Online Data Supplement

Respiratory Related Discharge of Genioglossus Muscle Motor Units

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1. Detailed Methods

Animals and surgical procedures. All procedures adhered to the Institutional Animal Care and Use Committee at the University of Arizona. Recordings were obtained from 17 spontaneously breathing anesthetized male Sprague-Dawley rats weighing 250-400g. Animals were initially anesthetized with isofluorane and a cannula was inserted into a femoral vein. Isofluorane anestehia was then discontinued, and animals were anesthetized with urethane, administered intravenously at a dose of 1.6 g/kg. Supplemental doses of 0.6 g/kg were given intravenously to maintain the depth of anesthesia, which was assessed by limb withdrawal and blood pressure responses to paw pinch. The animals were supine throughout the experiment. Rectal temperatures were maintained at 37^{o} C with a thermistor connected to a servo controlled heating pad (Haake Inc., model D1-L). Polyethylene catheters were inserted into a femoral artery and vein to monitor blood pressure and to administer fluids and drugs. The animal breathed spontaneously through a catheter inserted into the trachea, just below the larynx. The animals were euthanized at the end of the experiment with an intravenous bolus of sodium pentobarbital (100 mg/kg).

Measurement and analysis of respiratory airflow and blood gases. Respiratory airflow was recorded by attaching a pneumotachometer (Hans Rudolph, Series 8421) to the tracheal catheter. The two ports of the pneumotachometer were connected to either side of a differential pressure transducer (Validyne, model MP45-28-871). The resulting pressure signal provided a

record of instantaneous airflow into and out of the respiratory system. The rate of respiratory airflow was obtained by calibrating the pneumotachometer with a series of known, steady airflow rates (1, 2). To control the composition of the inspired gas delivered to the animal, we attached a t-tube to the inspiratory side of the pneumotach (2), and a steady bias flow of compressed gas was directed through the t-tube. The gas mixture was altered as needed by feeding compressed gas from CO_2, O_2 and N_2 tanks into a mixing rotameter (Matheson 7000 series), with the output of the rotameter connected to the t-tube. Inspired CO_2 concentrations were monitored with a CO_2 analyzer (Applied Electrochemistry, model CD3A) that was calibrated with a precision gas mixture (Matheson).

Arterial blood samples (150-200 μL) were withdrawn periodically and analyzed for PCO_2, PO_2 and pH at 37 ^oC with an Instrumentation Laboratories analyzer (Model 1640). The values were corrected to the animal's rectal temperature recorded at the time of sampling. These values were used to maintain HCO^-_3 levels between 23 and 26 mM, which is within the normal range for spontaneously breathing, urethane-anesthetized rats (1, 2). We did not sample arterial blood during the hypercapnic episodes for two reasons: first, the experiments were long and we wanted to conserve blood volume (for example, recording from 3 motor units in the same animal would require the withdrawal of 15, 150-200 μL blood samples, corresponding to about 10-12 % of the total blood volume in animals of this size); second, from our previous work we know the approximate levels of arterial CO_2 expected at each level of inspired CO_2 in this preparation (1). Based on these previous data, expected values for arterial PCO_2 would be as follows: 39.5 mmHg at 0 % inspired CO_2; 41.6 mmHg at 3 % inspired CO_2; 45.3 mmHg at 6 % inspired CO_2; 61.3 mmHg at 9 % inspired CO_2; and 72.3 mmHg at 12 % inspired CO_2.

Measurement of genioglossus muscle EMG activity. The genioglossus muscle was exposed bilaterally with a ventral approach after removing all overlying muscles, including the geniohyoid, as described in detail previously. Because the medial branch of the hypoglossal nerve innervates the genioglossus muscle, we sectioned the lateral branch bilaterally before recording commenced. The medial branch also innervates parts of the geniohyoid muscle and some of the intrinsic muscles of the tongue. However, by removing the geniohyoid muscle completely in each animal we insured that the recordings were not contaminated by activity from this muscle. And by using a ventral approach, dissecting down to the floor of the mouth, we remained well caudal and ventral to the tongue itself; this insured that we were not picking up intrinsic muscle motor unit activities (3, 4).

Two stainless steel fine wire electrodes (0.002 in diameter, California Fine Wire) were inserted into the belly of the genioglossus muscle as described in detail previously (1-4). Insulation was removed from the terminal 1-2mm of each electrode. The EMG was amplified with an alternating current differential amplifier (Grass, model 7WU16K), and filtered between 30 and 3,000 Hz. The amplified and filtered EMG was rectified and low-pass filtered using a time constant of 200 ms to give the rectified and integrated EMG waveform (iEMG).

Measurement of genioglossus muscle single motor unit potentials (SMUP). Single motor unit potentials were obtained with high impedance (10 Mohm) tungsten microelectrodes (Frederick Haer, Bowdoin, ME) inserted directly into the genioglossus. Single motor unit potentials were amplified (Grass, model 7WU16K), filtered between 300 and 10,000 Hz, and displayed on an oscilloscope. Potentials were viewed simultaneously on a computer monitor that displayed the digitally converted motor unit potential after passing it through a window discriminator that could be set for distinguishing both motor unit amplitude and shape, using the

Spike II template-matching algorithm, as described previously (5). This program requires the investigator to arbitrarily choose a percentage error that is based on the area and shape of the unit. This information is then used to make a template that each potential is compared to. We chose an error of 20%, which means that if the shape: amplitude parameter of a given motor unit differed from the template by more than 20%, the potential is considered to arise from a different motor unit. Figure 1 (located in the main manuscript) provides an example of our ability to discriminate individual genioglossus muscle motor units, under baseline and hypercapnic conditions. The recordings illustrate the unique shape of the three potentials, as well as the consistency of the shape from spike-to-spike even under hypercapnic conditions, when the muscle was contracting more vigorously.

Data analysis and statistics. All data were acquired and analyzed using Spike2 software (Cambridge Electronics Design, UK) and custom-designed programs. Tidal volume (V_T) and respiratory frequency (f_r) were computed from the flow signal, and pulmonary ventilation was calculated as the product of inspired V_T and f_r. The average genioglossus iEMG activity in each respiratory cycle was computed by calculating the total activity in each burst (the area under the iEMG burst), and then dividing total activity by the duration of the burst. Tidal volume, ventilation and the average iEMG were subsequently expressed as a percentage of the baseline values. We obtained average values by analyzing the data from 20 respiratory cycles taken in the last minute of the control period and at each level of hypercapnia.

We computed the interspike interval (ISI) and its reciprocal (the instantaneous firing frequency) for every spike in a burst (see Fig. 2, in the main manuscript), and this was done for each of ten bursts recorded within the last minute of each condition. We also computed the total number of spikes generated in each respiratory cycle, and computed the average value for each

condition and for each motor unit studied. Finally, we computed the standard deviation of the interspike intervals (ISI) of all spikes recorded in each condition, and quantified the variability of the ISI by computing the coefficient of variation and expressing it as a percentage [CV = (S.D/ Mean) x 100].

We used Friedman's non-parametric ANOVA on ranks if a given data set did not pass tests for equal variance and normality. In either case, post-hoc pair wise comparisons were evaluated with the Student-Neuman-Keuls procedure. The criterion for statistical significance for all analyses was $P < 0.05$. All statistical tests were done with InStat version 3.0 statistical software (GraphPad Software, San Diego, CA).

References

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