INTRODUCTION

Spinal Cord Injury (SCI) results in a loss of function such as mobility or sensation. SCI can be divided into two types of injury: complete and incomplete. A complete injury means that there is no function below the level of the injury; no sensation and no voluntary movement. An incomplete injury means that there is some function below the primary level of the injury. A patient with an incomplete injury may be able to move one limb more than another, may be able to feel parts of the body that cannot be moved, or may have more functioning on one side of the body than the other. With the advances in acute treatment of SCI, incomplete injuries are becoming more common. SCI usually induces swelling of the spinal cord, which result in spinal ischemia and a common cause of secondary damage at the time of injury. Although there is no cure for SCI at this time, the recent advances in acute treatment of SCI decrease in damage at the time of the injury. Steroid drugs such as methylprednisolone are often delivered to reduce loss of function in SCI patients. There are cases such as Frankel grade B [1] that motor disturbance does not improve, although feeling remains for sensory disturbance improve in a part of SCI patients. Moreover, it is reported that spinal cord ischemia or injury loses more motor functions than sensory functions. However, it is difficult to evaluate simply whether spinal sensory system is more vulnerable to ischemia or injury than motor system in SCI patients or SCI model animals, because of an anatomical complexity of the spinal cord and a variety of driving forces. Patch-clamp recording are recently developed and has been applied to a variety of spinal cord researches. In the present study, an ischemia-induced change in membrane currents was investigated in ventral and dorsal horn neurons of the rat spinal cord slices by using whole-cell patch-clamp technique to examine which is more vulnerable to ischemia at single cell level, spinal ventral horn neurons (motor neurons) or dorsal horn neurons (sensory neurons). The ischemia was stimulated by superfusing an oxygen and glucose-deprived medium (ISM), which is well established in brain slice preparations.

MATERIALS AND METHODS

Transverse spinal cord slices (500 μm in thickness) were prepared from L5 spinal cords of male Sprague-Dawley rats. A spinal cord slice was transferred to a recording chamber and placed on the stage of an upright microscope equipped with an infrared-differential interference contrast (IR-DIC) system. The slice was superfused (5-10 ml/min) with artificial ACSF solution contained (in mM): NaCl 117, KCl 3.6, CaCl2 1.2, MgCl2 1.2, NaHPO4 1.2, NaHCO3 25 and glucose 11; 95% O2 and 5% CO2, pH 7.3 at 36±1 °C. Spinal lamina regions were identified under lower magnification (with a 5X objective) and individual neurons were identified with a 40X objective under IR-DIC microscope and monitored by CCD camera on a video monitor screen. Whole-cell patch-clamp recordings were recently developed and has been applied to investigate a variety of spinal cord researches. In the present study, an ischemia-induced change in membrane currents was investigated in ventral and dorsal horn neurons of the rat spinal cord slices by using whole-cell patch-clamp technique to examine which is more vulnerable to ischemia at single cell level, spinal ventral horn neurons (motor neurons) or dorsal horn neurons (sensory neurons). The ischemia was stimulated by superfusing an oxygen and glucose-deprived medium (ISM), which is well established in brain slice preparations.

RESULTS

ISM superfusion for a several minutes produced robust increase in spontaneous excitatory postsynaptic current (sEPSC) frequency, following a huge inward current. Just after huge inward currents, membrane currents were unstable and synaptic activities could not be observed in all spinal neurons recorded, which indicated that ISM exposures induced cell death. The average latency to the huge inward currents after ISM exposures were 477±11s in 161 ventral horn neurons tested and 603±20s in 115 dorsal horn neurons tested. The average latency of dorsal horn neuron was significantly longer than that of ventral horn neurons. In addition, the average amplitude of the huge inward currents was 588±34 pA in 33 ventral horn neurons examined and 456±32 pA in 33 dorsal horn neurons examined. The average amplitude of the huge inward currents in ventral horn neurons was significantly bigger than that in dorsal horn neurons. Membrane capacitance was simultaneously measured in ventral horn and dorsal horn neurons. Average membrane capacitance was 62±3 pF in 40 ventral horn neurons and 39±1 pF in 54 dorsal horn neurons, which meant that ventral horn neurons are significantly bigger than dorsal horn neurons. Although the membrane capacitance in ventral horn neurons was much bigger than that in dorsal horn neurons, the membrane capacitance did not have any correlation with the latency of huge inward currents after ISM exposures. Furthermore, the recovery rate of membrane currents by superfusion of artificial ACSF solution was examined and compared between ventral and dorsal horn neurons. When ISM was immediately switched to normal Krebs solution at the peak of huge inward current, not only membrane current but also sEPSC frequency fully recovered in 60% of ventral horn neurons, 75% of dorsal horn neurons. When superfusion of artificial ACSF solution was performed 0.5, 1.0, and 1.5 min after the peak of huge inward currents, the recovery rate was 40% in ventral horn neurons and 54% in dorsal horn neurons, 18% in ventral horn neurons and 33% in dorsal horn neurons, 8% in ventral horn neurons and 27% in dorsal horn neurons, respectively. The recovery rate was dependently decreased in time. The recovery rate of dorsal horn neuron tended to be high compared with that in ventral horn neuron at immediately, 0.5, 1.0, and 1.5 min after the peak bottom of rapid inward current. When artificial ACSF solution was superfused at 2 min after the peak of huge inward current, all ventral horn and dorsal horn neurons recorded died.

DISCUSSION

Brain neurons in distinct region have different vulnerability to brain injury or ischemia, which is called “selective vulnerability of brain neurons” [4,5,6,7]. However, it is unclear which is more vulnerable to ischemia, spinal ventral horn neuron or dorsal horn neuron. In this study, the latency to the huge inward currents after ISM exposures in ventral horn neurons was much longer than that in ventral horn neurons. In addition, when superfusion of artificial ACSF solution was performed, the recovery rate in dorsal horn neurons was higher than that in ventral horn neurons. These results have suggested that ventral horn neuron is more vulnerable to ischemia than dorsal horn neuron at a single cell level. Consistently, the amplitude of huge inward currents in ventral horn neurons is bigger than that in dorsal horn neurons. It means that ISM exposures induced much more amount of cation, such as Ca2+, influx into ventral horn neurons than dorsal horn neuron. This may be a possible mechanism that motor disturbance is more severe than sensory disturbance in a part of SCI patients. There were some reports that the vulnerability of neurons was related to neuron size in brain [8]. However, rapid inward current latency and membrane capacitance were not significantly correlated in this study. Thus, differ from brain neurons, the vulnerability to ischemia in spinal neurons may not be related with the size of neurons. Distinct function in ion channels including glutamate receptors may be related to difference of vulnerability to injury or ischemia between spinal motor and sensory neurons.

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COMPARISON OF VULNERABILITY BY IN VITRO ISCHEMIA

IN VENTRAL AND DORSAL HORN NEURONS OF THE RAT SPINAL CORD

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