem to reach wild-type levels. We showed that this increase was not due to cellular proliferation. We propose that the MeCP2-deficient TH-expressing neurons lose their ability to synthesize TH at some point during their postnatal development. Our results suggest that a pharmacological stimulation of the noradrenergic system could be a promising approach for the treatment of the respiratory dysfunction which causes a significant proportion of death in RS patients.

Introduction
Rett syndrome (RS) is a neurological disorder primarily affecting girls, with an incidence of ~1/10 000 female births (Armstrong, 1997; Laurvick et al., 2006). Most cases are sporadic, although a few familial cases have been reported. RS is caused by mutations in the methyl-CpG binding protein 2 (MECP2) gene (Amir et al., 1999; Zoghbi, 2005; Philippe et al., 2006). Girls affected by RS are born after a normal pregnancy and uneventful delivery and have an apparently normal development throughout the first 6 months of life. Subsequently, they start to show symptoms including progressive loss of motor and cognitive skills, stereotypic hand movements, seizures and respiratory irregularities (Kerr & Julu, 1999; Julu et al., 2001; Shahbazian & Zoghbi, 2002). Twenty-six per cent of deaths in girls with RS are attributed to sudden cardiorespiratory arrhythmia (Kerr et al., 1997) although epidemiological data documenting the most frequent causes of death for RS patients are scarce. Using a mouse model of RS (Guy et al., 2001), we have previously shown that between 1 and 2 months of age all Mecp2-deficient mice exhibit breathing disturbances that worsen until fatal respiratory arrest (Viemari et al., 2005). These breathing troubles are mainly characterized by severe apneas increasing progressively in duration and intensity until the death of the mutant animals and are reminiscent of the clinical observations reported in several studies in RS patients (Julu et al., 2001; Weese-Mayer et al., 2006). We also showed that the Mecp2-deficient mice present developmental deficits of the bioaminergic systems developmentally correlated with the breathing difficulties. We observed that norepinephrine (NE) content is initially reduced in the medulla oblongata of Mecp2-deficient mice at 1 month of age and that, close to death, the serotoninergic contents is also affected. These neurochemical deficiencies are in part explained by a specific decrease in the number of medullary neurons expressing TH, a rate-limiting enzyme of catecholamine synthesis. Interestingly, we have shown in vitro using Mecp2-deficient brainstem slices that NE application was able to stabilize the firing pattern of the respiratory pacemaker neurons (Viemari et al., 2005). Therefore, our hypothesis was that breathing dysfunctions in Mecp2-deficient mice are due to a medullary bioaminergic defect and we hypothesized that a pharmacological treatment designed to stimulate NE metabolism could possibly improve breathing in the context of Mecp2 deficiency. The extracellular concentration of NE is dependent on neuronal activity (L’Heureux et al., 1986; Florin-Lechner et al., 1996) and is regulated by, among other factors, the reuptake of the neurotransmitter into the presynaptic neuron (Barker & Blakely, 1995) and presynaptic inhibitory α2-autoreceptors (Westfall, 1977). NE released at the synapse is taken up by the NE transporter that is found exclusively on the membrane of NE neurons (Lorang et al., 1994). DMI is a tricyclic antidepressant widely used in the treatment of depressive symptoms and is one of the agents most selective for the NE-selective transporter proteins, thereby increasing the availability of NE in the synaptic cleft (Sanchez & Hyttel, 1999; Hebert et al., 2001; Sacchetti et al., 2001). As we demonstrated that the noradrenergic system is deregulated in the brainstem of Mecp2-deficient mice, we decided to
investigate the effects of chronic in vivo stimulation of noradrenergic neurons by DMI on the breathing function, the number of catecholaminergic neurons and the lifespan of these mice.

Materials and methods

Animal breeding and genotyping

Experiments were performed using the mouse model (strain B6.129P2(C)-Mecp2<sup>tm1-1Bird</sup>) for RS developed by A. Bird (Guy et al., 2001). The mice were obtained from the Jackson Laboratory and maintained on a C57BL/6 background. The experimental procedures were carried out in keeping with the European guidelines for the care and use of laboratory animals (Council Directive 86/609/EEC). Moreover, our protocols were approved by the university Aix–Marseille Animal Research Committee. Hemizygous mutant males were generated by crossing heterozygous knockout females with C57BL/6 males. All our experiments were performed in hemizygous Mecp2-deficient males. Although RS in humans affects female patients, most researchers use Mecp2<sup>-/-</sup> male mice for their studies. This choice is dictated by the fact that the Mecp2 gene is X-linked in mouse and in humans, and hemizygous females will have a different number of Mecp2-expressing cells depending on their X-chromosome inactivation profile. As the proportion of Mecp2-deleted X chromosomes that will be inactivated in a given female animal is unpredictable we decided to use Mecp2<sup>-/-</sup> mice, which correspond to a complete absence of the Mecp2 gene product in all cells (i.e. a real null phenotype). Genotyping was performed by routine PCR techniques according to Jackson Laboratory protocols.

Plethysmographic recording of mouse breathing pattern

As reported elsewhere in detail, the breathing pattern was recorded from unrestrained mice by whole-body barometric plethysmography (Roux et al., 2000; Viemari et al., 2005). Breathing was recorded in a whole-body flow plethysmograph (EMKA Technologies, Paris, France) in which a constant flow pump connected to the animal chamber ensures proper and continuous inflow of fresh air, avoiding interruption of recording to flush air in the animal chamber. The animal and reference chambers (200 mL) were maintained in a temperature-regulated water bath, and maintained at 26–28 °C (temperature sensor Checktemp 1; Hanna Instruments, Lingolsheim, France). The spirogram was obtained by recording the pressure difference between the two chambers. The signal was amplified, filtered (DC–50 Hz), fed to an analogue-to-digital converter (sampling frequency 1 kHz) and stored on a PC disk via Spike 2 interface (Temeculit). The breathing cycle > 1 s.

Immunohistochemistry

Two-month-old mice were anaesthetized with a lethal pentobarbitone injection (300 mg/kg i.p.) and transcardially perfused (chilled saline for 1 min followed by 0.1 M PBS containing 4% paraformaldehyde for 10 min). Brains were postfixed for 5 h and placed overnight in PBS containing 20% sucrose and frozen at –80 °C. Medullary coronal sections (20 μm) were cut using a cryostat (Microm, France) and one in every five successive slices was arranged serially on a slide. The sections were permeabilized (0.15% Triton X-100), blocked with 7% normal goat serum, and incubated overnight at 4 °C with the primary antibody in PBS containing 3.5% serum and 0.15% Triton X-100. The sections were washed, incubated with the secondary antibody in PBS containing 3.5% serum and 0.15% Triton X-100, and re-washed. The slides were subsequently mounted in Prolong antifade (Thermo Electron). Tyrosine hydroxylase (TH) was probed with affinity-purified rabbit polyclonal antibodies (1:1000, Institut J. Boy, Reims, France). Cell proliferation was quantified by using a polyclonal rabbit antibody raised against an affinity-purified histone H3 phosphorylated at the serine 10 (histone H3-Ser10; Upstate Biotechnology) or a monoclonal antibody raised against an affinity-purified Ki-67 (BD Biosciences, Heidelberg, Germany, 550609). Goat antirabbit Alexa 488 (1:200) or Alexa 546 (1:200) and a goat antimouse Alexa 488 (1:200) were used as secondary antibodies (Molecular Probes, Eugene Oregon). The TH antibody was applied to only one every five successive sections. The nuclei of immunolabelled cell bodies were counted with digitized microphotographs recorded using a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany) equipped with a CoolSNAP camera (Princeton, Trenton, NJ, USA). The number of TH-expressing neurons in the ventral catecholaminergic cell group composed of noradrenergic A1 or adrenergic C1 nuclei (A1C1) and dorsal catecholaminergic cell group composed of noradrenergic A2 or adrenergic C2 nuclei (A2C2) was counted in every immunolabelled section. The anti histone H3-Ser10 antibody was applied to brainstem sections coming from Mecp2<sup>-/-</sup> and wild-type mice treated with DMI or placebo. In order to obtain positive controls containing stem/progenitor cells, we used sections coming from adult hippocampus and embryonic day 10.5 mice.

RNA extraction and PCR

Total RNA was extracted from the brainstem of mice using TRIzol reagent (Invitrogen). The RNA preparations were analysed for purity using the ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). DNase treatment was performed for 30 min with 1 μg of RNA, 1 U of RNase-free DNase I (Roche, Basel, Switzerland). Reverse transcription of 1 μg total RNA was performed in 20 μL of Superscript reaction buffer (Invitrogen) containing 12.5 ng/μL of RNase-free DNase I, 40 U of RNase inhibitor (Promega, Madison, WI, USA), 10 mM dNTP and 200 U of Superscript II reverse transcriptase (Invitrogen). For every reaction, one RNA sample was treated similarly without Superscript II reverse transcriptase (RT reaction) to provide a negative control. Real-time PCR reactions were performed in the LightCycler 480 system (Roche) using the SYBR Green I Master Kit (Roche) with 2 μL cDNA (1/3 dilution of the first-strand reaction) and 200 nM of each primer. Each reaction was performed in triplicate. To analyse TH mRNA expression, we used primers located in exon 5 (forward, 5'-CACAGCCGAGGATGTGTCCA-3') and exon 6 (reverse, 5'-TTCAGGGCACACGTACCTCGT-3') giving a 137-bp PCR product. To normalize our results we used GAPDH primers located in exon 7 (forward, 5'-TGGTCTGGTGGATGTCGA-3') and exon 8 (reverse, 5'-TCAAGAGGGTGTTGAGAG-3') giving a 77-bp PCR product.

Detection of apoptosis

A terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay was used to detect apoptosis on frozen sections. An in situ cell death detection kit (Roche Applied Science)
was used to label the nuclei of apoptotic cells with fluorescein. The cells were observed under a fluorescence microscope (Leica DMR). Negative controls were included by omitting the labelling buffer. Positive controls were obtained by digestion of the sections with deoxyribonuclease grade 1 for 10 min at 25 °C to induce DNA strand breaks prior to labelling procedures.

**Pharmacological treatment**

Mecp2-/- and wild-type mice received a daily i.p. injection of DMI (Sigma Aldrich) at 10 mg/kg or NaCl (placebo group) once a day. Each injection was performed between 09.00 and 10.00 h. The mice were assigned to each experimental group by an investigator who was blind to their genotype. Genotyping, treatment and immunohistochemistry experiments, and data analysis were performed by different investigators. To count TH-expressing neurons and to measure TH chemistry experiments, different groups by an investigator blind of their genotype. The first group was assigned to each experimental group by an investigator who was blind to their genotype. Genotyping, treatment and immunohistochemistry experiments, and data analysis were performed by different investigators. To count TH-expressing neurons and to measure TH mRNA levels after injection of DMI, Mecp2-/- and wild-type mice were treated starting at postnatal day (P)40 for 3 or 14 days. A treatment for 2 weeks has been selected because it is based on the minimal time required for an antidepressant treatment to induce therapeutic effects. The dose was selected on the basis of its ability to induce a major effect on NE metabolism (Daniel & Melzack, 1992; Invernizzi & Garattini, 2004).

**Statistical analysis**

Data are expressed as a mean ± SEM. The data were analysed with the SPSS software (SPSS Science Software GmbH., Erkrath, Germany). For all tests, the statistical significance was taken at \( P \leq 0.05 \). The Mann–Whitney U-test was used to assess the statistical significance of differential findings. Mouse survival was analysed using the Kaplan–Meier survival plot followed by a log-rank test, accepting \( P = 0.05 \) as statistically significant.

**Results**

**DMI treatment decreased the number of apneas in Mecp2-deficient mice**

We first investigated the effect of DMI treatment on the basal breathing parameters of five wild-type mice. DMI injection did not induce any significant modification of the mean values of tidal volume, respiratory frequency and ventilation (data not shown). As our previous results showed that the breathing function starts to be disturbed immediately after the first postnatal month, we decided to record the breathing pattern of Mecp2-deficient mice every 2 days from P20 until the sudden increase in the number of apneas. The period of the first injection was chosen in order to correspond to a specific stage of functional deficit rather than a particular age. Even if this sudden rise in the number of apneas was somewhat variable from one mouse to another (data not shown), we arbitrarily decided to initiate the treatment when the level reached 100 apneas/h. We found that this threshold approximately corresponds to the major period of reduction of the number of TH-expressing neurons (42 ± 7 days; data not shown). At this time, Mecp2-deficient mice were divided in two groups by an investigator blind of their genotype. The first group was treated with an intraperitoneal dose of DMI and the second group with a placebo (Fig. 1). In the placebo group, the number of apneas continued to increase 3 days after the first injection and it reached a plateau of \( \sim 350 \) apneas/h. Thereafter, the number of apneas stayed at an elevated level for 2 weeks until the death of the animal. In the group treated with DMI, the breathing was stabilized and the number of apneas was strongly reduced in comparison to the placebo group (\( \sim 75\% \) after 3 days of treatment). The breathing pattern of the DMI-treated mice remained stable for \( \sim 5 \) weeks and subsequently greatly increased again until their death (Fig. 1).

**TH neurons were not eliminated by apoptosis in Mecp2-deficient mice**

We next wanted to determine whether the TH-expressing neurons die or whether they lose their ability to synthesize the TH protein in the absence of Mecp2. In a previous study we have shown that the number of TH-expressing neurons in Mecp2-deficient mice was normal at birth but was decreased in the A2C2 cell group (\( \sim 40\% \)) at 1 month of age, and in both A1C1 (\( \sim 30\% \)) and A2C2 cell groups (\( \sim 37\% \)) at 2 months of age (Viemari et al., 2005). We tested whether this decrease was due to apoptosis using a TUNEL assay. We have analysed brainstem sections of 30-day-old (\( n = 3 \)), 45-day-old (\( n = 3 \)) and 60-day-old (\( n = 3 \)) Mecp2-deficient mice. No apoptotic cells were detected in the region containing A1C1 and A2C2 cell groups (Fig. 2).

**Desipramine treatment increased the number of TH-expressing neurons in Mecp2-deficient mice**

As we were not able to detect apoptosis in the brainstem of the Mecp2-deficient mice we hypothesized that the decrease in the number of TH-expressing neurons in these animals could be the consequence of a change in the phenotype of these cells. To test this hypothesis, we treated Mecp2-deficient and wild-type mice with a dose of DMI or placebo at P40. The first group was treated to determine whether the initial breathing stabilization by DMI at D + 3 (where D represents the day when the number of apneas reached 100 per hour) was correlated...
with an early increase in the number of TH-expressing neurons, and the second group was treated chronically for 2 weeks. In wild-type mice, both the DMI-treated and a placebo group showed no significant difference in the number of TH-expressing neurons in A1C1 or A2C2 neurons after 3 days (not shown) or 2 weeks (Fig. 3). This result is in good agreement with the fact that DMI injection in wild-type mice does not modify their basal ventilation (data not shown). In the group of Mecp2-deficient mice treated for 2 weeks with placebo, we confirmed a decrease in TH-expressing neurons in A1C1 and A2C2 catecholaminergic cell groups (−30% in A1C1, −50% in A2C2) as was previously described in untreated Mecp2-deficient mice (Viemari et al., 2005). Three days of DMI treatment in Mecp2-deficient mice increased the number of TH-expressing neurons (+25% in A1C1, \( P = 0.18 \); +20% in A2C2, \( P = 0.25 \)) compared to Mecp2-deficient mice injected with a placebo (\( n = 4 \) in both groups; data not shown). This increase in TH-expressing neurons at 3 days of treatment was correlated with an increase of TH mRNA (+15%) at the brainstem level (\( n = 3 \) in both groups; data not shown). Treatment for 2 weeks was able to increase the number of TH-expressing neurons in the A1C1 and the A2C2 cell groups of Mecp2-deficient mice to wild-type levels (Fig. 3).

Desipramine treatment did not induce cell proliferation in Mecp2-deficient mice

Neurogenesis in the brain of adult rats has been described once in the area of the dorsal vagal nucleus which includes the A1C1 and A2C2 catecholaminergic cell groups (Charrier et al., 2006). To determine whether DMI treatment was able to induce cellular proliferation in the A1C1 and A2C2 catecholaminergic cell groups, we used an antibody directed against histone H3-Ser10 or against Ki-67 to label brainstem sections of Mecp2-deficient mice. These two proteins are frequently used to identify cell proliferation in embryonic and adult rodents (Endl & Gerdes, 2000; Komitova et al., 2005). Embryonic sections of mouse brains and adult brain regions containing sites of neurogenesis, such as the dentate gyrus and the subventricular zone, were used as positive controls (Fig. 4). We counted positive cells in wild-type (\( n = 3 \)) or Mecp2-deficient (\( n = 3 \)) mice treated with DMI or with placebo. We failed to detect any proliferating cells, suggesting that the increase in the number of TH-expressing neurons after DMI treatment is not due to a proliferative mechanism. This is in good agreement with the results obtained in a previous experiment (Fig. 3) where we observed that treatment with DMI has no effect on the number of TH-expressing neurons in wild-type mice.

Treatment with desipramine extended the lifespan of Mecp2-deficient mice

As we found that treatment with DMI was able to decrease the number of apneas per hour in Mecp2-deficient mice and to increase the number of TH-expressing neurons in the brainstem of these animals, we measured the impact of the treatment on their lifespan. We treated Mecp2-deficient mice with DMI after the sudden increase (to 100 apneas/h) in the number of apneas as previously described until their death (Fig. 5). The first group of Mecp2-deficient mice was not treated and died at 56.7 ± 4.8 days (\( n = 11 \)), which is in good agreement with the mean lifespan previously reported with this model (Guy et al., 2001). The second group was treated with a placebo and survived until 60.8 ± 3.1 days (\( n = 7 \)). These two groups were not significantly different. The third group was treated chronically with DMI and survived for 90.8 ± 11.6 days (\( n = 6 \)). A Kaplan–Meier survival plot shows that the lifespan of the Mecp2-deficient mice treated with DMI was significantly increased (+48%) in comparison to Mecp2-deficient animals treated with a placebo.

Discussion

In a previous study we identified noradrenergic deficits leading to breathing dysfunctions in Mecp2-deficient mice (Viemari et al., 2005). These deficits were confirmed using HPLC measurements by another group (Ide et al., 2005). Here, we show that noradrenergic stimulation using a treatment with desipramine in Mecp2-deficient mice induces (i) an improvement of the breathing pattern lasting several weeks; (ii) a significant extension of their lifespan and (iii) an increase in the number of TH-expressing neurons in the A1C1 and A2C2 cell groups.

We selected desipramine as a pharmacological tool because it is the most selective agent for the noradrenergic neurons with a minimal effect on the serotoninergic and cholinergic systems (Westfall, 1977; Owens et al., 1997; Richelson, 2001). We used a dose of 10 mg/kg.
because it is within the range of doses (5–20 mg/kg) at which DMI has been previously shown to induce almost the complete inhibition of the cellular NE reuptake in rodents (Daniel & Melzacka, 1992; Invernizzi & Garattini, 2004). We have shown that the number and the intensity of the severe apneas affecting the Mecp2-deficient mice were significantly reduced after DMI treatment. However, even if the breathing function was improved by the treatment, the disturbances reappeared after several weeks of stabilization. Several hypotheses can be proposed to explain this transient effect. First, as serotonin is disturbed at late stages of disease progression in the brainstem of Mecp2-deficient mice, it could also participate in the reappearance of breathing dysfunctions. If this is the case, the association of desipramine and a ‘pharmacological stimulant’ of serotoninergic neurons could be beneficial. Second, the progressive increase in NE in the synaptic cleft could progressively induce desensitization of the adrenergic receptors, leading to a decrease in the NE effect. Third, we treated the Mecp2-deficient mice when the breathing dysfunction reached 100 apneas/h. As the bioaminergic deficits slightly precede this level of breathing dysfunction, an earlier treatment could be more effective. Finally, it has been extensively demonstrated that the extracellular concentration of NE is dependent on neuronal activity and is regulated both by a reuptake mechanism in the presynaptic neuron and by presynaptic inhibitory α2-autoreceptors (Westfall, 1977; Barker & Blakely, 1995; Florin-Lechner et al., 1996; Invernizzi & Garattini, 2004). Administration of α2-autoreceptor inhibitors with desipramine is able to markedly enhance the increase of extracellular NE (Invernizzi & Garattini, 2004) and this association could be beneficial.

In order to identify a potential cellular basis for the efficiency of the DMI treatment, we tried to understand why the number of TH-expressing neurons decreased in the brainstem of Mecp2-deficient mice. A problem during the development can be ruled out as the number of TH-expressing neurons is normal in 3-day-old Mecp2-deficient mice (Viemari et al., 2005). Consequently, two hypotheses can be proposed: (i) TH-expressing neurons die by apoptosis during the postnatal development; or (ii) TH-expressing neurons lose their ability to synthesize the TH protein. We ruled out the first hypothesis as no nuclei of apoptotic cells could be identified in the brainstem of 30-, 45- or 60-day-old Mecp2-deficient mice. For this reason, we favour the second hypothesis and propose that some TH neurons lose their phenotype at some point during the postnatal development of the Mecp2-deficient mice. A better understanding of the function of

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**Fig. 3.** Chronic treatment of Mecp2-deficient mice with desipramine increased the number of neurons expressing TH in the A1C1 and A2C2 catecholaminergic cell groups. (A) Photomicrographs of TH-expressing neurons in the A2C2 catecholaminergic nuclei in wild-type and Mecp2-deficient (mutant) littermates treated with DMI or placebo at 2 months of age after 2 weeks of DMI treatment. (B) The number of TH-expressing neurons was counted in the ventral A1C1 and dorsal A2C2 catecholaminergic nuclei in wild-type and Mecp2-deficient littermates at 2 months of age after 2 weeks of DMI treatment. Although there was no difference in the number of TH-expressing neurons after treatment of the wild-type animals, this number was significantly increased in the Mecp2-deficient mice treated with DMI in the two cell groups. *P < 0.05 between the different groups. The numbers of mice used for these experiments are n = 3 for wild-type placebo, n = 3 for wild-type desipramine, n = 6 for mutant placebo and n = 3 for mutant desipramine.
Fig. 5. Chronic treatment with desipramine extended the lifespan of Mecp2-deficient mice. The lifespan of the Mecp2-deficient mice was measured in animals either not treated or treated with a placebo or with DMI. Untreated mice survived for 56.7 ± 4.8 days (n = 11), the placebo group survived for 60.8 ± 3.1 days (n = 7) and the group treated with DMI survived for 90.8 ± 11.6 days (n = 6). The data were analysed using the Kaplan–Meier survival plot. There was a statistically significant difference (P = 0.05) between the group treated with DMI and the two other groups.
Mecp2 in neuronal cells will be necessary in order to characterize which mechanisms are involved in this phenomenon.

We have previously shown that Mecp2-deficient mice, after the first postnatal month, exhibit a significant decrease in the number of TH-expressing neurons in the A1C1 and A2C2 catecholaminergic cell groups, associated with a reduction in noradrenergic contents (Viemari et al., 2005). Here, we show that 3 days of DMI treatment, which is able to reduce the number of apneas of Mecp2-deficient mice, increase the number of TH-expressing neurons in a manner correlated with an increase in TH mRNA. Interestingly, a longer treatment (2 weeks) restored a normal number of TH-expressing cells in A1C1 and A2C2 cell groups of Mecp2-deficient mice. A putative mechanism for cellular proliferation upon DMI treatment was ruled out as we found no Ki-67- and no histone H3-Ser10-immunopositive cells in the brainstems of treated Mecp2-deficient mice. These proteins are markers of the mitotic cellular cycle (Juan et al., 1998; Endl & Gerdes, 2000). The brainstem catecholaminergic neurons of rodents are known to be developmentally regulated and selectively activated by pharmacological treatment as well as environmental modifications. During postnatal development in rats, noradrenergic neurons of the locus coeruleus (A6) exhibit a transient increase in the total number of TH-expressing neurons, associated with an increase in their catecholaminergic activity, at P14 compared to adult (Bezin et al., 1994; Roux et al., 2003). Moreover the sudden increase in TH-expressing cells does not result in neuronal proliferation as the peak of neuronal mitotic activity in A6 precursors has been dated at embryonic day 12.5 (Lauder & Bloom, 1974). Modifications in the environment of ‘quiescent’ cells could explain the temporal expression of the TH gene in these cells (Dreyfus et al., 1983; Robinson et al., 1993). Factors governing this epigenetic regulation of TH gene expression of ‘quiescent’ neurons would operate, for instance, in the target areas of these neurons (Robinson et al., 1993; Marcel et al., 1998). In adult rats and mice, pharmacological treatment with RU24722 (an eburnam-nin derivative) is known to increase the number of TH-expressing neurons in A6, showing not only a developmental plasticity but also an epigenetic regulation (Marcel et al., 1998; Bezin et al., 2000). The presence of TH-negative neurons expressing the homeodomain transcription factor Phox2a, specific for the determination of noradrenergic phenotype, has been demonstrated, providing further evidence that ‘resting-noradrenergic’ neurons exist in the adult rat A6 under basal conditions. Even though the plasticity of TH neurons has been extensively studied in the A6 noradrenergic neurons, such plasticity has also been found in other brainstem catecholaminergic neurons. A number of catecholaminergic neurons which did not express the TH mRNA or protein under normal conditions were detected in chronic hypoxic rats. This enlargement of the TH-immunopositive area was mainly observed in the A2C2 area (Dumas et al., 1996). This mechanism appears to be similar to the well studied ‘quiescent’ or sleeping cells that have been previously characterized in A6 noradrenergic neurons (Bezin et al., 2004).

In addition to the bioaminergic deficits, the brain of Mecp2-deficient mice exhibit a down-regulation of the brain-derived neurotrophic factor (BDNF) mRNA and disturbances of the glucocorticoid system (Nuber et al., 2005; Chang et al., 2006). DMI has been extensively described as a BDNF and glucocorticoid transcriptional modulator (Nibuya et al., 1995; Pariante et al., 2001; Jacobsen & Mork, 2004; Vinet et al., 2004). This could be of particular interest as BDNF and glucocorticoids are key factors involved in the maturation and the regulation of the TH neurons (Hagerty et al., 2001; Baquet et al., 2005; Guo et al., 2005). For instance, BDNF-knockout mice exhibit a decrease in the number of TH neurons in the A5 noradrenergic cell group of the brainstem and also in the A9 dopaminergic cell group (Baquet et al., 2005; Guo et al., 2005). Furthermore, a TH glucocorticoid-responsive element is directly involved in the regulation of the mouse TH promoter activity (Hagerty et al., 2001).

It has long been hypothesized that RS is associated with bioaminergic deficits, although early studies did not find any significant abnormalities in the levels of NE, dopamine or serotonin in the spinal fluid whereas brain autopsies revealed reduced levels of these substances and their metabolites (Nomura et al., 1985; Lekman et al., 1990; Nielsen et al., 1990; Dunn, 2001). Positron emission tomography identified a substantial alteration in TH activity (Segawa, 1997; Dunn & MacLeod, 2001). A pharmacological trial was performed on RS girls who received a supplement of tyrosine and tryptophan for up to 10 weeks. Unfortunately, this protocol did not provide significant improvement (Nielsen et al., 1990). Using an alternative strategy based on cellular observations, we were able to stimulate noradrenergic neurons using a pharmacological agent and to significantly improve the phenotype of Mecp2-deficient mice. We hope that our findings will promote new therapeutic developments for the treatment of RS children.

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Abbreviations

A1C1 and A2C2, catecholaminergic cell groups composed of noradrenergic A1, A2 or adrenergic C1, C2 nuclei; D, the day when the number of apneas reached 100 per hour; DMI, desipramine; histone H3-Ser10, histone H3 phosphorylated at serine 10; Mecp2, methyl CpG binding protein 2; NE, norepinephrine; P, postnatal day; RS, Rett syndrome; TH, tyrosine hydroxylase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling.

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