Time-dependent modulation of GABA<sub>\text{A}</sub>-ergic synaptic transmission by allopregnanolone in locus coeruleus neurons of MeCP2-null mice

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Rett syndrome (RTT) is a neurodevelopmental disorder with symptoms starting 6–18 mo after birth, while what underlies the delayed onset is unclear. Allopregnanolone (Allop) is a metabolite of progesterone and a potent modulator of GABA<sub>\text{A}</sub>-ergic currents whose defects are seen in RTT. Allop changes its concentration during the perinatal period, which may affect central neurons via the GABA<sub>\text{A}</sub>-ergic synaptic transmission, contributing to the onset of the disease. To determine whether MeCP2 disruption affects Allop modulation, we performed studies in brain slices obtained from wild-type (WT) and MeCP2<sup>-/-</sup> mice. Allop dose dependently suppressed locus coeruleus (LC) neuronal excitability in WT mice, while MeCP2-null neurons showed significant defects. Using optogenetic approaches, channelrhodopsin was specifically expressed in GABA-ergic modulator of GABAA-ergic currents whose defects are seen in RTT. In our previous study, we have also found a significant defect in GABAA-ergic transmission of locus coeruleus (LC) neurons in MeCP2<sup>-/-</sup> mice at an early developmental age (27), which may contribute to the electrophysiological abnormalities of LC neurons (27, 54). The GABA<sub>\text{A}</sub> receptors are ligand-gated ion channels. In addition to the natural ligand GABA, the receptors are modulated by neuroactive steroids such as allopregnanolone (Allop). Allop derived from progesterone by 5α-reductase and 3α-hydroxysteroid dehydrogenase is known to contribute to neuroprotection in fetal brain (24). The levels of the neurosteroid change during perinatal periods (7, 10, 17, 19). Allop levels drop immediately after birth, continue to decline until a temporary elevation at 10 to 14 days postnatal in rats, then decrease to a steady low level at ~3 wk of postnatal periods (20), a time period when RTT-like symptoms start in mice. The coincidence suggests that Allop may play a role in the onset of RTT symptoms.

In MeCP2<sup>-/-</sup> mice, electrophysiological defects have been previously found in NE-ergic neurons of the LC, which also receive GABA<sub>\text{A}</sub>-ergic inhibitory inputs (4, 27, 45, 47, 54). The defects are accompanied by several autonomic dysfunctions, including cardiac regulation, arousal status, and breathing activity (8, 21, 49, 55). We have recently found that the MeCP2 knockout causes significant disruption in GABA<sub>\text{A}</sub>-ergic currents in LC neurons (27). Hence, it is possible that the modulation of GABA<sub>\text{A}</sub>-ergic synaptic transmission by neurosteroids may be affected by MeCP2 disruption, contributing to the delayed onset of RTT-related disorders. To address this possibility, we studied the effects of Allop on GABA<sub>\text{A}</sub>-ergic synaptic transmission in wild-type (WT) and MeCP2<sup>-/-</sup> mice during the first 3 wk of postnatal life.

METHODS

Animals. Female heterozygous MeCP2<sup>tm1.1Bird</sup> mice on the C57BL/6 background were purchased from Jackson Laboratory (Bar Harbor, ME) with the genotype MeCP2<sup>+/−</sup>. The MeCP2<sup>-/-</sup> male mice were produced by cross-breeding the MeCP2<sup>−/−</sup> females with WT C57BL/6 males. To confirm the absence of the MeCP2 gene, offspring were routinely genotyped with a polymerase chain reaction (PCR) protocol provided by the Jackson Laboratory. The MeCP2<sup>-/-</sup> males (7 to 25 days of age) were used in the present study as a mouse model of RTT, while their male littermates served as the WT control. Experiments were conducted in six groups: 1-wk-old (7–9 days) for WT and MeCP2<sup>-/-</sup>, 2-wk-old (14–15 days) for WT and MeCP2<sup>-/-</sup> mice.
days) for WT and MeCP2−/−, and 3-wk-old (21–25 days) for WT and MeCP2−/−, respectively. Only male F1-generation mice were used in the present study. All animals were used for experiments before weaning. To optogenetically stimulate GABA-ergic neurons, channelrhodopsin (ChR2) with enhanced yellow fluorescent protein (EYFP) was expressed specifically in GABA-ergic neurons by cross-breeding the glutamic acid decarboxylase (GAD)-IRESCre strain mice, Gad2tm2(CAG-COP4-H134R/EYFP)H12/J (SN 010802), with ChR2-EYFP-Loxp strain mice, B6;129S-Gt(Rosa)26Soim12(CAG-COP4-H134R/EYFP)H2/J (SN 12569) (Jackson Laboratory). All animal experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Georgia State University Institutional Animal Care and Use Committee.

Brain slice preparation. Brain slices were prepared as described previously (27, 54). In brief, all animals (7–25 days old) were decapitated after deep anesthesia with inhalation of saturated isoflurane. The brain stem was obtained rapidly and placed in ice-cold, sucrose-rich artificial cerebrospinal fluid (sucrose aCSF) containing (in mM) 200 sucrose, 3 KCl, 2 CaCl2, 2 MgCl2, 26 NaHCO3, 1.3 Na2HPO4, and 10 d-glucose. The solution was bubbled with 95% O2 and 5% CO2 (pH 7.40). Transverse pontine sections (300 μm) containing the LC area were obtained using a vibratome sectioning system. The slices were transferred to submerged recording bath chambers (volume, 170 mm3; Warner Instruments) and maintained at 32–35°C.

Identification of LC neurons. The LC neurons were identified by 1) location in the LC area, 2) morphological characteristics, and 3) electrophysiological properties, including the less negative membrane potential (Vm), spontaneous firing, delayed excitation following a hyperpolarizing pulse, weak spike frequency adaptation, and lack of postinhibition rebound (27). Additionally, our previous studies have shown that all these LC neurons express tyrosine hydroxylase in postinhibition rebound (27). Additionally, our previous studies have shown that all these LC neurons express tyrosine hydroxylase in postinhibition rebound (27). Additionally, our previous studies have shown that all these LC neurons express tyrosine hydroxylase in postinhibition rebound (27). Additionally, our previous studies have shown that all these LC neurons express tyrosine hydroxylase in postinhibition rebound (27).

Optogenetic stimulation of GABA-ergic neurons. The optical stimulators were performed by using a xenon arc lamp with a high-speed switcher (Lambda GD-4, Sutter Instruments). The light source was connected to the incident-light illuminator port of the microscope. The light passed through a 470-nm band-pass filter and provided ∼20 mW/mm2 light intensity. The 10-ms pulse trains were generated with a Digitimer D4030 pulse generator. The light intensity was estimated with a MRDS50 photodiode (Motorola).

Electrophysiology. Whole cell patch clamp was performed in brain slices with cell visualization using a Zeiss Axioskop 2 microscope and a near-infrared charge-coupled device (CCD) camera. Patch pipettes were pulled with a Sutter pipette puller (model P-97, Novato, CA). The pipette resistance was 3–5 MΩ. The internal (pipette) solution for current-clamp recording contained (in mM) 130 K gluconate, 10 KCl, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP, and 0.4 EGTA (pH 7.3). The internal pipette solution for voltage clamp contained (in mM) 135 CsCl, 2 MgCl2, 2 Mg-ATP, 1 Na-GTP, and 10 HEPES (pH 7.30). The aCSF solution was applied to the bath, which contained (in mM) 124 NaCl, 3 KCl, 1.3 Na2HPO4, 2 MgCl2, 10 d-glucose, 26 NaHCO3, and 2 CaCl2 (pH 7.4). The slices were continuously perfused with the external solution with superfusion of 95% O2 and 5% CO2 at 33°C. In voltage-clamp recording, the GABA(A) receptor-mediated, light-evoked, and spontaneous inhibitory postsynaptic currents (sIPSCs) were pharmacologically isolated by addition of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), the N-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonoventanoic acid (DL-APV, 10 μM), and the glycine receptor antagonist strychnine (1 μM) to the external solution. All sIPSC recordings were performed at a holding potential of −70 mV. When sIPSCs were tested, the control solution was perfused for at least 5 min before the 10-min allopregnanolone perfusion. Recordings signals were amplified with an Axopatch 200B amplifier (Molecular Devices, Union City, CA), digitized at 10 kHz, filtered at 2 kHz, and collected with Clampex 8.2 data acquisition software (Molecular Devices). The temperature was maintained by a dual automatic temperature controller (Warner Instruments). Only neurons with a stable resting membrane potential (Vm) more negative than −40 mV and an action potential with amplitude >65 mV were used in the studies. These cells were usually recorded for >45 min, a time period that was adequate for our experimental protocol. During the whole recording,
access resistance was monitored. Only recordings with stable resistance values (<20% changes) were used for data analysis.

The electrophysiological data were analyzed with Clampfit 10.3 software (Molecular Devices) and Mini Analysis Program 6.07 software (Synaptosoft). Data are presented as means ± SE. Statistical analysis of other parameters was performed using ANOVA, two-tailed Student’s t-test, or the Mann-Whitney test. Differences were considered significant when \( P \leq 0.05 \).

RESULTS

Inhibition of LC neurons by Allop in WT and Mecp2\(^{-/}\)\(^\gamma\) mice. We first tested the effects of two concentrations of Allop on the firing activity of LC neurons in 3-wk-old mice. In WT mice, the LC neurons showed steady spontaneous firing activity (Fig. 1A). The firing rate, membrane potential \( (V_m) \), and input resistance \( (R_m) \) averaged 4.2 ± 0.5 Hz \((n = 6)\), −45 ± 0.4 mV \((n = 6)\), and 465 ± 21 MΩ \((n = 6)\), respectively, in WT cells. Application of 500 and 1,000 nM Allop to the perfusion solution produced dose-dependent inhibition of the activity of the LC neurons. The inhibition of LC neuron firing activity by Allop was washed out slowly after 40 min of control solution (aCSF) perfusion (Fig. 1A). The firing rate of the LC neurons was inhibited by 32 ± 4.2% with 500 nM Allop \((P < 0.01, n = 6)\) and 56 ± 10% with 1,000 nM Allop \((P < 0.001, n = 6)\) (Fig. 2A). In the current-voltage relation:

![Graphical representation of data](attachment:image.png)
ship, $R_m$ dropped significantly when the cell was exposed to Allop (Fig. 1C). In comparison with basal levels, $R_m$ was reduced by $19 \pm 2.1\%$ with 500 nM Allop ($P < 0.01, n = 6$) and $31 \pm 5.6\%$ with 1,000 nM Allop ($P < 0.001, n = 6$) (Fig. 2B); the membrane potential hyperpolarized to $-46 \pm 0.7$ with 500 nM ($n = 6$) and $-48 \pm 0.2$ with 1,000 nM ($P < 0.01, n = 6$) from $-45 \pm 0.5$ mV (Fig. 2C). In the same age group (3 wk), application of 500 nM Allop did not have significant inhibition on firing activity of LC neurons in Mecp2−/− mice (Fig. 1B and Fig. 2, D–F). With 1,000 nM Allop exposure, the firing rate of the LC neurons was suppressed by $20.1 \pm 6.1\%$ ($P < 0.05, n = 6$) (Fig. 2D), and $R_m$ dropped by $13.4 \pm 3.2\%$ ($P < 0.05, n = 6$) (Fig. 2E). In comparison with WT mice, LC neurons in Mecp2−/− mice showed slightly greater $R_m$ (486 ± 35, $n = 6$) and slightly less negative $V_m$ ($-45 \pm 0.6, n = 6$), although none of these membrane property differences were statistically significant. The inhibitory effects on LC firing rate of two concentrations of Allop were lower in Mecp2-null mice than in WT neurons by $\sim 26\%$ (500 nM, $P < 0.01, n = 6$) and $\sim 36\%$ (1,000 nM, $P < 0.05, n = 6$), respectively (Fig. 2G). Similarly, their effects on $R_m$ was reduced by $\sim 20\%$ (500 nM Allop, $P < 0.01, n = 6$) and $\sim 18\%$ (1,000 nM, $P < 0.01, n = 6$) (Fig. 2H). As shown in Fig. 3, 10 μM bicuculline (BIC), a selective GABA$\text{A}$ receptor inhibitor, completely abolished the inhibitory effects of 1,000 nM Allop on firing rate (Fig. 3C), $R_m$ (Fig. 3D), and $V_m$ (Fig. 3E) of LC neurons in 3-wk-old WT mice.

Enhancement of GABA$\text{A}$-ergic currents by Allop. Because Allop significantly suppressed the LC neuronal activity in current-clamp recording, we asked whether Allop-induced inhibitions were caused by enhanced GABA$\text{A}$-ergic synaptic inputs to LC neurons. By taking advantage of optogenetics, we expressed channelrhodopsin (ChR2) in GABA$\text{A}$-ergic neurons and tested the effects of Allop on light-evoked GABA$\text{A}$-ergic IPSCs in LC neurons in brain slices. In the transgenic mice, GABA$\text{A}$-ergic neurons were identified on the basis of the expression of green fluorescent protein (GFP). GFP labeling revealed that dense GABA$\text{A}$-ergic neurons are located in the ventromedial peri-LC area and rostral ventral medulla, which is consistent with previous studies (4, 15).

Optical stimulation with 470-nm blue light pulses (10 ms) reliably evoked depolarization and action potentials in current clamp (Fig. 4A), the latency of action potential varying from $\sim 8$ to 14 ms after light pulse onset. The blue light pulses also produced rapid inward photocurrents in voltage clamp (Fig. 4B).

Subsequently, inward Cl$^-$ currents were studied in LC neurons at a holding potential of $-70$ mV with symmetric Cl$^-$ concentrations in internal and external solutions in voltage clamp, when ionotropic glutamate receptors and glycine receptors were pharmacologically blocked. Under this condition,
optical stimulation of GABA-ergic neurons with 10-ms light pulses evoked large IPSCs in LC neurons (Fig. 4C), which exhibited an onset latency varying from 10 to 16 ms after onset of light pulses. The averaged amplitude of light-evoked IPSCs was 12.4 ± 2.3 pA (n = 8; Fig. 4F), and the averaged decay time constant (τ) of IPSCs was 31.1 ± 3.0 ms (n = 8; Fig. 4G). Considering the latency between the light-evoked action potentials in GABA-ergic neurons (8 – 14 ms) and the IPSCs (10 – 16 ms) in LC neurons, these IPSCs are likely to be monosynaptic. All IPSCs were completely blocked by 10 μM bicuculline, a selective antagonist of GABAA receptor (Fig. 4D). Perfusion of the cells with 1,000 nM Allop significantly increased the amplitude of the IPSCs (21.2 ± 3.5, n = 8, P < 0.05) and prolonged the decay τ to 93.6 ± 9.9 ms (n = 8, P < 0.01; Fig. 4G).

Defects in Allop-mediated potentiation of GABA<sub>A</sub>-ergic IPSCs in Mecp2<sup>−/−</sup> mice. The effect of Allop on spontaneous GABA<sub>A</sub>-ergic IPSCs (sIPSCs) was studied in LC neurons from WT and Mecp2<sup>−/−</sup> mice at 3 wk of age. In voltage clamp at a holding potential of −70 mV with symmetric Cl<sup>−</sup> concentrations in internal and external solutions, the GABA<sub>A</sub>-ergic sIPSCs were isolated by pharmacological blocking of glutamate and glycine receptors. Application of 500 and 1,000 nM Allop to the perfusion solution increased both frequency and amplitude of the sIPSCs in WT neurons (Fig. 5A). Analysis of the cumulative fraction showed that Allop caused shifts of the
interevent interval and amplitude of the sIPSCs toward smaller interval range and greater amplitude (Fig. 6, A and B). Consistently, 1,000 nM Allop increased the overall frequency of the sIPSCs by 99.0 ± 30.1% (P < 0.001, n = 8; Fig. 6C), the IPSC amplitude by 49.2 ± 21.7% (P < 0.01, n = 7) in WT neurons, respectively (Fig. 6D). The Allop-induced potentiation of sIPSCs was accompanied by a significant increase in the time constant of the sIPSC decay (Fig. 6E).

LC neurons in MeCP2−/Y mice showed fewer and smaller GABAergic sIPSCs than those in WT mice (Fig. 5B). No significant effects on frequency or amplitude of the sIPSCs were found with 500 nM Allop in MeCP2−/Y mice (Fig. 5). Application of 1,000 nM Allop increased the sIPSC frequency and prolonged the decay τ but failed to affect the amplitude of sIPSCs in LC neurons in MeCP2−/Y mice (Fig. 6, F–J). When the effects of Allop on GABAergic sIPSCs were compared between WT and MeCP2-null neurons, sIPSCs from MeCP2−/Y mice had a much poorer response to Allop (Fig. 7). In response to 1,000 nM Allop, the sIPSC frequency increased by ~100% in WT neurons, but by ~40% in MeCP2-null neurons (Fig. 7A). The modulation of the GABAergic sIPSC amplitude was impaired even more severely in MeCP2-null neurons. Instead of potentiation seen in the WT, MeCP2−/Y mice showed a slight reduction in sIPSC amplitude when the cell was exposed to Allop (Fig. 7B). The trend was the same with 500 nM Allop though to a lesser degree (Fig. 7, A and B).

**Effects in postnatal period.** The age dependence of the Allop-mediated modulation of sIPSCs was studied during the postnatal period of 1–3 wk and compared between WT and MeCP2−/Y mice. At 3 wk the frequency of GABAergic sIPSCs was slightly increased in WT mice. Instead, the frequency of sIPSCs of LC neurons in 3-wk-old MeCP2−/Y decreased significantly (3-wk-old: 0.38 ± 0.05 Hz, n = 9 vs. 2-wk-old: 0.62 ± 0.25 Hz, n = 9, P < 0.05). The amplitude of sIPSCs showed a slight increase during 3 wk of development in WT mice. In contrast, there was a significant decrease in the amplitude of sIPSCs in 3-wk-old MeCP2−/Y mice (3-wk-old: 9.7 ± 1.4 pA, n = 9 vs. 2-wk-old: 11.3 ± 1.1 pA, n = 9, P < 0.05). At 3 wk of age, the frequency (WT: 0.85 ± 0.18 Hz, n = 10 vs. MeCP2−/Y: 0.38 ± 0.05 Hz, n = 9, P < 0.01) (Fig. 8A) and amplitude (WT: 13.0 ± 0.9 pA, n = 10 vs. MeCP2−/Y: 9.7 ± 1.4 pA, n = 9, P < 0.01) (Fig. 8B) of sIPSCs in MeCP2−/Y mice were significantly lower and smaller than those in WT mice. The decay time constant of sIPSCs was not affected by age in both WT and MeCP2−/Y mice (Fig. 8C).

During the first 2 wk of development, the effects of two concentrations of Allop on sIPSCs did not show significant difference between WT mice and MeCP2−/Y mice. At 3 wk of age, the potentiation of sIPSCs by Allop was significantly greater in WT mice than in MeCP2−/Y mice. The percentage controls in frequency and amplitude with 500 nM Allop were 138 ± 8% (n = 9) and 113 ± 4% (n = 9) in
3-wk-old WT mice and 114 ± 7% (P < 0.05, n = 8) and 88 ± 4% (P < 0.05, n = 8) in 3-wk-old Mecp2-null mice; with 1,000 nM Allop, 200 ± 30% and 149 ± 22% in 3-wk-old WT mice (n = 9) and 138 ± 10% (P < 0.05, n = 8) and 91 ± 5% (P < 0.05, n = 8) in 3-wk-old Mecp2-null mice (Fig. 9).

DISCUSSION

In the present study, we found evidence for the time-dependent modulation of GABA<sub>A</sub>-ergic synaptic transmission by Allop in LC neurons of Mecp2-null mice. Like the WT neurons, Allop augments GABA<sub>A</sub>-ergic IPSCs and suppresses LC neuronal excitability in Mecp2-null mice during the first 2 wk of the postnatal period. Such an effect deteriorates abruptly at 3 wk when RTT-like symptoms have fully developed in the Mecp2<sup>−/−</sup> mice. Concerning the onset time of RTT-like symptoms in the mice and the changes in Allop concentrations during early postnatal life, these findings suggest that the alteration in the Allop modulation by the Mecp2 disruption may contribute to the delayed onset of RTT.

NE-ergic neurons in the LC project to the forebrain, spinal cord, and other brainstem nuclei and play an important role in cognitive processes, motor function, cardiorespiratory regulation, arousal status, and stress response (12, 16, 46, 48). The defects in the NE-ergic system contribute in a major way to autonomic disorders in RTT, including breathing irregularities (49, 55). In our previous studies, we have observed defects in GABA<sub>A</sub>-ergic transmission of LC neurons in Mecp2-null mice (27). Consistently, the irregular breathing pattern of Mecp2<sup>−/−</sup> mice responds to benzodiazepine treatment (50). In addition to LC neurons, Chao et al. (9) have shown that the disruption in forebrain GABA<sub>A</sub>-ergic neurons also leads to RTT phenotypes. All these lines of evidence indicate that the change in the function of the GABA<sub>A</sub>-ergic system during development may play a crucial role in the development of RTT.

Allop, a major metabolite of progesterone, can be synthesized de novo in the brain and has been considered as the most potent endogenous modulator of the GABA<sub>A</sub>-ergic system through interaction with GABA<sub>A</sub> receptors (26, 42). Using an optogenetic approach to specifically stimulate GABA-ergic neurons, we have found that Allop increases the amplitude and decay time of GABA<sub>A</sub>-ergic IPSCs. Consistent with the observation, Allop augments both frequency and amplitude of GABA<sub>A</sub>-ergic sIPSCs. These results thus show for the first time that Allop enhances GABA<sub>A</sub>-ergic synaptic transmission in LC neurons. These results are consistent with previous

**Fig. 6.** Summary of Allop effects on sIPSCs of LC neurons in WT and Mecp2<sup>−/−</sup> mice. A and B: in WT mice, 500 and 1,000 nM Allop shifted the cumulative fraction curves of sIPSCs to a higher frequency (A) and larger amplitude (B). C and D: consistently, two concentrations of Allop significantly increased the averaged frequency (C) and amplitude (D), accompanied by a significant increase in decay τ (E). F and G: in Mecp2<sup>−/−</sup> neurons, Allop shifted the cumulative fraction of inter-event interval toward the high-frequency range (F) without changing the fraction of the amplitude (G). H: no significant effect was seen with 500 nM Allop, while 1,000 nM Allop had a significant effect on averaged frequency of sIPSCs. I: two concentrations of Allop did not affect the averaged amplitude. J: Allop also significantly prolonged the decay τ in Mecp2<sup>−/−</sup> mice. Cumulative distribution and bar graphs are derived from all of the events detected in the final 5 min under control or Allop conditions. Bar graphs are presented as means ± SE; n = 8 cells for both the WT and Mecp2<sup>−/−</sup> groups (one-way ANOVA).

**Fig. 7.** Comparison of the effects of Allop on sIPSCs of LC neurons between WT and Mecp2<sup>−/−</sup> mice. Both enhancements of frequency (A) and amplitude (B) by two concentrations of Allop were significantly reduced in Mecp2<sup>−/−</sup> mice. Data are presented as means ± SE (two-way ANOVA followed by Bonferroni post hoc tests); n = 6 cells for both the WT and Mecp2<sup>−/−</sup> groups.
studies showing that Allop stimulates GABA-ergic currents in other brain regions, including the hippocampus (33, 38), cerebellum (11), and neocortex (40). The GABA\textsubscript{A}-ergic system provides the main inhibitory inputs to LC neurons (4, 45, 47), regulating their firing activity and cellular responses to presynaptic inputs (3, 47). In whole cell recordings, we indeed have observed a significant inhibition in firing activity of LC neurons when the cells were exposed to Allop. All these findings indicate that GABA\textsubscript{A}-ergic synaptic transmission can be manipulated by the neurosteroid.

The GABA\textsubscript{A}-ergic synaptic transmission is defective in LC neurons of Mecp2\textsuperscript{2\textsuperscript{-/-}} mice. Our results suggest that these defects do not appear in the first 2 wk of postnatal age. The GABA\textsubscript{A}-ergic synaptic transmission deteriorates abruptly at 3 wk when RTT-like symptoms are clear in Mecp2\textsuperscript{2\textsuperscript{-/-}} mice. At the same time, the potentiation of GABA\textsubscript{A} receptors by Allop becomes significantly lower in Mecp2-null neurons than in WT. Two potential mechanisms may underscore the abrupt change in Allop modulation of GABA\textsubscript{A} receptors. Progesterone is produced in the ovary, adrenal gland, and placenta. The blood concentrations of progesterone increase drastically during pregnancy, reaching submicromolar levels at term (37). Allop biosynthesis is known to change during postnatal periods (2, 18, 19), which may result from the decline in progesterone concentrations after birth. Therefore, it is possible that the defects in GABA\textsubscript{A}-ergic synaptic transmissions occur when the progesterone concentration drops to a critical level that is inadequate for the potentiation of GABA\textsubscript{A} receptors, leading to insufficient GABA\textsubscript{A}-ergic inhibition seen in Mecp2-null mice.

Second, the subunit composition of the GABA\textsubscript{A} receptors can change in given neurons during development as well as in certain pathophysiological states (41), which may also alter with Mecp2 knockout and influence the GABA\textsubscript{A} receptor sensitivity to neurosteroids. The GABA\textsubscript{A} receptors are pentamers consisting of 1, 2, 3, 4, and 5 subunits with 1, 2, 3, 4, and 5 the most common type in the brain (22, 23, 36). Belleti et al. (5) have reported that different isoforms of 1-subunits in GABA\textsubscript{A} receptors can cause a change of the neurosteroid effects. The GABA\textsubscript{A} receptors incorporating the 5-subunit produce both negative and positive effects on neurosteroid-mediated potentiation of GABA\textsubscript{A}-ergic synaptic transmission (53, 57). In addition, the 5-subunit that has been found in LC neurons (4, 44) may affect the neuronal sensitivity to Allop. In the HEK cell expression system, Davies et al. (13) indicate that expressing the 5-subunit decreases the GABA\textsubscript{A} receptor poten-

Fig. 8. Developmental changes in GABA-ergic inputs of LC neurons in WT and Mecp2\textsuperscript{2\textsuperscript{-/-}} mice. A: during the first 3 postnatal weeks the frequency of GABA\textsubscript{A}-ergic sIPSCs was increased in WT mice. The frequency of sIPSCs of Mecp2\textsuperscript{2\textsuperscript{-/-}} LC neurons decreased significantly in 3-wk-old Mecp2\textsuperscript{2\textsuperscript{-/-}} mice. B: a slight increase in amplitude was observed during the first 3 wk of development in WT mice. In contrast, there was a significant decrease in the amplitude of sIPSCs in 3-wk-old Mecp2\textsuperscript{2\textsuperscript{-/-}} mice. At 3 wk of age, both frequency and amplitude of sIPSCs in Mecp2\textsuperscript{2\textsuperscript{-/-}} mice were significantly lower and smaller than those in WT mice. C: there was no developmental trend in decay time constant of sIPSCs in both WT and Mecp2\textsuperscript{2\textsuperscript{-/-}} mice. Data are presented as means ± SE (Student’s t-test); 1-wk-old, n = 8 for WT and n = 7 for Mecp2\textsuperscript{2\textsuperscript{-/-}}; 2-wk-old, n = 9 for WT and n = 9 for Mecp2\textsuperscript{2\textsuperscript{-/-}}; 3-wk-old, n = 10 for WT, n = 9 for Mecp2\textsuperscript{2\textsuperscript{-/-}}.

Fig. 9. Comparison of the effects of Allop on sIPSCs of LC neurons between WT and Mecp2\textsuperscript{2\textsuperscript{-/-}} mice during the first 3 wk of postnatal development. A–D: the effects of 500 nM (A and B) and 1,000 nM (C and D) Allop on frequency (A and C) and amplitude (B and D) of sIPSCs did not show any significant difference between WT mice and Mecp2\textsuperscript{2\textsuperscript{-/-}} mice during the first 2 wk of development; at 3 wk, the potentiation of sIPSCs by Allop was significantly increased in WT mice compared with Mecp2\textsuperscript{2\textsuperscript{-/-}} mice. Data are presented as means ± SE (Mann-Whitney test); 1-wk-old, n = 8 for WT and n = 7 for Mecp2\textsuperscript{2\textsuperscript{-/-}}; 2-wk-old, n = 9 for WT and n = 9 for Mecp2\textsuperscript{2\textsuperscript{-/-}}; 3-wk-old, n = 10 for WT, n = 9 for Mecp2\textsuperscript{2\textsuperscript{-/-}}.
neurons may be also attributable to the defects in the Allop.

It is well known that the early postnatal development is a critical period for neurogenesis and synapse formation. Remarkable changes in both expression and function of neurotransmitter receptors in addition to the GABA_A-receptors, including serotonin and glutamate receptors, have been found in rat motoneurons during the first 3 postnatal weeks (29, 32).

The temporal and regional changes in GABA_A receptor subunits during development and pathological conditions have been found in both rats (30, 31, 34) and mice (1, 14, 39). All these alterations of subunit expression contribute to the changes in the GABA_A receptor functions during early postnatal development (14, 34). Therefore, developmental alterations in the expression of GABA_A receptor subunits in LC neurons may be also attributable to the defects in the Allop modulation as well as the deficiency in the GABA_Aergic system. Although the detailed molecular and cellular processes are still unclear, the finding of the defect in Allop modulation in our current studies is encouraging, which as a potential mechanism for the delay onset of RTT symptom warrants further studies to elaborate what GABA_A receptor subunits are indeed affected by the Mecp2 disruption, and when the effects take place in postnatal development.

In conclusion, Allop enhances GABA_Aergic synaptic transmission and inhibits firing activity of LC neurons. Such a synaptic modulation is impaired in Mecp2−/− mice. Strikingly, the impairment manifests itself at 3 wk of postnatal age when the RTT-like symptoms have fully developed in the mice. Although the delayed onset of the RTT symptoms is still not understood, the demonstration of the time-dependent Allop modulation of GABA_Aergic synaptic transmission in Mecp2−/− mice seems to be an important step toward increasing our understanding of RTT pathophysiology.

REFERENCES


25. Hogart A, Nagarajan RP, Patzel KA, Yasui DH, Lasalle JM. 15q11–13 GABA_A receptor genes are normally biallelically expressed in brain yet...


