CO₂ decreases membrane conductance and depolarizes neurons in the nucleus tractus solitarii

J.B. Dean¹, W.L. Lawing¹, and D.E. Millhorn¹,²
¹ Department of Physiology and ² Curriculum for Neurobiology, The University of North Carolina, Chapel Hill, N.C. 27599, USA

Summary. To identify central sites of potential CO₂/H⁺-chemoreceptive neurons, and the mechanism responsible for neuronal chemosensitivity, intracellular recordings were made in rat tissue slices in two cardiopulmonary-related regions (i.e., nucleus tractus solitarii, NTS; nucleus ambiguus, AMBc) during exposure to high CO₂. When the NTS was explored slices were bisected and the ventral half discarded. Utilizing such “dorsal” medullary slices removed any impinging synaptic input from putative chemoreceptors in the ventrolateral medulla. In the NTS, CO₂-induced changes in firing rate were associated with membrane depolarizations ranging from 2–25 mV (n = 15). In some cases increased e.p.s.p. activity was observed during CO₂ exposure. The CO₂-induced depolarization occurred concomitantly with an increased input resistance ranging from 19–23 MΩ (n = 5). The lower membrane conductance during hypercapnia suggests that CO₂-induced depolarization is due to a decreased outward potassium conductance. Unlike neurons in the NTS, AMBc neurons were not spontaneously active and were rarely depolarized by hypercapnia. Eleven of 12 cells tested were either hyperpolarized by or insensitive to CO₂. Only 1 neuron in the AMBc was depolarized and it also showed an increased input resistance during CO₂ exposure. Our findings suggest that CO₂/H⁺-related stimuli decrease potassium conductance which depolarizes the cell and increases firing rate. Although our in vitro studies cannot guarantee the specific function of these cells, we believe they may be involved with brain pH homeostasis and cardiopulmonary regulation.

Key words: Central chemoreceptor – Carbon dioxide – Nucleus tractus solitarius – Nucleus ambiguus – Cardiopulmonary control – Brain slice – Intracellular recording – Rat

Introduction

The predominant effect of hypercapnia on nerve cells is hyperpolarization and a decreased firing rate (Gill and Kuno 1963; Krnjevic et al. 1965; Carpenter et al. 1974). Exceptions to this generalization are special cells in the carotid body and brainstem that function as CO₂/H⁺-chemoreceptors in the regulation of the cardiopulmonary systems. The precise location of chemosensitive cells in the brainstem remains unknown (see Millhorn and Eldridge 1986). However, the early work of Mitchell et al. (1963) and Loeschcke et al. (1970) showing that acidic and hypercapnic solutions stimulated respiration only when placed onto the ventral surface of the medulla oblongata (VLM) led to the now widely accepted hypothesis that the central chemoreceptors reside exclusively at or near the VLM (Loeschcke 1982; Bruce and Cherniack 1987). The exclusion of other chemosensitive sites within the brainstem by this hypothesis has been challenged by recent findings (Kiley et al. 1985; Malcolm et al. 1980) that the respiratory response elicited by application of these stimulants onto the VLM represents only a fraction of the overall response to CO₂ inhalation in glomectomized animals (see also Comroe 1943; Lipscomb and Boyarsky 1972; Cragg et al. 1977; Miles 1983; Millhorn and Eldridge 1986; Dean and Millhorn 1987).

In recent years there has been a renewed interest in identifying brainstem sites that contain chemosensitive neurons. Thus far, the only area out-
side the VLM for which there is evidence for chemosensitivity is the nucleus tractus solitarii (NTS), a major integrative area for cardiopulmonary control, in the dorsal aspect of the medulla. Miles (1983) using an in vitro brain slice preparation found that a substantial number of single-units in the NTS showed an increased firing rate in response to acidic perfusion medium. Dean and Millhorn (1987) using a coronal medullary slice preparation in which the ventral half of the slice had been removed found that 29% of the cells tested in the NTS increased their firing rate during high CO2 exposure.

The present work was undertaken to extend these observations by performing intracellular recordings and measuring the effect of hypercapnia on the passive membrane properties of NTS neurons in vitro. All recordings were made from cells in “dorsal” medullary slices; i.e., coronal slices that were bisected and the ventral halves discarded. This eliminated the possibility of any impinging synaptic input from putative chemoreceptors in the VLM. We found that neurons in the NTS were depolarized by hypercapnia and showed a concomitant increase in input resistance (Rn) across the membrane. Thus, our findings show that a subpopulation of cells in the NTS are indeed CO2/H+ chemosensitive and that the stimulatory effect of CO2 on these cells might be due to a decreased conductance of potassium (gK).

Methods

Brain slices (400 μm thick) were prepared from male Sprague-Dawley rats (100–400 gm) using standard procedures (Dean and Boullant 1988). The coronal slices encompassed an area extending from obex rostrally for approximately 4 mm. Each coronal slice was bisected and the ventral half discarded. The dorsal half (i.e., “dorsal” slice) was maintained in an interface chamber (Kelso et al. 1983) with continuous nutrient medium perfusion (1–1.5 ml/min, 35.5–37°C). The nutrient medium (Dean and Boullant 1989) was bubbled with 5% CO2/95% O2 to maintain medium pH at 7.4 and slice oxygenation. Cells were tested for chemosensitivity by changing the CO2 concentration (7, 10 and 15% CO2, balance O2) in the gas mixture bubbling the medium and blowing across the slices. Two, 5 and 10% increases in CO2 over control caused medium pH to decrease 0.1, 0.24 and 0.47 units, respectively. These CO2 (and pH) levels were chosen based on measured brain extracellular and intracellular pH values during hypercapnia (Siesjo et al. 1972) and the stimulatory effects hypercapnia has on respiration in rodents (Holloway and Heath 1984; Harada et al. 1985). Preliminary experiments showed that after switching to high CO2, the firing rate response usually began within 0.5–1 min and peaked after 1.5–2 min (Dean and Millhorn 1987).

Intracellular measurements were made with microelectrodes (70–150 MΩ) that contained a Ag/Cl wire and 3 M K+-acetate. Recordings were amplified with a high voltage electrometer equipped with a constant current source for injecting brief current pulses (10–500 ms, ± 0.1 to 0.5 nA) in the bridge-balanced mode. Following impalement cells were usually hyperpolarized below threshold to facilitate microelectrode-membrane sealing. A stable membrane potential (Em) ≥ −45 mV was typically achieved 2–15 min after impalement. Stable recordings with action potentials of ≥55 mV typically lasted from 20 min to 6 h. Intracellular waveforms were monitored continuously on a storage oscilloscope and recorded on tape (A. R. Vetter, VCIR model 420-D). Cells were analyzed off-line using a digital storage oscilloscope, x/y plotter and polygraph. Firing rate (impulses/s) was determined with a window discriminator/integrator.

In another series of experiments, intracellular recordings were made during hypercapnia from neurons in the compact portion of the nucleus ambiguus (AMBc) (Bieger and Hopkins 1987) in intact coronal slices.

Results

Cells in the NTS displayed resting Em of −54 ± 10 mV (± S.D.), input resistances (Rn) of 128 ± 56 MΩ (± S.D.) and spontaneous firing rate that ranged from <0.1 to 10 impulses/s. A total of 31 NTS neurons was studied during high CO2. Thirteen cells (42%) were insensitive to hypercapnia and displayed no changes in Em, firing rate or Rn. Three cells (10%) were hyperpolarized (by 8 or 9 mV) during hypercapnia, Rn was not determined for these cells. Fifteen (48%) neurons were depolarized during hypercapnia; 13 spiking neurons and 2 non-spiking silent cells. CO2-induced changes in firing rate were associated with membrane depolarizations ranging from 2 to 25 mV. Employing 15% CO2 was not essential for evoking a significant response; equivalent depolarizations were observed in several neurons with 7 or 10% CO2. CO2-excited neurons were located primarily in the commissural, medial and lateral subnucleus of the NTS.

Figure 1 shows a typical response for a CO2-excited neuron; i.e., a neuron depolarized during hypercapnia. This cell was located in the commissural subnucleus of the NTS in a dorsal slice. In Fig. 1a, resting Em was −56 mV the cell showed occasional action potentials (0 to 0.1 impulses/s) during the control period. A hyperpolarizing current pulse (−0.2 nA, 200 ms) was applied via the microelectrode to measure Rn during hypercapnia. Exposure to 15% CO2 decreased Em to −52 mV (see Fig. 1b, 2) and increased firing rate to 6 impulses/s. Rn increased from 66 to 90 MΩ (+35% change) (this effect is superimposed on the depolarization and is therefore not obvious). It generally took 4–8 min following hypercapnia for CO2-induced changes in Em, Rn and firing rate to return to control levels. Representative action potentials recorded before (1), during (2) and after (3) 15%