Serotonin release acts on 5-HT2 receptors in the dorsomedial medulla oblongata to elicit airway dilation in mice

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Summary. Serotonin (5-hydroxytryptamine; 5-HT) excites neurons in the hypoglossal and solitary tract nuclei through 5-hydroxytryptamine 2 (5-HT2) receptors, and contributes to genioglossal muscle activation, hypotension and bradycardia. This study investigated the influence of 5-HT2 receptor-mediated 5-HT action in the hypoglossal and solitary tract nuclei on respiratory variables, particularly airway resistance. Adult male mice were subjected to microdialysis and placed in a double-chamber plethysmograph. 5-HT release and respiratory variables were assessed in response to fluoxetine perfusion or fluoxetine plus LY-53857 coperfusion of the dorsomedial medulla oblongata (DMM), which includes the hypoglossal and solitary tract nuclei. 5-HT release in the DMM was increased but respiratory rate was not affected by fluoxetine perfusion with or without LY-53857. Specific airway resistance was significantly larger with fluoxetine plus LY-53857 coperfusion than at baseline or during perfusion with fluoxetine. Conversely, tidal volume was significantly lower with fluoxetine plus LY-53857-coperfusion than at baseline. These results suggest that 5-HT release in the DMM is regulated by a suppressive effect of local 5-HT transporter activity, which elicits airway dilation and increases tidal volume through local 5-HT2 receptors without affecting respiratory rate.

Key words. Airway resistance, Double-chamber plethysmograph, 5-HT, Hypoglossal nucleus, Microdialysis

1 Introduction

Most patients with obstructive sleep apnea experience upper airway closure in the retropalatal and retroglossal regions. Increased volume of the soft palate, tongue, parapharyngeal fat pads and lateral pharyngeal walls has been demonstrated in these patients (Schwab and Gefter, 2002). During nose breathing, nasal resistance accounts for nearly half of the total airway resistance. During mouth breathing, upper airway resistance (from

the mouth, pharynx, glottis, larynx and upper trachea) accounts for a third of the total airway resistance (Ferris et al., 1964). The upper airway is influenced by many dilator muscles (Kubin and Davies, 2002). Further clarifying the control mechanism of airway resistance is important for elucidating the mechanism underlying obstructive sleep apnea.

Several studies have shown the role of serotonin (5-hydroxytryptamine; 5-HT) in the hypoglossal nucleus (nXII) in the activation of the hypoglossal nerve and genioglossal muscle activity, particularly when mediated by 5-HT2 receptors (Kubin et al., 1992; Jelev et al., 2001; Fenik and Veasey, 2003). 5-HT in the nXII modulates genioglossus activity across natural sleep-wake states (Jelev et al., 2001), suggesting that the basal degree of serotonergic activity is important in studying serotonergic physiology.

In the solitary tract nucleus (nTS), 5-HT evokes hypotension and bradycardia (Laguzzi et al., 1984), mediated by 5-HT2 receptors (Merahi et al., 1992). 5-HT2 receptor activity can both excite and inhibit neurons in the nTS (Sévoz-Couche et al., 2000). The nTS is part of the respiratory network, included in the dorsal respiratory group. In mice, afferent convergences in the nTS from pulmonary C-fibers, cardiac receptors, and peripheral chemoreceptors have been shown (Paton, 1998). These findings suggest that 5-HT neurons in the nTS affect respiration.

In the present study, the effects of 5-HT2 receptor activation in the dorsomedial medulla oblongata (DMM), including the nXII and the nTS, on airway resistance and respiratory variables were investigated under local perfusion with fluoxetine, a selective serotonin reuptake inhibitor (SSRI), with or without LY-53857, a 5-HT2 receptor antagonist. 5-HT release was concomitantly measured to confirm 5-HT transporter activity and enhanced-serotonergic transmission in the DMM.

2 Methods

2.1 General

Experiments were performed on 10 male C57BL/6N mice of 10.7 $\pm$ 0.8 weeks of age weighing 25.4 $\pm$ 0.7 g (mean $\pm$ SEM) (CLEA Japan, Tokyo, Japan). Experiments were approved by the Showa University Animal Experiments Committee. The procedure for surgical implantation of a microdialysis probe in the DMM has been described in detail previously (Kanamaru and Homma, 2007). Briefly, each mouse was anesthetized intraperitoneally with pentobarbital sodium (0.5 mg/0.1 ml saline/10 g body weight). Rectal temperature was kept at 37 °C. The head region was cleaned with an antiseptic, isodine, and locally anesthetized by 2%

xylocaine injection. The medulla oblongata was exposed dorsally. A microdialysis probe (CUP7; membrane length, 1 mm; Carnegie Medicin, Stockholm, Sweden) was ocularly inserted into the medulla oblongata, 0.45 mm lateral, 0.8 mm rostral and 1 mm ventral to the obex. The probe was fixed to the cranial bone with dental cement and the skin incision was closed. Mice were placed in a double-chamber plethysmograph. The microdialysis probe was flushed with artificial cerebrospinal fluid (aCSF; in mM: 121.1 NaCl, 5 KCl, 24 $NaHCO_3$, and 1.5 $CaCl_2$ adjusted to pH 7.4 with 95% O_2 and 5% CO_2) at 5 μl/min for 40 min and equilibrated at 1.2 μl/min for 80 min. During these periods the mice could recover from the anesthesia and acclimatize to the chamber. Dialysate was collected every 25 min into vials containing 10 μl of 0.02 M acetic acid. Respiratory flow curves for the head and body chambers were plotted with pneumotachographs and pressure transducers (TV-241T and TP-602T, Nihon Kohden) at a 10 kHz sampling rate, and analyzed using PowerLab (ADI Instruments, NSW, Australia). Baseline samples were taken to confirm 5-HT release. After the second baseline sample was taken, the aCSF perfusion was changed to 10^{-5} M fluoxetine (Sigma-Aldrich, St. Louis, MO, USA) or 10^{-5} M fluoxetine plus 10^{-5} M LY-53857 (Sigma-Aldrich).

2.2 5-HT analysis

5-HT concentration was analyzed with an ECD-HPLC (BMA-300; EiCOM, Kyoto Japan) equipped with an EICOMPAK (CA-5ODS 2.1 mm x 150 mm, EiCOM). The mobile phase was sodium phosphate buffer (pH 6.0, 0.1 M) containing 5% methanol, 50 mg/l EDTA·2Na and 100 mg/l sodium pentanesulfonate. The flow rate was 0.23 ml/minute. Column temperature was maintained at 25 °C. 5-HT was oxidized at 400 mV with Ag-AgCl on a graphite electrode. Of each 40-μl sample, 35 μl was injected into the HPLC using an autosampler (NANOSPACE SI-2; Shiseido, Tokyo Japan). Chromatographs were recorded and analyzed with PowerChrom (EPC-300; EiCOM).

2.3 Measurement of specific airway resistance and respiratory variables

Specific airway resistance (sR_{aw}) was calculated as: $R_{aw} * TGV = \tan\{2\pi * (\text{delay time})/(\text{total time})\} * (P_{atm}-47) * 1.36 * (\text{total time})/2\pi$, where R_{aw} is the airway resistance, TGV is the thoracic gas volume, delay time is the duration between the head and body flows, total time is the duration of one

respiratory cycle, and P_{atm} is the ambient pressure (mmHg) (Pennock et al., 1979). Respiratory rate (breaths/min), tidal volume (BTPS; ml/10 g body weight) and minute ventilation (BTPS; ml/10 g body weight) were calculated from the respiratory flow curve for the head chamber calibrated with a 0.5-ml injection of air. The probe sites were verified with 50-μm-thick coronal sections.

2.4 Data analysis

Respiratory variables and sR_{aw} were analyzed for 5 s every 5 min and were averaged for 5 measurements per collection period. 5-HT release and sR_{aw} were expressed as percentages of the mean baseline value. The data were analyzed by one-way repeated-measurement ANOVA, with Greenhouse -Geisser correction (Kanamaru et al., 2001). $P < 0.05$ was considered statistically significant.

3 Results

3.1 5-HT release in the DMM

Baseline 5-HT release in the DMM was the same in both groups (6.76 ± 1.70 fmol/35 μl injection in the fluoxetine group and 5.57 ± 1.49 fmol/35 μl injection in the fluoxetine plus LY-53857 group ($n = 5$ each)). 5-HT release significantly increased above baseline in both groups (1.9-fold in the fluoxetine group and 2.1-fold in the fluoxetine plus LY-53857 group), but this was not significantly different between the two groups (Fig. 1A).

The microdialysis probe sites were distributed in the DMM, including in the nXII, the nTS, and the dorsal motor nucleus of the vagus, around 7.48 mm posterior to bregma. The locations of probe sites were similar between the fluoxetine and the fluoxetine plus LY-53857 groups (Fig. 1B).

3.2 Specific airway resistance and respiratory variables

sR_{aw} was not significantly affected by fluoxetine, but was significantly increased by 1.9-fold by fluoxetine plus LY-53857 in the fourth collection period. The difference between the two groups was significant (Fig. 2A).

Respiratory rate was not significantly affected by fluoxetine perfusion or fluoxetine plus LY-53857 coperfusion. There was no difference in respiratory rate between the two groups (Fig. 2B).

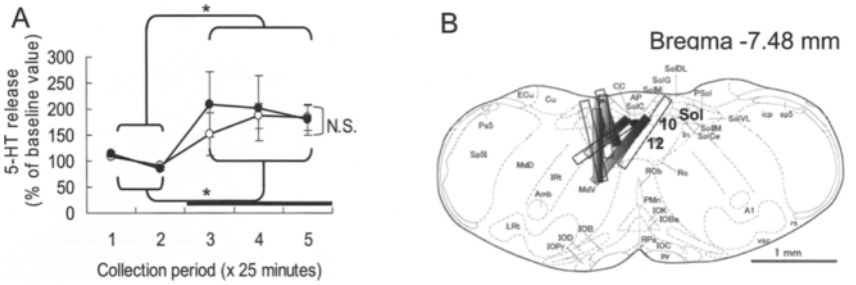

Fig. 1. A: 5-HT release in the DMM in fluoxetine-perfused (open circles, n = 5) and fluoxetine plus LY-53857-coperfused (filled circles, n = 5) groups. Mean ± SEM. The collection period was 25 min.; the drug-perfusion period is indicated by a black bar; *, $p < 0.05$; N.S., no significance. B: microdialysis probe sites on mouse brain atlas (Franklin and Paxinos, 1997). Open bars, fluoxetine-perfused group; solid bars, fluoxetine plus LY-53857-coperfused group. Sol = solitary tract nucleus; 10 = dorsal motor nucleus of vagus; 12 = hypoglossal nucleus.

Tidal volume was not affected by fluoxetine perfusion into the DMM, but was significantly decreased by fluoxetine plus LY-53857 coperfusion. There was no significant difference between the two groups (Fig. 2 C).

Minute ventilation was obtained by multiplying the respiratory rate with the tidal volume. Minute ventilation tended to decrease during fluoxetine plus LY-53857 coperfusion, but the decrease was not significant. There were no significant differences between the two groups (Fig. 2D).

4 Discussion

In the present study, perfusion of the DMM with fluoxetine, an SSRI, increased local 5-HT release. Perfusion with a 5-HT2 receptor antagonist significantly increased airway resistance and slightly decreased tidal volume without affecting respiratory rhythm. These results suggest that: 1) 5-HT release is regulated by a suppressive effect of local 5-HT transporter activity in the DMM, and 2) the 5-HT release elicits airway dilation and a slight increase in tidal volume by 5-HT2 receptors in the DMM.

5-HT outflow from the hippocampus and frontal cortex of mice is enhanced by systemic administration of fluoxetine, although this is limited by autoreceptors such as 5-HT1A and 5-HT1B (Malagié et al., 2002). There is some evidence that 5-HT transporters are distributed in the nTS, (Huang and Pickel, 2002; Paterson et al., 2004; Nakamoto et al., 2000) and the nXII (Paterson et al., 2004). The present results suggest that the 5-HT dynamics in the DMM are regulated by local 5-HT transporters in mice, i.e. 5-HT transporters take up 5-HT and maintain 5-HT release in the DMM.

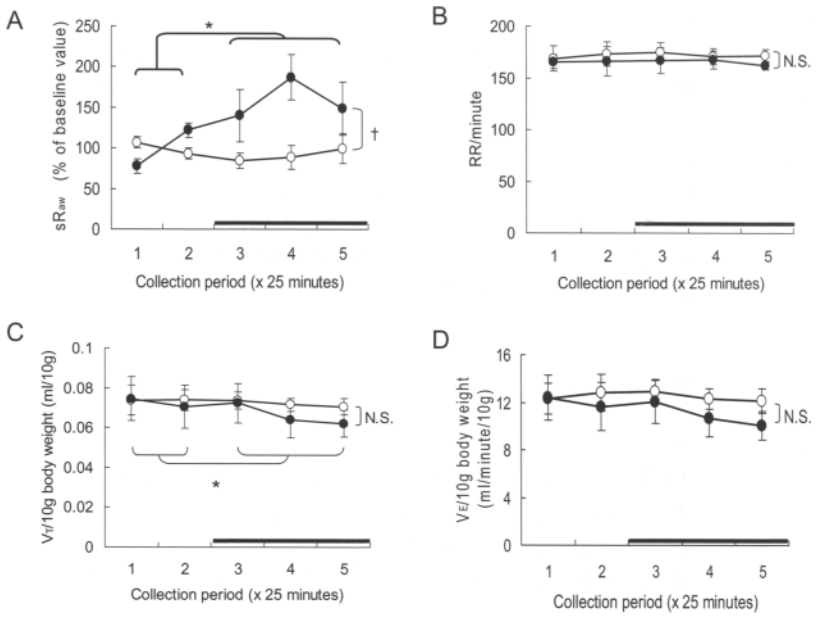

Fig. 2. Respiratory variables obtained for fluoxetine perfusion (open circles, n = 5) and fluoxetine plus LY-53857 coperfusion (filled circles, n = 5) in the DMM. A: specific airway resistance (sR_{aw}). B: respiratory rate (RR). C: tidal volume (V_T). D: minute ventilation ($\dot{V}_E$). Mean ± SEM. The drug-perfusion period is indicated by a black bar; *, $p < 0.05$; †, $p < 0.05$; N.S., no significance.

Similar increases in 5-HT release during fluoxetine perfusion and fluoxetine plus LY-53857 coperfusion demonstrate similar degrees of serotonergic stimulation. Therefore, the different responses with and without a 5-HT2 receptor antagonist depend on different activity levels of the 5-HT2 receptors, not the serotonergic neurons.

In this study, a microdialysis probe for the DMM was ocularly inserted to a depth of 1 mm from the dorsal surface of the medulla oblongata at the level of the area postrema. Only a membrane of the probe was inserted into the brain, which minimized brain damage and raised the accuracy of the probe position. However, there was some difficulty in rigid fixation of the probe onto the cranial bones and in free movement of neck. Therefore, acute experiments were performed.

5-HT perfusion of the nXII increases sleeping genioglossal muscle activity to normal waking levels in rats (Jelev et al., 2001). Hypoglossal motoneurons in cats and hypoglossal nerve activity in rats are stimulated by 5-HT2 receptors in the nXII (Kubin et al., 1992; Fenik and Veasey, 2003). Paroxetine, an SSRI, augments genioglossal electromyographic

activity in normal humans (Sunderram et al., 2000). Some SSRIs decrease the apnea-hypopnea index during REM sleep in obstructive sleep apnea patients (Hanzel et al., 1991; Kraiczi et al., 1999). In the present study, 5-HT2 receptor-mediated 5-HT activity in the DMM decreased airway resistance, suggesting that 5-HT2 receptor activity in the nXII excites the hypoglossal nerve, contracts the genioglossal muscle, and elicits airway dilation.

During REM sleep, airflow resistance above the larynx is twice as high as during wakefulness in normal humans (Hudgel et al., 1984). In our study, the sR_{aw} with a SSRI and a 5-HT2 receptor antagonist was twice as high as during stimulation by an SSRI alone. This suggests that airway dilation is functionally influenced by 5-HT2 receptor activity in the nXII.

In vagotomized and anesthetized rats, the contribution of serotonergic receptors in the nXII to hypoglossal nerve activity is 35% (Fenik et al., 2005) and to genioglossal muscle activity is 50% (Sood et al., 2005). This was similar to the degree of 5-HT2 receptor contribution to airway resistance in our experiments. The serotonergic drive modulating genioglossal muscle activity, however, was little observed in vagi-intact rats (Sood et al., 2005), which indicates that a minimal endogenous 5-HT drive in the nXII modulates genioglossal muscle activity in rats unless augmented by neural input. Further experiments are needed to clarify the physiological conditions activating serotonergic input to the nXII.

Tidal volume was significantly decreased by fluoxetine plus LY-53857 coperfusion, but the effect was not statistically different from the effect of fluoxetine perfusion alone. Thus manipulation of only 5-HT2 receptors in the DMM has a minor effect on the regulation of tidal volume.

Respiratory rhythm generator is distributed throughout the ventral respiratory group (Ballanyi et al., 1999; Onimaru and Homma, 2003). Stimulation of 5-HT2 receptors in the DMM of resting mice may not affect the respiratory rhythm generators.

The increase in 5-HT release during fluoxetine perfusion did not affect sR_{aw} and tidal volume. Hypoglossal 5-HT decreases glutamate release from the raphe pallidus to the nXII through presynaptic 5-HT1A or 1B receptors (Bouryi and Lewis, 2003). This suggests that spontaneous 5-HT release in the DMM has already elicited airway dilation and increased tidal volume before fluoxetine perfusion; stimulates the inhibitory 5-HT1A and 5-HT1B receptors and excitatory 5-HT2 receptors; or both.

In conclusion, 5-HT release in the DMM is regulated by a suppressive effect of local 5-HT transporters. The 5-HT release in the DMM elicits airway dilation and a slight increase in tidal volume without affecting respiratory rhythm. Those effects are mediated by 5-HT2 receptors. The inactivation of 5-HT2 receptor activity in the DMM doubles the airway resistance. It is useful to measure airway resistance for elucidation of mechanisms underlying upper airway control and obstructive sleep apnea.

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