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Short communication

Contribution of 5-HT_{2A} receptors on diaphragmatic recovery after chronic cervical spinal cord injury

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ABSTRACT

Unilateral C2 spinal cord hemisection (C2Hx) interrupts bulbospinal respiratory pathways innervating ipsilateral phrenic motoneurons, resulting in cessation of ipsilateral diaphragm motor output. Plasticity within the spinal neural circuitry controlling the diaphragm can induce partial recovery of phrenic bursting which correlates with the time-dependent return of spinal serotonin (5-HT) immunoreactivity in the vicinity of phrenic motoneurons. The 5-HT_{2A} receptor subtype is present on phrenic motoneurons and its expression is up-regulated after cervical spinal cord injury; however the functional role of these receptors following injury has not been clearly defined. The present study evaluated the functional role of 5-HT_{2A} receptors by testing the hypothesis that pharmacologic blockade would attenuate diaphragm activity in rats with chronic cervical spinal cord injury. Bilateral diaphragm electromyography (EMG) was performed in vagal-intact and spontaneously breathing rats before and after intravenous administration of the 5-HT_{2A} receptor antagonist Ketanserin (1 mg/kg). Intravenous ketanserin significantly attenuated ipsilateral diaphragm EMG activity in C2Hx animals but had no impact on diaphragm output in uninjured animals. We conclude that 5-HT_{2A} receptor activation contributes to the recovery of ipsilateral phrenic motor output after chronic cervical spinal cord injury.

1. Introduction

Unilateral hemisection of high cervical spinal cord (i.e., C2Hx) results in the immediate cessation of ipsilateral phrenic activity and paralysis of the hemidiaphragm. Phrenic motor output ipsilateral to the lesion gradually recovers over weeks to months post-injury due to activation of previously latent crossed spinal pathways (i.e., crossed phrenic phenomenon) (Lee and Hsu, 2017; Lee et al., 2014). Considerable evidence suggests a role for serotonin in this recovery. For example, Hadley et al. (1999) demonstrated that treatment with a serotonin (5-HT) depleting drug (*para*-chlorophenylalanine) prior to injury can block the expression of the crossed phrenic phenomenon evoked by asphyxia during the acute injury phase (Hadley et al., 1999a). Similarly, a study by Golder et al. (2001) showed that rats treated with the serotonin toxin 5,7-dihydroxytryptamine also demonstrated a reduced incidence of recovery of ipsilateral phrenic bursting (Golder et al., 2001). These results suggest that 5-HT is necessary for recovery of ipsilateral phrenic activity after cervical spinal cord injury. However, both the 5-HT depleting drug and 5-HT neurotoxin mentioned in above studies were applied prior to the spinal cord injury surgery. Thus it remains unclear whether the role of 5-HT is to induce activation of crossed spinal pathway and/or maintain the phrenic bursting after cervical spinal cord injury. Golder and Mitchell (2005) observed that phrenic burst amplitude after cervical spinal cord injury correlated with time-dependent return of spinal 5-HT immunoreactivity (Golder and Mitchell, 2005). Moreover, protein and mRNA expression of 5-HT_{2A} receptor within the phrenic nucleus was elevated after cervical spinal hemisection (Fuller et al., 2005; Mantilla et al., 2012). Thus, we hypothesize that the recovery of 5-HT that occurs over several weeks after injury may act on 5-HT_{2A} receptors to facilitate phrenic bursting during the chronic injury phase.

2. Methods

All experimental protocols were approved by the Institutional Animal Care and Use Committees at National Sun Yat-sen University.

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Nineteen male Sprague-Dawley rats at age of 7–8 weeks were purchased from BioLasco Taiwan Co., Ltd (Taiwan) and assigned to uninjured (n = 8) and C2Hx (n = 11) group.

2.1. Cervical spinal cord hemisection

At 9–10 weeks of age, rats in the C2Hx groups were anesthetized with xylazine (10 mg/kg, s.c., Rompun®, Bayer) and ketamine (140 mg/kg, i.p., Ketalar®, Pfizer) The C2 spinal cord was exposed by dorsal midline incision followed by C2 laminectomy. Using a microscalpel, the left C2 spinal cord was incised and a lesion cavity was created by gentle aspiration using a micropipette connected to a suction pump. The dura was sutured with 10-0 nylon (UNIK) sutures, and the overlying muscles and skin were closed with 4-0 chromic (UNIK) and 4-0 nylon sutures (UNIK) sutures, respectively. Animals were then given injections of yohimbine (1.2 mg/kg, s.c., Tocris) to reverse the effect of xylazine, lactated Ringer's solution (5 ml, s.c., Nang Kuang Pharmacerutical Co., Ltd) to prevent dehydration, and buprenorphine (0.03 mg/kg, s.c., Shinlin Sinseng Pharmaceutical Co., Ltd.) for analgesia. Oral supply of Nutri-cal (1–3 ml, EVSCO pharmaceuticals) and lactated Ringer's solution injection (5 ml, s.c.) were administered daily until recovery of volitional eating and drinking.

2.2. Experimental preparation and protocol

At 8–9 weeks post-injury, terminal neurophysiology experiments were performed as previously described (Hsu and Lee, 2015). Briefly, animals were anesthetized with an injection of urethane (1.6 g/kg, i.p., Sigma). An adequate plane of anesthesia was confirmed by the absence of the toe-pinch withdrawal reflex. Rats were placed in a supine position and the rectal temperature was maintained at 37 ± 1 °C by a servo-controlled heating pad (model TC-1000, CWE Inc.). The trachea was cannulated with an endotracheal tube (PE-240, Clay Adams) and connected to a respiratory flow head (MLT1L, ADInstruments) and a spirometer (FE141, ADInstruments) for respiratory flow measurement. A hyperoxic gas mixture (50% O_2, balance N_2; flow rate: 2 l/min) was delivered to the animal via a T-piece tube. The femoral artery and vein were catheterized for blood pressure measurement (Transducer: DTX-1; Amplifier: BPM-832, CWE Inc.) and drug administration, respectively.

The abdominal surface of the diaphragm were exposed by laparotomy, and the bipolar silver electrodes (coated silver wire with exposed tips, #786000, A-M system) threaded through 26 gauge needles were inserted into the medial costal region of bilateral diaphragm. The diaphragm EMG signals were amplified (1000x) and band-pass filtered (0.3–10 kHz) by a differential A/C amplifier (Model 1700, A-M Systems), and processed with the rectified and smoothed function (time constant: 25 ms) by Spike2 software (Cambridge Electronic Design Limited). Physiological signals were digitized using the CED Power 1401 (Cambridge Electronic Design Limited) at sampling rate of 100 Hz (e.g., airflow and blood pressure) or 10 KHz (e.g., diaphragm EMG activity) and recorded in a computer by Spike 2 software.

After stable recording of the respiratory airflow and diaphragm EMG signals, a single bolus injection of ketanserin, a 5-HT_{2A} receptor antagonist (1.0 mg/kg, 0.5 ml/kg, Tocris) was delivered intravenously to evaluate the functional role of 5-HT_{2A} on the respiratory and cardiovascular parameters.

2.3. Data analyses

Respiratory frequency was calculated from the respiratory airflow trace. Tidal volume data were derived from the integrated inspiratory airflow using a Spike 2 script. The amplitude of inspiratory activity of the diaphragm was defined as the difference between the maximum and minimum values of the rectified and smoothed diaphragm EMG signals within a single breath. EMG data were expressed in arbitrary unit (a.u.) or as a percentage of the baseline activity (% BL; i.e., before ketanserin administration). All physiological parameters were averaged over 30 s prior to ketanserin administration (e.g. baseline) and over 10 s at ~1 min post-ketanserin administration. Student's *t*-tests were used to compare the age and body weight between uninjured and C2Hx animals, and the amplitude of diaphragm EMG activity between the contralateral and ipsilateral sides. The influence of ketanserin administration on diaphragm output, respiratory frequency, and blood pressure was evaluated by a two-way mixed-design measures analysis of variance followed by the Dunnett post hoc test [factor one: group (uninjured vs. C2Hx); factor two: condition (before vs. after ketanserin administration)]. Differences were considered statistically significant when $P < 0.05$. Data are expressed as the mean ± standard error of the mean.

3. Results

3.1. Animals

Eight uninjured (age: 137 ± 4 days; weight: 588 ± 35 g) and eleven C2Hx animals (age: 137 ± 4 days; weight: 497 ± 17 g) were studied to examine the influence of intravenous ketanserin on the cardiorespiratory pattern in chronically injured rats. Age was similar between two groups; however, rats with chronic C2Hx weighed significantly less than uninjured rats at the time of terminal neurophysiology procedures ($P < 0.05$).

3.2. Cardiorespiratory pattern following chronic cervical spinal cord injury

Representative examples of the cardiorespiratory pattern and diaphragm EMG activity were shown in Fig. 1A. Mean arterial pressure and heart rate were similar between uninjured (89 ± 4 mmHg; 363 ± 23 beats/min) and C2Hx rats (83 ± 6 mmHg; 381 ± 19 beats/min) at baseline; however, the pattern of respiratory efforts differed between groups. Specifically, in rats with C2Hx, respiratory frequency was higher and tidal volume was lower than uninjured animals ($P < 0.05$, Fig. 1Ba and Bb). As shown in Fig. 1A, robust bilateral diaphragm EMG output was recorded in uninjured animals at baseline. By comparison, although bilateral diaphragm output was evident in C2Hx animals, the amplitude of ipsilateral EMG signals was significantly attenuated (0.30 ± 0.13 a.u.) as compared to the contralateral diaphragm (0.81 ± 0.14 a.u.) ($P < 0.01$).

3.3. Alteration of cardiorespiratory pattern and diaphragm EMG activity following administration of ketanserin

Respiratory frequency was significantly reduced, from 124 ± 10 breaths/min to 110 ± 7 breaths/min after administration of ketanserin in uninjured animals ($P < 0.05$, Fig. 1Ba). Similarly, C2Hx animals exhibited a lower respiratory frequency following ketanserin injection (before vs. after: 158 ± 8 vs. 136 ± 6 breaths/min, $P < 0.01$, Fig. 1Ba). Tidal volume was not significantly affected by ketanserin delivery in spinal intact animals though it was slightly enhanced in C2Hx animals (before vs. after: 2.1 ± 0.1 vs. 2.3 ± 0.1 ml, $P < 0.05$, Fig. 1Bb). Mean arterial pressure was significantly decreased in response to ketanserin delivery in both spinal intact (from 89 ± 4 to 65 ± 4 mmHg) and C2Hx animals (from 83 ± 6 to 67 ± 5 mmHg) ($P < 0.01$). Intravenous injection of ketanserin also reduced heart rate in C2Hx animals (from 381 ± 19 to 352 ± 24 beats/min) ($P < 0.05$).

Intravenous administration did not significantly alter diaphragm EMG on either side in uninjured rats. Diaphragm EMG activity was maintained at 93 ± 4% of BL and 93 ± 2% of BL, respectively (Fig. 1Bc and Bd) for the contralateral (i.e., right side) and ipsilateral (i.e., left side) diaphragm. Similarly, intravenous ketanserin did not significantly alter contralateral diaphragm EMG activity in C2Hx rats, however it did attenuate the activity of the ipsilateral diaphragm to