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Isolation and electrical characterization of the rat spinal dorsal horn neurons

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The spinal dorsal horn is the area where primary afferent fibers terminate and cutaneous sensory information is processed. A number of putative neurotransmitter substances, including excitatory and inhibitory amino acids and peptides, are present in this region and sites and cellular mechanisms of their actions have been a target of numerous studies. In this study, single neurons were acutely isolated and the properties of whole cell current and responses to excitatory and inhibitory neurotransmitters were studied by the patch clamp method.

Young rats (7-14 days) were anesthetized with diethyl-ether, and the lumbar spinal cord was excised and cut transversely at a thickness of 300 μm by Vibroslicer. The treatment of spinal slices with low concentration of proteases (pronase and thermolysin 0.75 mg/ml) and mechanical dissociation yielded isolated neurons with near intact morphology. Multipolar, ellipsoidal and bipolar, and pyramidal cells were shown. By applying step voltage pulses to neurons held at -70 mV, two types of inward currents and one outward currents observed. The fast activating and inactivating inward current was the Na^+ current because of its fast kinetics and blocking by 0.5 μM TTX, a specific blocker of Na^+ channel. The second type of inward currents were sustained. Based on their kinetics and current-voltage relations, it was likely that the second type of inward current was the voltage-dependent Ca^{2+} current. In the presence of TTX, the steady-state currents mainly represented outward K^+ current which looked like the delayed rectifier K^+ current. In addition, the membrane currents produced by agonist of excitatory amino acid (EAA) receptor and the endogenous transmitter candidate L-glutamate were recorded in isolated whole-cell voltage clamped neurons as well as responses to inhibitory amino acids (γ-amino butyric acid, glycine). Drugs were applied by a method that allows complete exchange of the solution within 1 sec; an infinite number of solutions can be applied to a single cell.

Glutamate and NMDA induced inward currents. The amplitudes of currents were 116.7 ± 12.40 pA (n=5) and 49.30 ± 6.90 pA (n=3) in glutamate (30 μM) and NMDA (30 μM), respectively. Glycine (1 μM) potentiated glutamate-induced currents to 4-5 times and NMDA-induced currents to 8-10 times. In the high internal Cl^- (143 mM), inward currents were induced by 30 μM glycine. These currents reached 31.35 ± 6.12 nA (n=2) and rapidly desensitized after peak and reached steady-state. At the same condition, inward currents were induced also by 1 μM GABA. These currents reached 225.77 ± 41.64 pA (n=3) and decreased continuesly after peak of currents. Our data indicate that there is reasonable agreement between many of the responses of isolated neurons and those studied in *in vivo* and *in vitro* slice and culture preparations. Therefore these results mean that isolated neuron cells were healthy. This method will be used with usefulness investigation of responses of neurotransmitters and peptides

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Key Word: spinal dorsal horn neurons, excitatory-inhibitory amino acids