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Developmental effects of ketamine on inspiratory hypoglossal nerve activity studied in vivo and in vitro

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Abstract

The effects of the anesthetic ketamine on properties of inspiratory bursts (I-bursts) in mouse hypoglossal nerve activity were studied in vivo and in vitro. In urethane anesthetized mice we observed rhythmic I-phase activity in only one of eight pups at P9 days. In contrast in older mice rhythmic I-phase hypoglossal activity was almost always observed. Ketamine caused a reduction in I-burst frequency and an increase in peak integrated hypoglossal nerve activity in all three age groups studied (P10–P13, P15–P20 and adult mice). In these mice I-phase oscillations, due to hypoglossal motoneurons firing clusters of action potentials at a particular frequency, were observed in control and after ketamine. Ketamine did not change the frequency of the dominant spectral peak determined from power spectra examined from 0 to 200 Hz. The effects of ketamine were also studied in vitro in the mouse rhythmic medullary slice preparation. Ketamine reduced hypoglossal I-burst frequency and I-burst peak integrated amplitude. Oscillations were observed in I-phase activity, and as in the in vivo studies ketamine did not shift the dominant spectral peak frequency. These results demonstrate that in vivo and in vitro ketamine results in significant changes in I-burst frequency and peak integrated hypoglossal nerve activity, but changes in the oscillation frequency are minimal.

Keywords: Control of breathing; In vivo; In vitro; Oscillations; Inspiration; Ketamine

1. Introduction

The availability of in vivo and in vitro animal models to study the effects of pharmacological agents on respiration has enabled important new insights to be gained into the mechanisms of action of these substances. For example, the widely used anesthetic and analgesic ketamine, which is a phencyclidine derivative whose primary action is as a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, has been reported to have "beneficial" effects on the respiratory system, which include minimal respiratory depression and protection of airway reflexes (Hass and Harper, 1992; Kohrs and Durieux, 1998). Yet previous studies in spontaneously breathing neonatal and adult animals have shown that ketamine at clinically relevant doses markedly depresses overall ventilation, having its strongest effect by reducing respiratory rate (Jaspar et al., 1983; Saetta and Mortola, 1985; Sarton et al., 2001; Lydic and Baghdoyan, 2002). Thus a comprehensive study of the effects of ketamine on respiration, both in vivo and in vitro in the same animal species is warranted.

Studies of respiration have generally characterized respiratory motor output by recording inspiratory-phase (I-phase) related neural discharge in respiratory motor nerves, such as the phrenic and hypoglossal nerves. From these recordings effects of various manipulations have been characterized both by their effects on long-time-scale and short-time-scale properties of inspiratory motor discharge. Long-time-scale properties include: I-burst duration, I-burst frequency and peak integrated inspiratory activity. Short-time-scale properties are associated with oscillations that are routinely observed during the I-phase in these motor discharges (Funk and Parkis, 2002). Short-time-scale properties include: the frequency of the dominant peak observed in the I-phase power spectrum and the relative power of this dominant peak. Aside from the brief mention that ketamine affects phrenic and recurrent laryngeal nerve activity by Richardson and Mitchell (1982), there is a dearth of information on its effect on short-time-scale properties of I-phase motor discharge. In the present study we determined the effects of ketamine on long- and short-time-scale I-phase hypoglossal nerve activity recorded both in vivo, in urethane anesthetized

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neonatal and adult mice, and in vitro in experiments performed using the rhythmically active neonatal mouse medullary slice preparation.

2. Methods

2.1. Preparations

In vivo experiments were performed on Swiss–Webster mice, both neonatal (P9–P20) and adult female mice were used in these studies. All mice were initially anesthetized with a brief exposure to air containing isoflurane. Mice were then deeply anesthetized by an intraperitoneal injection of urethane (dose 2 mg/g; see O'Neal et al., 2005) dissolved in saline solution. This anesthetic was supplemented when the mice showed reflex responses to a strong interdigitary pinch. Robust short-time-scale oscillations in phrenic and hypoglossal nerve inspiratory-phase (I-phase) activity have been routinely seen in mice anesthetized with urethane (O'Neal et al., 2005). During the experiments the rectal temperature was measured and maintained at approximately 36–37 °C using a heated water pad on which the mice were placed. Using a ventral approach in the neck we performed a tracheotomy and installed a piece of plastic tubing in the lumen of the distal trachea so that the animal could spontaneously ventilate its lungs through this tube. Using the same approach we dissected and cut the left hypoglossal nerve. Mineral oil was then applied to the region around the nerve, so that the nerve resided in a pool of mineral oil. The cut central end of the nerve was placed upon a bipolar platinum wire recording electrode. During experiments the animals spontaneously breathed room air. At the end of the experiments animals were killed with an intracardiac injection of saturated KCl solution.

In vitro experiments were performed on rhythmically active medullary slices derived from Swiss–Webster mice (P4–P7) that were from the same litters as those used in the in vivo experiments. We have previously described the methods used for obtaining these rhythmic medullary slices (Sebe et al., 2006) (see also Funk et al., 1993). Briefly, neonatal mice were initially anesthetized with isoflurane and then decapitated in accordance with the regulations of the University of Washington Institutional Animal Care and Use Committee (IACUC). Following decapitation the brain stem and upper cervical spinal cord were separated and removed from the mouse. The tissue was placed on a Sylgard platform and cut using a vibratome (Pelco). Slices were cut (200 μm) from rostral to caudal until the facial nucleus was no longer visible and the rostral most end of the inferior olive was visible. Immediately following identification of these and other ventrally and dorsally located landmarks, the rhythmic medullary slice (700 μm) was cut. The slices were then placed in a recording chamber maintained between 27 and 28 °C and superfused with artificial cerebrospinal fluid containing 8 mM K^+ as described below.

2.2. Recording and experimental procedures

In the in vivo experiments activity from the central end of the hypoglossal nerve was recorded, amplified and band-pass filtered (10 Hz–3 kHz) with an AC preamplifier (Grass model P15). The output of the preamplifier was digitally sampled at 5 kHz and the digitized signal stored on the hard drive of a computer (Molecular Devices—PClamp8 software). "Leaky" integrated hypoglossal rootlet activity was also measured from the band-pass filtered rootlet activity using a custom built "leaky" integrator (time constant = 100 ms), and this signal was also digitally stored. Following the recording of sufficient control I-phase activity to calculate long- and short-time-scale properties (see below) we injected ketamine (Ketamine HCl—Phoenix Pharmaceuticals, St. Joseph, MO) intramuscularly (in the gastrocremius muscle). The dose of ketamine injected depended on the age group from which the mice were drawn, since we found that spontaneous breathing in younger mice was more sensitive to ketamine. We empirically arrived at the dose of ketamine used based on its ability to significantly slow the inspiratory burst frequency. Specifically, in the group of adult mice we administered ketamine at a dose of 0.2 mg/g. In the two neonatal age groups (second (P10–P13) and third (P15–P20) weeks old), ketamine was administered at a dose of 0.015 and 0.10 mg/g, respectively. In all cases within 1–2 min of ketamine administration inspiratory burst frequency slowed. When this slowing reached a stable level we recorded a sufficient number of inspirations (more than 20) in order to compute both the long-time-scale and short-time-scale properties.

In the in vitro experiments slices were bathed in a high K^+ artificial cerebrospinal fluid that contained (in mM): 118 NaCl, 8 KCl, 1 $MgCl_2$, 1 NaH_2PO_4, 25 $NaHCO_3$, 30 D-glucose and 1.5 $CaCl_2$. This solution was superfused over the preparation at 2–3 ml/min. We used glass suction electrodes to record neural activity from the cut central end of hypoglossal rootlets. This rootlet activity was then amplified and band-pass filtered (0.1 Hz–2 kHz) using a Cyber Amp 320 instrument (Molecular Devices). This signal was then digitally sampled at 5 kHz and the digitized signal stored on the hard drive of a computer (Molecular Devices—PClamp8 software). "Leaky" integrated hypoglossal rootlet activity was also measured and this signal was digitally stored. Following acquisition of control data ketamine was washed in for 10 min in the bathing solution before rootlet activity was recorded in ketamine.

2.3. Data analysis

Long-time-scale properties of the inspiratory burst (I-burst) were measured from the digitized recordings using Clampfit (Molecular Devices). These long-time-scale properties included: I-burst duration, I-burst frequency and peak integrated inspiratory activity. For each experiment (either whole animal or slice) these were visually measured using on-screen cursors and then averaged based on data from 10 sequential I-bursts of activity. Inspiratory-burst duration was measured using the raw nerve recording; I-burst frequency and peak integrated I-burst amplitude were measured using the "leaky" integrated trace.

Short-time-scale properties were determined from I-phase power spectra that were computed in Clampfit. For each animal and in each condition (control versus ketamine) 20 I-bursts were selected and used to compute 20 absolute power spectra.