Conditional depletion of methyl-CpG-binding protein 2 in astrocytes depresses the hypercapnic ventilatory response in mice

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Garg SK, Lioy DT, Knopp SJ, Bissonnette JM. Conditional depletion of methyl-CpG-binding protein 2 in astrocytes depresses the hypercapnic ventilatory response in mice. J Appl Physiol 119: 670–676, 2015. First published July 23, 2015; doi:10.1152/japplphysiol.00411.2015.—Mice that are deficient in the transcription factor methyl-CpG-binding protein 2 (MeCP2) have a depressed hypercapnic ventilatory response (HCVR). The expression of MeCP2 can be selectively removed from astrocytes or neurons, thus offering a tool to dissect the role of this transcription factor in astrocytes from that in neurons. Studies were carried out in the progeny of mice that were a cross between those harboring a tamoxifen (TAM)-inducible Cre recombinase transgene driven by the human astrocytic glial fibrillary acidic protein (hGFAP) promoter, or Cre recombinase under control of the synapsin promoter, with mice containing a Cre-excisable exon III in the MeCP2 gene. The TAM-conditional excision of the MeCP2 exon allowed the respiratory CO2 response to be studied in the same animals before and after selective depletion of MeCP2 in astrocytes. Immunohistochemistry showed that following TAM treatment only ~20% of GFAP-labeled cells in the retrotrapezoid nucleus and in the raphé magnus were positive for MeCP2. The slope of the relative increase in minute ventilation as a function of 1, 3, and 5% inspired CO2 was depressed in mice with depleted astrocyte MeCP2 compared with wild-type littersmates. In contrast, selective depletion of MeCP2 in neurons did not significantly affect slope. While neurons which constitute the respiratory network ultimately determine the ventilatory response to CO2, this study demonstrates that loss of MeCP2 in astrocytes alone is sufficient to result in a dramatic attenuation of the HCVR. We propose that the glial contribution to HCVR is under the control of the MeCP2 gene.

HYDROGEN ION HOMEOSTASIS IS largely dependent on CO2/pH regulation of minute ventilation. Thus the cellular and molecular mechanisms that transduce changes in blood and brain parenchymal CO2/pH to increase the rate and depth of breathing are important areas of current study (12, 17, 18, 21, 26, 27, 40). While the generally accepted view holds that response to CO2 is a property of neurons, recent work has pointed to an important contribution from astrocytes (14, 15, 18, 21, 27). Respiratory CO2 ventilatory response is significantly depressed in mice that lack or are deficient in the X-linked transcription factor methyl-CpG-binding protein 2 (MeCP2) (6, 50, 58). Mutations in this nuclear protein cause the neurological disorder Rett syndrome (RTT) (3).

A previous study suggested that activation of chemosensitive neurons of the retrotrapezoid nucleus (RTN) could affect CO2 levels by both their intrinsic pH sensitivity of as well as by non-cell-autonomous activity of neighboring astrocytes (19). The extent of astrocyte contribution by release of ATP to signal RTN neurons is unsettled. Photoactivation of astrocytes on the ventral surface of the medulla in anesthetized rats increased the output of phrenic nerve activity, and the effect was markedly reduced (~90%) by the addition of the ATP receptor antagonist MRS2179 (19). In contrast, the phrenic response to increasing end-expiratory CO2 from 5 to 10% was only reduced by 30% after bilateral injection of pyridoxalphosphate-6-azophenyl-2,4′-disulfonic acid into the RTN of anesthetized vago-sino-aortic-denervated rats (56). In addition, dispersed Phox2b-expressing RTN neurons have been shown to be intrinsically chemosensitive (53). Thus the relative contributions of neurons vs. astrocytes to the overall respiratory response to CO2 in vivo are not presently known. Here we have taken advantage of RTT mouse models that exhibit depressed respiratory CO2 responses and in which the associated mutation can be selectively targeted to astrocytes or to neurons to evaluate their relative importance in HCVR. The perturbations in partial pressure of CO2 during quiet breathing revealed a critical role of astrocytes in the mechanisms of the HCVR and suggested that the CO2/pH-sensitive mechanism of astrocytes contains components that are under the transcriptional control of MeCP2.

METHODS

The protocols were approved by the Oregon Health and Science University Institutional Animal Care and Use Committee, and were in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals. MeCP2Jaenisch.Flox (MeCP2lox/lox) (8) mice were obtained from the Mutant Mouse Medical Resource Center at the University of California, Davis. Male mice harboring a tamoxifen (TAM)-inducible Cre recombinase transgene driven by the hGFAP promoter (23) were crossed with female mice containing a Cre-excisable transcriptional sequence in the MeCP2 gene that flanked exon III. The progeny that inherited both alleles are referred to as MeCP2lox/lox-hGFAPcreT2 mice. These crosses also yielded MeCP2lox/lox, MeCP2lox/+ and hGFAPcreT2 genotypes. SynCre mice were obtained from the Kohwi-Shigematsu laboratory at Lawrence Berkeley National Laboratory. For neuronal MeCP2 knockout experiments, male SynCre mice were crossed with female MeCP2lox/lox, and the F1 males were used in the experiments. These crosses yielded MeCP2lox/lox-, SynCre, MeCP2loxloxT2, MeCP2lox+ and SynCre offspring. Genotypes were determined by tail or ear biopsies as previously described (35). All mice were on a C57BL/6 background. All studies were performed using male mice between 40 and 170 days of age.

Tissue preparation, immunohistochemistry, and cell counts. Mice were terminally anesthetized by intraperitoneal injection of Avertin (2-2-tribromoethanol; 15 µl/g of a 2% solution) and killed by transcardial perfusion of 4% paraformaldehyde in PBS. The brains were removed and incubated in 30% sucrose overnight at 4°C.
Coronal sections (20-40 μm) were cut at −20°C using a cryostat (Leica, Buffalo Grove, IL), placed in antifreeze, and stored at −20°C. Sections were immunolabeled overnight at 4°C using the following primary antibodies: rabbit anti-Mecp2 (1:500, Covance, Princeton, NJ), chicken anti-Mecp2 (1:500, Millipore), mouse anti-GFAP (1:500, Millipore, Billerica, MA), mouse anti-NeuN (1:200, Chemicon, Billerica, MA), mouse anti-tryptophan hydroxylase (1:500, Chemicon), goat anti-somatostatin (1:200, Santa Cruz, Dallas, TX), and mouse anti-Phox2b (1:200; a generous gift from Dr. Jean Francois Brunet). Sections were then incubated in the appropriate Alexa Fluor secondary antibodies (1:500, Invitrogen, Carlsbad, CA) for 1 h at room temperature. 4,6-Diamidino-2-phenylindole was present in the ProLong Gold Antifade (Invitrogen) mounting reagent. All images were collected using a Zeiss confocal microscope (model LSM 510; Zeiss, Germany). MeCP2-expressing cells were identified as previously described (35).

**TAM treatments.** TAM (Sigma) was made fresh weekly by dissolving in 90% sunflower seed oil-10% ethanol solution by bath sonication for 20–30 min at 4°C with intermittent vortexing. Final concentration of TAM for injections was 20 mg/ml. Following baseline CO2 respiratory response studies in P40 to P45 mice, the animals were injected intraperitoneally with 100 mg/kg of TAM for 7 days. The first post-TAM CO2 response study was evaluated 2 wk after the treatment completion and then repeated at 4, 8, 12, and 16 wk.

**Plethysmography.** Respiratory frequency, tidal volume (VT), and minute ventilation (V˙E) were determined using the method of body plethysmography described in detail previously (5, 38). Briefly, individual unanesthetized animals were placed in a 65-ml chamber with the head exposed through a close-fitting hole in Parafilm. A pneumotachograph (38) was connected to the chamber and a differential pressure transducer (model PT5A; Grass Instruments, West Warwick, RI). The pressure signal was integrated to give readings in cm H2O. Volume changes were calibrated by injecting known amounts of air into the chamber. The analog signal from the transducer was amplified, converted to digital, displayed on a monitor, and stored on their product minute ventilation (V˙E) were determined using the method of body plethysmography described in detail previously (5, 38).

**RESULTS**

**Effect of MeCP2 depletion in astrocytes on the HCVR.** Because of their prominent role in central respiratory CO2 chemosensitivity, we focused on the medullary RTN and raphé magnus (RM) for assessing the efficiency of MeCP2 depletion in astrocytes. Similar to our previous study (35) following TAM treatment of Mecp2JFlox/y-hGFAPcreT2 mice, MeCP2 was only expressed by 23.1% of GFAP-positive RTN cells compared with 74.8% in Mecp2JFlox/y mice and in 22.1% of astrocytes in RM compared with 78.6% in Mecp2JFlox/y mice (Fig. 1).
Baseline respiratory parameters of Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-hGFAPcreT2</sup> mice did not differ from those in Mecp2<sup>+/y</sup> animals. TAM treatment of wild-type (WT) mice (<i>n</i> = 9) did not affect baseline respiratory frequency (234 ± 30 vs. 239 ± 18 breaths/min), <i>V</i><sub>T</sub> (5.78 ± 2.43 vs. 5.87 ± 2.07 μl/g), and <i>V</i><sub>E</sub> (1.33 ± 0.78 vs. 1.33 ± 0.42 ml·min⁻¹·g⁻¹). Similarly, TAM treatment had no effect on resting respiratory frequency (236 ± 13.2 vs. 231 ± 18.5 breaths/min), <i>V</i><sub>T</sub> (5.12 ± 1.75 vs. 5.72 ± 1.93 μl/g), or <i>V</i><sub>E</sub> (1.13 ± 0.4 vs. 1.43 ± 0.37 ml·min⁻¹·g⁻¹) in Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-hGFAPcreT2</sup> animals (<i>n</i> = 7). Following TAM treatment, the ventilatory response of Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-hGFAPcreT2</sup> mice to CO<sub>2</sub> was significantly depressed at all tested levels of hypercapnia (Fig. 1, A and C). The effect of MeCP2 depletion in astrocytes was greater at 1% and 3% inspired CO<sub>2</sub> than it was at 5% inspired CO<sub>2</sub> (64, 64, and 56%, respectively). The slope of the relative increase in minute ventilation as a function of inhaled CO<sub>2</sub> concentration after TAM was significantly depressed in Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-hGFAPcreT2</sup> mice compared with that in Mecp2<sup>+/y</sup> littermates (5.18 ± 2.09 vs. 8.42 ± 1.59, <i>P</i> = 0.001; Fig. 1C). TAM treatment had no effect on the respiratory responses to CO<sub>2</sub> in Mecp2<sup>+/y</sup> mice (Fig. 1C) as well as in Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub></sup> or hGFAPcreT2 mice (not shown). Four out of seven Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-hGFAPcreT2</sup> mice had depressed CO<sub>2</sub> respiratory responses at 2 wk after the completion of TAM treatment, while all seven animals exhibited reduced CO<sub>2</sub> sensitivity at 4 wk after the treatment. This is consistent with the 2-wk half-life of MeCP2 protein (9). Once established, the depressed respiratory CO<sub>2</sub> sensitivity in Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-hGFAPcreT2</sup> mice persisted for at least 16 wk after TAM treatment (Fig. 2A). The dramatically reduced ventilatory response to CO<sub>2</sub> in conditions of MeCP2 deletion in astrocytes was entirely due to the inability of these animals to mount an adequate <i>V</i><sub>T</sub> increase with inhaled CO<sub>2</sub> (Fig. 2C). CO<sub>2</sub>-induced increases in the respiratory frequency were similar to that recorded in WT mice (Fig. 2B).

**Effect of MeCP2 depletion in neurons on the HCVR.** Due to the noninducible nature of neuronal MeCP2 depletion by Cre driven by the synapsin promoter, ventilatory CO<sub>2</sub> responses could not be evaluated before and after MeCP2 depletion using the same experimental strategy as described above for astroglial deletion. In the Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-SynCre</sup> mice only 4.6% of Phox2b-positive neurons in the RTN (putative central respiratory chemosensors) were immuno-positive for MeCP2 (Fig. 3A). In RM only 10.8% of NeuN-positive, and tryptophan hydroxylase-2-positive cells expressed MeCP2 (Fig. 3A). The baseline respiratory pattern in Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-SynCre</sup> mice (<i>n</i> = 7) was found to be different from WT littermates (<i>n</i> = 9) with an elevated <i>V</i><sub>T</sub> (5.91 ± 0.58 vs. 4.17 ± 0.57 μl/g, <i>P</i> < 0.0001), and trends toward a reduced respiratory frequency (213 ± 13 vs. 234 ± 24 breaths/min, <i>P</i> = 0.058) and a higher <i>V</i><sub>E</sub> (1.25 ± 0.16 vs. 0.97 ± 0.18 ml·min⁻¹·g⁻¹, <i>P</i> = 0.058). In response to inhalation of 1% CO<sub>2</sub> in the inspired air, the increase in <i>V</i><sub>E</sub> for Mecp2<sup>Fl<sub>F</sub>ox<sub>y</sub>-SynCre</sup> mice (8.67 ± 8.84%) was significantly less than that in WT littermates (18.84 ± 8.7%, <i>P</i> = 0.033; Fig. 3B). At 3 and 5% inspired CO<sub>2</sub>, however, the increase in <i>V</i><sub>E</sub> in mice with depletion of MeCP2 in neurons did not show a significant difference from that in WT animals (3% CO<sub>2</sub>: 25.52 ± 7.17 vs. 35.81 ± 3.3%, <i>P</i> = 0.101; 5% CO<sub>2</sub>: 38.7 ± 9.45 vs. 56.72 ± 16.05%, <i>P</i> = 0.112; Fig. 3B). In contrast to the result of MeCP2 depletion in astrocytes, the slope of the relative increase in minute ventila-

![Figure 2](http://japl.physiology.org/Downloadedfrom)
on the resultant respiratory response exceeded that in neurons (3%: 58.7 ± 9.8 vs. 28.7 ± 5.7%, P < 0.01; 5%: 42.9 ± 8.9 vs. 31.8 ± 9%, P < 0.05). In addition, the slope of the relative increase in minute ventilation as a function of inhaled CO2 concentration was significantly depressed in mice with depleted MeCP2 confined to astrocytes while it was not with neuronal depletion (Figs. 1C and 3B). There was a trend for this slope (5.18 ± 2.09) to be less steep with astrocyte depletion than that with loss of MeCP2 in neurons (7.51 ± 2.91; P = 0.075). This greater effect of MeCP2 depletion in astrocytes was observed despite the finding that MeCP2 was absent from neurons in Mecp2JFlox/y-SynCre mice to a larger extent than from astrocytes in Mecp2JFlox/y-hGFAPcreT2 mice.

DISCUSSION

Selective depletion of MeCP2 in astrocytes resulted in a marked depression of the ventilatory responses to increases in the level of inhaled CO2. The magnitude of this reduced response in minute ventilation (62, 59, and 43% less than that of WT at 1, 3, and 5% inspired CO2, respectively) was similar to that observed in conditions of global MeCP2 deficiency (50, 38, and 30%) (50), also reported in separate studies at 1, 2, and 3% inspired CO2 (38, 39, and 48%) (58). In addition, the extent of depression in the HCVR was comparable to that seen with either elimination or silencing of chemosensitive serotonin (5-HT) raphé neurons or removal of Phox2b-expressing neurons in the RTN, summarized in Table 1. With two exceptions

Table 1. Summary of the literature data comparing decreases in hypercapnic ventilatory response when chemosensitive RTN or 5-HT raphé neurons are targeted

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>CO2 (%)</th>
<th>Decrease From Control (%)</th>
</tr>
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<tbody>
<tr>
<td>25</td>
<td>Lmx1bKO of 5-HT neurons</td>
<td>3 and 5</td>
<td>44 and 32.5</td>
</tr>
<tr>
<td>33</td>
<td>5-HT transporter KO</td>
<td>5</td>
<td>49 (males)</td>
</tr>
<tr>
<td>48</td>
<td>Saporin (70% decrease Phox2B &quot;TH&quot; RTN neurons)</td>
<td>9 (end expiratory)</td>
<td>41</td>
</tr>
<tr>
<td>36</td>
<td>Allanostatin receptor transfection in RTN (50–64% Phox2B neurons with carotid and aortic body denervation)</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>44</td>
<td>Acute hyperpolarization of 5-HT neurons</td>
<td>5</td>
<td>47</td>
</tr>
<tr>
<td>42</td>
<td>Phox2b27Ala mutation targeted to RTN</td>
<td>8</td>
<td>60 (adults)</td>
</tr>
<tr>
<td>24</td>
<td>Pet-1 KO (70% decrease 5-HT neurons)</td>
<td>3,5,7, and 10</td>
<td>42 (male at 7%)</td>
</tr>
<tr>
<td>45</td>
<td>Acute hyperpolarization of Egr2 neurons</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>47</td>
<td>Saporin RTN (94% decrease Phox2B &quot;TH&quot; neurons)</td>
<td>7</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>+ carotid body denervation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Acute hyperpolarization of Egr2-Pet1 serotonin neuron subtype</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>32</td>
<td>Deletion of the proton-activated receptor GPCR4 in RTN</td>
<td>6 and 8</td>
<td>65</td>
</tr>
<tr>
<td>Present</td>
<td>MeCP2 depletion in astrocytes</td>
<td>1.3 and 5</td>
<td>64, 64 and 56</td>
</tr>
</tbody>
</table>

RTN, retrotrapezoid nucleus; 5-HT, serotonin; KO, knockout; MeCP2, methyl-CpG-binding protein 2.
(47), all these studies were conducted in animals with intact chemoreception in the carotid bodies. When elimination of the majority of Phox2b-expressing neurons in RTN was combined with carotid body nerve transection, the magnitude of depression of the CO2 response exceeded that seen with loss of RTN Phox2b-expressing neurons alone (47), indicating a contribution from the peripheral chemoreceptors. Studies that used allanostatin to inhibit RTN neurons were conducted with carotid and aortic nerves transected in anesthetized rats and intact in conscious animals. The depression in CO2 response was greater in the latter (36). Selective depletion of MeCP2 in neurons resulted in a lesser depression of the CO2 response than that seen with the loss of MeCP2 in astrocytes, despite the extent of MeCP2 excision being greater. The two strategies, however, are not identical. Neuronal MeCP2 depletion occurred in embryos while that in astrocytes was induced in adult mice. Thus, compensatory mechanisms may have developed in the MeCP2 \(^{\text{PHOX2B27Ala}}\) mice. Indeed mice with the PHOX2B \(^{\text{PHOX2B27Ala}}\) mutation targeted to the RTN (42) showed no ventilatory response to 8% CO2 at postnatal days 2–9, but as adults it was 40% of that in the control animals.

There are a number of mechanisms that could be proposed for the role of astroglial dysfunction in reducing the HCVR of MeCP2-deficient mice. Using slice preparations, Wenker et al. (55) showed that ~20% of RTN astrocytes are chemosensitive. Chemosensitivity was associated with a decrease in K\(^+\) current in response to CO2. This current was blocked by barium and desipramine, consistent with the involvement of Kir4.1 and Kir5.1 channels. There are, however, conflicting data with respect to the expression of Kir4.1 in MeCP2-deficient mice as well as its relative levels of expression in neurons compared with astrocytes. Western blots of tissue from locus coeruleus in null males showed overexpression of the Kir4.1 channel (58). In contrast, immunohistochemistry studies reported that Kir4.1 expression is lower in cortical astrocytes of MeCP2-depleted mice (10). Kir4.1 expression in astrocytes genetically labeled with eGFP was confined predominantly to this cell type (41). In addition, conditional depletion of Kir4.1 appears to be associated with depressed CO2 chemosensitivity (22). Chemosensitivity of Kir4.1 at physiological CO2/pH is only present when the channel is a heteromere with Kir5.1. Mice with loss of Kir5.1 have a depressed HCVR (51). Taken together, these reports suggest a probable mechanism involving altered Kir4.1 expression in astrocytes that underlies the effect of MeCP2 deficiency on HCVR. Activation of glial sodium/bicarbonate cotransporters leading to depolarization has also been proposed as one putative mechanism underlying their CO2 chemosensitivity (39). In addition, studies in the nucleus of the solitary tract have proposed that extracellular acidification regulates synaptic transmission by compromising glial glutamate uptake (29).

Opening of connexin 26 hemichannels in response to CO2 is also an important mechanism for the release of ATP (28, 37). The connexins (Cx43, Cx40, Cx45, Cx32) have been found to be expressed at normal levels in MeCP2-deficient mice (57). Connexin 26, however, was not investigated in this study.

In addition to their signaling functions, astrocytes also provide metabolic support to neurons by producing and supplying them with a readily available source of energy in the form of lactate. It has recently been shown that basal lactate metabolism and facilitation of lactate release in response to hypoxia are intact in Mecep2 null mice (52). In addition, oxygen consumption in male mice lacking MeCP2 is not different from that in WT mice (16). Imaging of calcium signaling in ventral medullary astrocytes, however, revealed that astroglial responses to CO2 are markedly depressed (52). Importantly, the response of these astrocytes to ATP is the same as that seen in WT mice. Thus the defect in RTT mouse astrocytes resides in their inability to sense physiological increases in CO2, whereas the downstream signaling pathways activated by released ATP appear to be intact (52).

The depressed respiratory response with the depletion of MeCP2 both in astrocytes and in neurons was entirely due to the inability of experimental animals to increase V\(_T\) in response to the increases in the level of inspired CO2. The respiratory frequency response was not affected (Fig. 2, B and C, and Fig. 3, C and D). This response pattern is similar to that observed in global MeCP2 null male mice where V\(_T\) increased only 0, 9, and 22% at 1, 3, and 5%, respectively, compared with 9, 16, and 22% increases in frequency (50), and resembles what has been seen when RTN signaling was experimentally manipulated as opposed to that in medullary raphé nuclei. For example, unilateral lesioning of RTN with ibotenic acid depressed V\(_T\) with little change in frequency (2). Similarly, muscimol application in the RTN only affected V\(_T\) (34). Selective inhibition of Phox2b-expressing RTN neurons also depressed the response to CO2 primarily through an effect on V\(_T\) (36). Bilateral RTN administration of saporin conjugated to substance P depressed HCVR primarily through an effect on V\(_T\) (47). When RTN lesions were combined with carotid sinus denervation, frequency was also affected, albeit to a lesser extent than V\(_T\). There are, however, exceptions to this pattern. When the toxic PHOX2B \(^{\text{PHOX2B27Ala}}\) mutation was targeted to the RTN, adult mice had a markedly depressed response to CO2 mostly due to a reduction of frequency (42). At P2 and P9, however, when these animals were unresponsive to CO2, the defect was from a lack of V\(_T\) response. In addition, when these RTN-Phox2b neurons were transfected with channel rhodopsin 1, photoactivation produced a larger increase in frequency than in V\(_T\) (1). Recently, it has been shown that deletion of the proton-activated receptor GPCR4 in mice depressed the HCVR by ~65%. GPCR4 is localized primarily to the RTN. The depressed HCVR to 6 and 8% CO2 was primarily due to a failure in respiratory frequency (32).

In contrast, genetic or pharmacological silencing and/or depletion of 5-HT neurons in the medullary raphé appears to primarily affect the respiratory frequency responses to CO2. Pet-1 null male mice that were ~70% depleted in central 5-HT neurons displayed a significantly depressed HCVR confined to reduced frequency responses (24). Lower respiratory frequency also accounted for the depressed HCVR in Lmx1b \(^{-/-}\) mice that lack 5-HT neurons (25). However, when the 5-HT 1a receptor agonist 7-(Dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol was given in medullary raphé, V\(_T\) response was also affected (49). A pharmacogenetic approach that allowed acute and reversible silencing of 5-HT neurons markedly reduced the HCVR (44). Both V\(_T\) and frequency responses were impaired with V\(_T\) effect being greater. Recently, this strategy for acute reversible silencing of 5-HT neurons has been refined so that separate subtypes can be targeted (7). Only the Egr2-Pet1 subtype of 5-HT neurons when inhibited resulted in a reduced respiratory CO2 response. This subtype of 5-HT
neurons is mainly concentrated in RM, which prompted us to focus on this area for immunohistochemistry. Interestingly, depression of the CO₂ response associated with silencing of the Erg2-Pet1 subtype of neurons primarily affected Vr responses.

The depressed HCVR in RTT may be an important contributor to the disordered respiration in this condition that is characterized by periodic breathing and frequent apneas (31, 43). Congenital central hypoventilational syndrome that also has a depressed chemosensitivity shows apnea in patients (54) and when the defect is generated in mice (13). In addition, apnea was a frequent occurrence in the recent report of mice with depressed chemosensitivity due to deleted GPCR4 (32). Augmented CO₂ has been shown to decrease apnea in mouse models of RTT (4) (30) and in a case report of a patient (46).

Chemosensitivity of Phox2b-expressing neurons in the RTN has been proposed to include two distinct mechanisms: 1) direct chemosensory responses of neurons and 2) release of ATP from astrocytes in the brain that turn activate neurons (15, 20, 21, 39, 56). At 1% CO₂, depletion of MeCP2 has a similar effect when removed from astrocytes or neurons. Thus both are contributing to the increase in minute ventilation. At 3 and 5% CO₂, however, astrocyte MeCP2 depletion has a greater effect, and the response in Mecp2\textsuperscript{JFlox\textsuperscript{\textast}}-SynCre mice was not significantly different from that in WT littermates. This suggests that, at higher levels of CO₂, astrocytes are the dominant contributors to the rise in minute ventilation. The predominant role of astrocytes in the HCVR is also supported by the observation that the slope of relative increase in minute ventilation as a function of added inspiratory CO₂ is depressed with selective removal of MeCP2. In contrast, the removal of MeCP2 confined to neurons did not affect the slope in the HCVR.

In summary, selective deletion of MeCP2 in astrocytes and neurons has allowed an estimate of their relative contributions to HCVR. These studies suggest that astrocytes play a major role in the mechanisms of respiratory CO₂ chemosensitivity, which link increases in brain parenchymal PCO₂/[H\textsuperscript{+}] and a modified pattern of breathing.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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