A selective 5-HT$_{1a}$ receptor agonist improves respiration in a mouse model of Rett syndrome

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Levitt ES, Hunnicutt BJ, Knopp SJ, Williams JT, Bissonnette JM. A selective 5-HT$_{1a}$ receptor agonist improves respiration in a mouse model of Rett syndrome. J Appl Physiol 115: 1626–1633, 2013. First published October 3, 2013; doi:10.1152/japplphysiol.00889.2013. Rett syndrome is a neurological disorder caused by loss of function mutations in the gene that encodes the DNA binding protein methyl-CpG-binding protein 2 (Mecp2). A prominent feature of the syndrome is disturbances in respiration characterized by frequent apnea and an irregular interbreath cycle. 8-Hydroxy-2-dipropylaminotetralin has been shown to positively modulate these disturbances (Abdala AP, Duttschmann M, Bissonnette JM, Paton JF, Proc Natl Acad Sci USA 107: 18208–18213, 2010), but the mode of action is not understood. Here we show that the selective 5-HT$_{1a}$ biased agonist 3-chloro-4-fluorophenyl-(4-fluoro-4-[(5-methylpyrimidin-2-ylmethyl)-amino]-methyl)-piperidin-1-yl)-methanone (F15599) decreases apnea and corrects irregularity in both heterozygous Mecp2-deficient female and in Mecp2 null male mice. In whole cell voltage-clamp recordings from dorsal raphe neurons, F15599 potently induced an outward current, which was blocked by barium, reversed at the potassium equilibrium potential, and was antagonized by the 5-HT$_{1a}$ antagonist WAY100135. This is consistent with somatodendritic 5-HT$_{1a}$ receptor-mediated activation of G protein-coupled inwardly rectifying potassium channels (GIRK). In contrast, F15599 did not activate 5-HT$_{1b/d}$ receptors that mediate inhibition of glutamate release from terminals in the nucleus accumbens by a presynaptic mechanism. Thus F15599 activated somatodendritic 5-HT$_{1a}$ autoreceptors, but not axonal 5-HT$_{1b/d}$ receptors. In unanesthetized Mecp2-deficient heterozygous female mice, F15599 reduced apnea in a dose-dependent manner with maximal effect of 74.5% at 0.25 mg/kg and improved breath irregularity. Similarly, in Mecp2 null male mice, apnea was reduced by 62 ± 6% at 0.25 mg/kg, and breathing became regular. The results indicate respiration is improved with a 5-HT$_{1a}$ agonist that activates GIRK channels without affecting neurotransmitter release.

apnea; Rett syndrome; serotonin

RETT SYNDROME (RTT) is an autism spectrum disorder caused by loss of function mutations in the X-linked gene that encodes the transcription factor methyl-CpG-binding protein 2 (MeCP2) (2). Respiratory disturbances characterized by frequent apnea and an irregular breath cycle are common and disturbing features of the syndrome (31, 35). This respiratory pattern is faithfully mimicked in mouse models of RTT (reviewed in Refs. 16, 18, 28). Previous work has shown that irregular and prolonged activity in postinspiratory (post-I) nerves is a major factor of respiratory disturbances in mouse models of RTT (1, 32). Building on earlier studies that demonstrated inhibition of expiratory neurons with serotonin 5-HT$_{1a}$ agonists (17, 29), Abdala and coauthors (1) showed that 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) decreased the incidence of apnea and corrected irregular breathing in mouse models of RTT. It was proposed that 8-OH-DPAT acted at 5-HT$_{1a}$ somatodendritic autoreceptors to stimulate G protein-coupled inward-rectifying potassium channels (GIRKs), and that the resulting hyperpolarization inhibited post-I neurons. 8-OH-DPAT, however, also has presynaptic effects (reviewed in Ref. 10), may inhibit serotonin re-uptake (5), and activates 5-HT$_{7}$ receptors (15). To determine whether the effects of 8-OH-DPAT on respiration are due to selective activation of 5-HT$_{1a}$ receptors, we have used an agonist, F15599, that preferentially activates these receptors.

F15599, 3-chloro-4-fluorophenyl-(4-fluoro-4-[(5-methylpyrimidin-2-ylmethyl)-amino]-methyl)-piperidin-1-yl)-methanone, is chemically distinct from 8-OH-DPAT (25). Both in vivo and in vitro studies have shown the selectivity and specificity of F15599. In rat cortex and striatum, F15599 was 1,000-fold selective for 5-HT$_{1a}$ receptors over other 5-HT, dopamine, and adrenoreceptors (25). In addition, the compound did not interact with a wide range of other receptors, ion channels, or monoamine transporters (25). F15599 increased extracellular dopamine concentration, as determined by microdialysis, in rat medial prefrontal cortex. The dose of F15599 required to increase dopamine release was eightfold lower than that required to decrease 5-HT in ventral hippocampus, an effect thought to be dependent on 5-HT$_{1a}$ autoreceptors (19). In addition, low doses of F15599 stimulated pyramidal neuron activity in the medial prefrontal cortex, whereas much higher doses were necessary to inhibit electrical activity of serotoninergic neurons in the dorsal raphe. The interpretation of these results was that low concentrations of F15599 act selectively on 5-HT$_{1a}$ heteroreceptors (19).

Somatodendritic 5-HT$_{1a}$ autoreceptors on dorsal raphe neurons increase a potassium conductance. Using brain slice electrophysiology and in vivo plethysmography, F15599 was found to potently activate somatodendritic 5-HT$_{1a}$ autoreceptors and to correct the respiratory abnormalities in MeCP2-deficient mice.

METHODS

The experiments 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. Plethysmography studies were conducted with B6.129P2(C)-MeCp2tm1Bird (stock no. 003890, Jackson Laboratory, Bar Harbor, ME) mice. Heterozygous Mecp2-deficient female mice (6.1–17.4 mo old, n = 7), wild-type C57Bl6J female mice (6.0 mo old, n = 6), and Mecp2 null male mice.
mice (postnatal days 43–72, n = 12) were used. Electrophysiology experiments used adult (2–4 mo) male and female C57BL/6J wild-type mice.

**Electrophysiology.** Acute brain slices containing dorsal raphe or nucleus accumbens were prepared as described previously (23). Briefly, adult (2–4 mo) male and female C57Bl/6J wild-type mice were killed, and the brain was removed, blocked, and mounted in a vibratome chamber (Leica VT 1200S). Coronal slices (230–240 μm) were prepared in ice-cold artificial cerebrospinal fluid (ACSF) containing the following (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 2.6 CaCl₂, 1.2 NaH₂PO₄, 11 t-glucose, and 21.4 NaHCO₃ (equilibrated with 95% O₂/5% CO₂). Slices were stored at 34°C in glass vials with oxygenated (95% O₂/5% CO₂) ACSF. MK801 (10 μM; Tocris, Ellisville, MO) was included in the cutting and initial incubation solution to block N-methyl-D-aspartate receptors. After an incubation period of at least 30 min, slices were transferred to the recording chamber, which was perfused with 34°C ACSF at a rate of 1.5–3 ml/min, and cells were visualized with an upright microscope with infrared illumination.

Dorsal raphe neurons were identified on the midline and ventral to the aqueduct in slices that were rostral to the level where the aqueduct begins to meet the fourth ventricle and caudal to the decussation of the cerebellar peduncle. Whole cell recordings were made from dorsal raphe neurons with an Axopatch 1D amplifier in current or voltage-clamp mode (holding potential = −60 mV). Recording pipettes (1.7–2.1 MΩ) were filled with internal solution containing the following (in mM): 115 potassium gluconate, 20 KCl, 1.5 MgCl₂, 10 HEPES (K), 1 BaPTA, 10 Na₂-phosphocreatine, 2 Mg-ATP, 0.2 Na-GTP, pH 7.4, 275–280 mosM. Series resistance was monitored without compensation and remained <15 MΩ for inclusion. Serotonin neurons were initially identified by midline location and large cell body (13). Recorded neurons had the following electrophysiological properties: membrane capacitance = 30 ± 1 pF; input resistance = 396 ± 28 MΩ; resting membrane potential = −64 ± 2.6 mV. In current-clamp mode, neurons were not spontaneously active, but injection of current (50–250 pA, 2 s) produced slow, regular firing of long-duration action potentials (action potential half-width = 1.20 ± 0.03) that increased in frequency with larger current injections (50 pA = 5.5 Hz; 100 pA = 11.1 Hz; 150 pA = 17.4 Hz; 200 pA = 21.7 Hz; 250 pA = 25.6 Hz). These properties are consistent with previously reported properties of non-GABAergic, tryptophan hydroxylase positive, medial dorsal raphe neurons from mice (13). For current-voltage analysis, voltage steps (50 to 130 mV, 200 ms) were applied as the compound was washed from the slice (Fig. 1A). A series of voltage steps (50 to 130 mV, 200 ms) were applied as the compound was washed from the slice (Fig. 1A). A series of voltage steps (50 to 130 mV, 200 ms) were applied as the compound was washed from the slice (Fig. 1A). A series of voltage steps (50 to 130 mV, 200 ms) were applied as the compound was washed from the slice (Fig. 1A). A series of voltage steps (50 to 130 mV, 200 ms) were applied as the compound was washed from the slice (Fig. 1A). A series of voltage steps (50 to 130 mV, 200 ms) were applied as the compound was washed from the slice (Fig. 1A).

**RESULTS**

**Platystmography studies.** Respiratory frequency, tidal volume, and their product minute ventilation were determined in a body plethysmograph (7, 24). Briefly, individual unanesthetized animals were placed in a 65-ml chamber with their head exposed through a close fitting hole in Parafilm. A pneumotachograph (24) was connected to the chamber, and a differential pressure transducer (model PT5A, Grass Instrument, West Warwick, RI). The pressure signal was integrated to give tidal volume. 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 to disk by computer for later analysis. The studies were begun after the animal was given time to become adjusted to the chamber. The respiratory studies were conducted between 1100 and 1700.

Baseline respiratory pattern was obtained during a 30-min period. The mice then received an intraperitoneal injection of F15599 (0.02–0.25 mg/kg for female mice and 0.02–0.5 mg/kg for males). Twenty minutes following the F15599 injection, the 30-min study was repeated. The concentration of F15599 was 0.02–0.5 mg/ml. Mice were used in a number of plethysmograph studies after receiving F15599. The minimal interval between dosages was 3 days (average = 4.8 ± 0.8 days).

**Open-field studies.** Oral administration of F15599 in rat at high doses (ED₅₀ 7.2 mg/kg) induced flat body posture (4). To rule out effects of the drug on locomotion, we performed open-field studies following the dose that was effective in improving respiration. Motor activity studies were carried out at the same time of day (1200–1800), for both F15599 and vehicle administration, which were studied on separate days, and in the same dedicated observation room. Heterozygous Mecp2-deficient mice were placed singly into a standard open-field box for 20 min with side-viewing and top-viewing cameras (Clever Systems, Reston, VA). Mice were recorded for 20 min after an approximate 1-min delay from initial placement in the center of the open field. Activity traces were acquired in real time using StereoScan Software (Clever Systems) and processed on a Dell computer.

**Analysis.** Data are reported as means ± SE. Apnea was defined as total respiratory cycle time (Ttot) ≥ 1.0 s. Irregularity score was calculated from absolute (Ttot − Ttot + 1)/Ttot + 1 and is reported as the variance. The entire 30-min period for baseline or post-F15599 treatment was used for analysis. Effect of F15599 in Mecp2-deficient animals when evenly distributed was determined with one-way repeated-measures ANOVA. Post hoc testing used the Tukey method. Wilcoxon rank-sum test was used for unevenly distributed data. p = 0.05 was taken as significant. Effect of F15599 in C57Bl/6J wild-type female mice at a single dose was determined with paired t-test. Sigma Stat 3.1 was used for statistical evaluation. F15599 concentration-response curves were fit by nonlinear regression with variable slope using GraphPad Prism software.

**RESULTS**

**F15599 activates somatodendritic 5-HT₁₆ autoreceptors on dorsal raphe neurons.** Somatodendritic 5-HT₁₆ autoreceptors on serotonergic neurons in the dorsal raphe activate GIRK channels. The ability of F15599 to activate these 5-HT₁₆ receptors was tested using whole cell voltage-clamp recordings from dorsal raphe neurons in acute mouse brain slices. Perfusion of F15599 (1 μM) produced an outward current that reached steady state in 1–2 min and slowly returned to baseline as the compound was washed from the slice (Fig. 1A). A series of voltage steps (–50 to –130 mV, 200 ms) were applied before and during F15599 (1 μM) perfusion to determine the identity of the F15599-mediated outward current. The current produced by F15599 at each voltage was determined by subtracting the corresponding control (i.e. baseline) current. The F15599-mediated current displayed inward rectification and
reversed at the potassium equilibrium potential, consistent with activation of GIRK channels (Fig. 1A, n = 3).

Electrical stimulation in dorsal raphe evokes endogenous 5-HT release. This 5-HT activates somatodendritic 5-HT$_{1a}$ receptor-mediated G protein-coupled inwardly rectifying potassium channels (GIRK) conductance in dorsal raphe neurons. Whole cell voltage-clamp recordings are from dorsal raphe serotonin neurons in acute mouse brain slice. A: bath perfusion of F15599 (1 μM) caused outward current (I). Voltage steps (−50 to −130 mV, 200 ms) were performed before and during F15599 perfusion. Graph of F15599-mediated current (pA) vs. potential (mV) showed inward rectification and reversal at the predicted potassium equilibrium potential, consistent with activation of GIRK. B: 5-HT-inhibitory postsynaptic currents (IPSCs) were evoked by pairs of electrical stimuli (500 μs, 50 Hz) applied when indicated by arrows (stim). Perfusion of BaCl$_2$ (100 μM) to block GIRK reduced 5-HT-IPSCs and F15599-mediated current. C: summary of the amplitude of 5-HT-IPSCs or F15599 (100 nM)-mediated current in control (C) or 100 μM BaCl$_2$ (Ba). Current amplitude was normalized to the capacitance of the neuron and reported as current density (pA/pF). Each symbol represents an individual data point; the line and error bars are means ± SE. Barium significantly reduced the amplitude of 5-HT-IPSCs (P = 0.007, paired t-test) and F15599 (100 nM)-mediated current (P = 0.0002, unpaired t-test). D: electrically evoked 5-HT-IPSCs were blocked by the 5-HT$_{1a}$ antagonist WAY100135 (1 μM; WAY). Examples are an average of 3 sweeps. n = 7. E: concentration-response curves of F15599-mediated GIRK current in control dorsal raphe slices or in slices that were pretreated with the 5-HT$_{1a}$ antagonist WAY100135 (1 μM) for at least 5 min. Current amplitude was normalized to cell capacitance and reported as pA/pF (means ± SE; n = 3–7 per data point). Data were fit by nonlinear regression with variable slope. EC$_{50}$ in control = 37 nM (95% confidence interval: 21–65 nM); EC$_{50}$ with WAY100135 = 1,400 nM (95% confidence interval: 370–5,500 nM).
Presynaptic 5-HT_{1b/d} receptors on glutamatergic terminals in the nucleus accumbens can inhibit glutamate release (23). The 5-HT_{1b/d} agonist sumatriptan (1 μM) reduced the amplitude of EPSCs by F15599 (1 μM) did not change the amplitude of EPSCs. The 5-HT_{1b/d} agonist sumatriptan (sumat, 1 μM) reduced the amplitude of EPSCs from medium spiny neurons in nucleus accumbens. EPSCs were evoked by electrical stimulation (100 μs). F15599 (1 μM) did not change the amplitude of EPSCs.

Effect of F15599 in Mecp2-null male mice. Before treatment, null male animals had 86.4 ± 9.0 apneas/h of 1.32 ± 0.03 s duration. Respiratory frequency was 262 ± 19 beats/min, and their irregularity score was 0.248 ± 0.028. F15599 had a

Effect of F15599 in Mecp2-deficient heterozygous female mice. Under baseline conditions, Mecp2-deficient heterozygous female mice had 162 ± 25 apneas/h, lasting 1.34 ± 0.04 s. The irregularity score was 0.425 ± 0.057, and respiratory frequency was 167 ± 8.6 breaths/min. Treatment with F15599 reduced the incidence of apnea in a dose-dependent manner. At 0.1 mg/kg, F15599 reduced apnea by 75.4 ± 6.9% from 123 ± 52 to 31 ± 12 apneas/h (P = 0.031 Wilcoxon). The average duration of apnea decreased from 1.33 ± 0.03 to 1.19 ± 0.04 s (P = 0.023 ANOVA with Tukey posttest). The irregularity score was lowered to 0.083 ± 0.028, a value comparable to that in wild-type females (0.027 ± 0.003) (Fig. 3). Respiratory frequency was not significantly affected (baseline, 173 ± 13.9 vs. F15599, 188 ± 9.8 beats/min). A 20-min open-field video recording in heterozygous Mecp2-deficient female mice following the 0.1 mg/kg dose of F15599 showed that, compared with vehicle, there was no effect on total distance covered, velocity, nor time spent in the center square (Fig. 4).

Effect of F15599 in C57BL6/J female mice. Apnea (2 per hour) was seen in one of six wild-type mice. The female mice were studied following the effective F15599 dose seen in the Mecp2-null male mice. F15599 treatment in wild-type females showed an insignificant decline in irregularity from 0.03 s (baseline) to 0.028 s (P = 0.234, paired t-test).

Effect of F15599 in Mecp2 null male mice. Before treatment, null male animals had 86.4 ± 9.0 apneas/h of 1.32 ± 0.03 s duration. Respiratory frequency was 262 ± 19 beats/min, and their irregularity score was 0.248 ± 0.028. F15599 had a

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maximal effect at 0.25 mg/kg ip, with a 61.9 ± 6.6% reduction in the incidence of apnea from 85 to 33 apneas/h ($P = 0.0018$ ANOVA with Tukey posttest) (Fig. 6). There was no significant effect on the duration of apnea, 1.24 ± 0.06 vs. 1.30 ± 0.19 s. The basal frequency in the null males was considerable faster than in the heterozygous Mecp2-deficient females (262 ± 19 vs. 162 ± 25 beats/min), thus by using a single definition of apnea (≥1.0 s), their respective incidence of apnea may not be comparable. With respect to the effect of F15599, however, this is not a factor, because each drug study was preceded by a 30-min control and the drug effect reported as the relative decrease in apnea compared with that control. The null males consisted of two separate populations with respect to frequency: lower frequency, $n = 7$ median 218 beats/min, range 204–253; higher frequency, $n = 5$ median 324 beats/min, range 281–426. At the effective dose of F15599, 0.25 mg/kg, apnea was reduced by 61.2% of control in the low-frequency mice and 62.6% of control in the high-frequency animals. Irregularity score at 0.25 mg/kg F15599 fell from 0.264 ± 0.026 to 0.114 ± 0.031 ($P = 0.0005$ ANOVA with Tukey posttest). Irregularity was also significantly reduced by F15599 at 0.1 mg/kg from 0.229 ± 0.058 to 0.123 ± 0.023 ($P = 0.022$ ANOVA with Tukey posttest). Respiratory frequency was not significantly changed with the 0.25 mg/kg dose: 221 ± 16 to 236 ± 20 beats/min.

**DISCUSSION**

The heterozygous Mecp2-deficient female and null male mice used in these studies were of sufficient maturity to have developed robust respiratory phenotypes characterized by frequent apnea and an irregular interbreath period. The null male mice had baseline higher respiratory frequency, lower incidence of apnea, and lower irregularity than the heterozygous female mice. This less severe respiratory phenotype may be due to the younger age (1.5–2.5 mo) of the males compared with the females (6–17 mo). The selective 5-HT$_1_a$ agonist F15599 positively modulated both apnea and the irregular breath cycle in both females and males.

In addition, C57BL6/J, the background strain for the mutant mice, female animals were treated with F15599 at the dose that was affected in Mecp2$^{-/-}$ mice. Their low baseline incidence of apnea precluded identifying an effect on this parameter. The selective agonist did not significantly affect irregularity or respiratory frequency in wild-type female mice.

It has been reported that male C57BL6/J mice have a very high incidence of spontaneous apnea (33, 34): 85–115/h. Additionally, 8-OH-DPAT at 0.01 mg/kg resulted in a 40% reduction in these apneas (33). We did not observe frequent apnea in the C57BL6/J females presently studied. Our laboratory’s previous studies in this strain of mice (1, 12) also found a low, ∼5/h, occurrence of apnea in both female and male...
C57BL6/J mice, as have others (8). The Stettner et al. studies (33,34) defined apnea as a break in Ttot that lasted more than two cycles. At the respiratory frequency of the mice studied, this would equate to 0.7–0.8 s, somewhat shorter than the 1.0 s used by Derecki and coauthors (8) and ourselves. It is unlikely, however, that the different definitions of apnea fully explain the marked difference in the observed incidence of apnea. Although the mice are of the same strain, they may have been obtained from different suppliers.

F15599 has been identified as a functionally selective biased agonist that preferentially activates 5-HT1a heteroreceptors (25). However, here F15599 potently activated somatodendritic 5-HT1a autoreceptors that are coupled to GIRK on serotonergic dorsal raphe neurons. The F15599-mediated GIRK current was 10-fold more potent than previously reported F15599-in-mediated GIRK current reported here resembles the EC50 of F15599-mediated activation of Galpha3 reported previously (25).

5-HT1a agonists have been shown to correct the respiratory depression caused by opiates (9, 21, 22, 30) and that associated with surgical resection of an astrocytoma situated in the pons and medulla (36). In addition, buspirone, a partial 5-HT1a agonist, has been used either alone (3) or in combination with the selective serotonin-reuptake inhibitor fluoxetine (14) in case reports of RTT patients. Buspirone alone reduced the percent time that oxygen saturation was below 90% from an incidence of 90% to 52% in one subject (3). The combined treatment reduced the number of hyperventilation/apnea episodes from ~70/day to 5/day in a separate individual (14). The present findings indicate that a selective approach to drug design of 5-HT1a agonists that retain GIRK channel activity without other effects may be beneficial for treatment of the respiratory disorders in RTT.

The anatomical localization(s) and cellular mechanisms that underlie the corrective effects of 5-HT agonists are not completely understood. Ultrastructural analysis has demonstrated that 5-HT1a receptors are localized both pre- and postsynaptically (6). Immunohistochemistry has shown that 5-HT1a receptors are abundant in the nucleus of the solitary tract, pre-Bötzinger complex, Bötzinger complex, pontine parabrachial complex, and Kölliker-Fuse nucleus (9). An initial proposal suggested that 8-OH-DPAT acting at 5-HT7 receptors, for which it has an affinity, antagonizes the depressive effects of opiates on cellular cyclic-AMP (29). Direct testing of this hypothesis has failed to add confirmation. Using an in situ rat brain stem preparation (27), Manzke and coauthors (21) found that a 5-HT7 receptor agonist depressed, rather than stimulated, output of the phrenic nerve. Furthermore, a 5-HT7 receptor antagonist did not block 8-OH-DPAT stimulation of phrenic nerve activity. The present results showing that F15599, which does not bind to 5-HT7 receptors, improves respiration in Mecp2-deficient mice also argues against this proposed mechanism.

Recently, attention has been directed to the role of glycine inhibitory neurons as mediators of the 5-HT1a agonist stimulation of respiration. The glycineric antagonist strychnine occluded the stimulatory effects of 8-OH-DPAT on phrenic nerve output (21). In addition, 8-OH-DPAT reverses the respiratory depression caused by fentanyl in wild-type mice, but not in mice that lack the glycine receptor alpha3 subtype (22). A majority of pre-Bötzinger complex neurons are glycineergic and express 5-HT1a receptors (21). 5-HT1a agonist inhibition of post-I and/or late expiratory neurons may explain the modulation of respiratory abnormalities in Mecp2-deficient mice. During apnea, post-I activity, as reflected in recordings from the central vagus nerve, is greatly

Fig. 6. Effect of F15599 on respiration in Mecp2 null males. A: group data (n = 4 for 0.02 mg/kg, n = 5 for 0.5 mg/kg, and n = 6 for 0.1 and 0.25 mg/kg) for various doses for apnea. *P = 0.018 (ANOVA). B: irregularity. *P = 0.0005. **P = 0.022. C: respiratory frequency.
enhanced and prolonged (1, 32). Apnea is also characterized by activity in abdominal nerves indicating late expiratory neuron discharge (1). Ionophoretic application of 8-OH-DPAT to late expiratory neurons resulted in relative hyperpolarization, a decrease in intensity, and shortening of duration of their discharge (17). The potent activation of GIRK current by F15599 is consistent with hyperpolarization of post-I and/or late expiratory neurons as a potential mechanism underlying its attenuation of apnea and irregularity in mouse models of RTT.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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