Short communication

Ionic Currents in the Somatic Membrane of Identified T-Mechanosensory Neurons Isolated from Segmental Ganglia of the Medicinal Leech

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Based on physiological investigations, Nicholls and Baylor (1968) identified and described three types of mechanosensory neurons in segmental ganglia of medicinal leech: T-cells receiving touch information, P-cells sensitive to changes in pressure applied on the skin, and N-cells affected by nociceptive stimuli. The electrical excitability and the passive electrical parameters of the somatic membrane of mechanosensory neurons have been thoroughly investigated (Baylor and Nicholls 1969a, b, c; Jansen and Nicholls 1973; Fuchs et al. 1981). Differences have been observed in the electrical pattern between different types of mechanosensory neurons. T-cells were found to differ from the other types of the leech neurons also by their sensitivity to some drugs that alter the function of the voltage-dependent ionic channels (Kleinhaus and Prichard 1983; Johansen and Kleinhaus 1985). Attempts to determine the characteristics of the different types of leech neurons under voltage-clamp conditions using single- or double- barrelled microelectrodes came up against some difficulties (Kleinhaus and Prichard 1983). We applied the methods of intracellular perfusion and voltage-clamp to study the ionic mechanisms of excitability of the somatic membrane of leech neuron under controlled external and internal ionic conditions.

The experiments were carried out on type T-mechanosensory neurons isolated from segmental ganglia of medicinal leech. Ganglia from *Hirudo medicinalis* were treated with 0.3 % Pronase E, dissolved in standard leech Ringer solution (Table 1, bottom), for 20 min at 18—20 °C. After the treatment, the ganglia were transferred to the standard solution and kept there for 60 min at 4 °C. Using finely sharpened metal needles, the connective tissue sheaths covering the ganglion were torn in several places as far as possible from the selected neurons and carefully removed from the glial packets with nerve cell bodies

Table 1. The composition of the solutions mmol/l $^{-1}$, pH 7.4

Extracellular solution	NaCl	KCl	$CaCl_2$	$BaCl_2$	Tris-Cl	MGA
Normal leech Ringer	115	4	1.8	—	10	—
Ba^{2+}-enriched	103	4	1.8	10	10	—
Na^+-free	—	4	1.8	—	10	115
Na^+-free, Ca^{2+}-enriched	—	4	10	—	10	107
Na^+-free, Ba^{2+}-enriched	—	4	1.8	10	10	105

Intracellular solution	KCl	EGTA	Tris-Cl	Tricine	MGA
Normal	120	0.2	10	—	—
K^+-free	—	0.2	—	30	100

attached. The axons of the neurons were cut off, the isolated neuronal somata were collected with a micropipette and allowed to recover over 1 h in standard leech saline.

Intracellular perfusion of isolated nerve cell bodies was performed on a V-shaped polyethylene micropipette by the technique originally developed in Kostyuk's laboratory (Krishtal and Pidoplichko 1975). With the help of a micromanipulator and using a thin needle, a pore was made under a microscope in the top of a pipette; the inner diameter of the pore was 15—20 μm, i.e. about one-third of the cell diameter (45—50 μm), giving a series resistance of 200—250 kΩ Electrodes made of Ag wire coated with AgCl were used to connect the electronic equipment to the experimental chamber.

Bridges filled with 3 mol.l^{-1} KCl solution in agar connected the two electrodes to artificial intracellular and extracellular solution circuits. The experimental arrangement and the electronic circuitry allowed studying somatic membrane action potentials under current-clamp conditions and the corresponding transmembrane ionic currents under voltage-clamp conditions. The current-clamp circuit used in our experiments did not permit measurement of the cell body resting potential; due to this, it was used only for neuron identification. Correction of the suction pipette potential for series resistance was made as described by Osipchuk and Timin (1984). After the compensation, the transient capacitive current time varied from 100 to 300 μs in different cells. The linear components of the leakage currents associated with the transmembrane ionic currents were subtracted during the experiments.

Throughout the experiments the isolated neurons were perfused with external and internal solutions. The ionic composition of the basic solutions is shown in Table 1. The normal leech Ringer fluid was prepared as described by Jansen et al. (1981). For the study of net inward currents, extracellular Na^+ and intracellular K^+ were substituted by N-methyl-D-glucamine (Mitra and Morad 1985). To avoid possible nonspecific outward currents (Byerly and Hagiwara

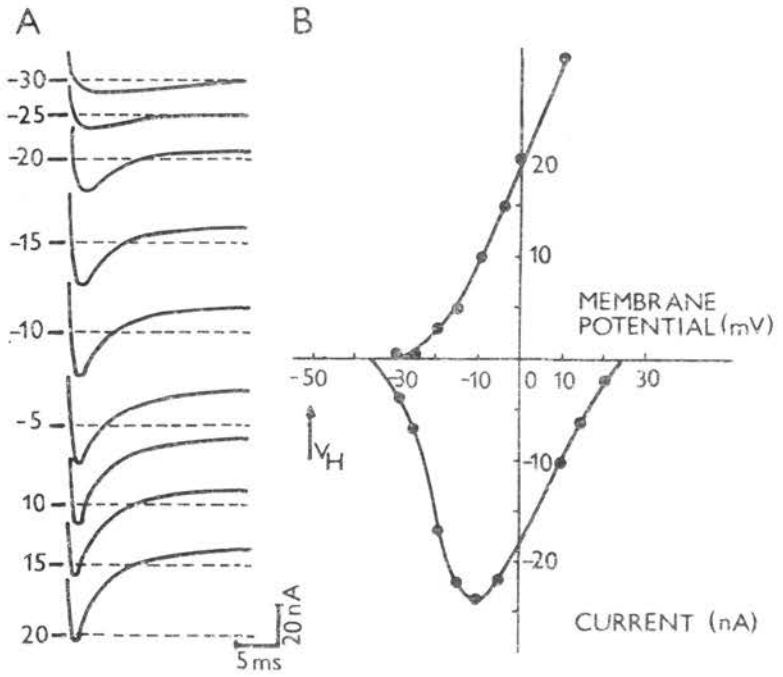

Fig. 1. Ionic currents in the somatic membrane of T-mechanosensory neurons of the leech. *A*. Currents elicited by membrane potential steps from holding potential (V_H) −50 mV to values indicated at each trace (in mV). *B*. Current-voltage relationship for inward currents (determined at the most negative current value) and for outward currents (measured as a positive current value at 25 ms from the pulse beginning). The external solution was the standard leech Ringer, the internal solution was normal (Table 1). Holding potential was −50 mV. The single suction pipette method for internal perfusion and voltage clamping.

1982; Valkanov and Boev 1986a), the K^+-free intracellular solution was buffered with Tricine. The presence of Ca^{2+}-channels was examined in Na^+-free extracellular solutions with increased concentrations of Ca^{2+} or Ba^{2+}.

The experiments were carried out at 18—20 °C.

The isolation procedure used provided high yields of undamaged excitable T-nerve cell bodies. No vesicles and vacuoles reported by other authors (Veprintsev et al. 1984) were seen. Under current-clamp conditions the cell bodies usually responded to stimulation by square wave electric pulses with single action potentials. Occasionally, upon a longer depolarizing stimulus, the T-cell bodies fired repetitively. Both the pattern and the duration of the action potentials corresponded to those described by Nicholls and Baylor (1968) in cell bodies of T-mechanosensory neurons of the medicinal leech.

Under voltage-clamp conditions, with normal extracellular and intracellular solutions (Table 1), a fast transient inward current followed by an outward

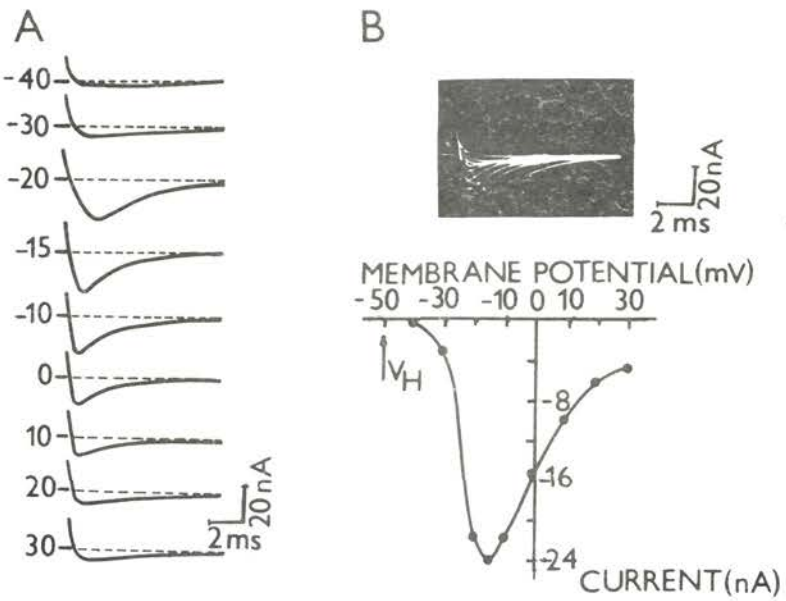

Fig. 2. Net inward currents in an internally perfused T-cell body. *A*. Currents evoked by depolarizing pulses. The figure at the traces show pulse potentials (in mV). *B*. Plot of the peak values of the voltage-clamp currents (from the family shown on the top) against fixed membrane potential. Holding potential (V_H) −50 mV. The extracellular solution was normal leech Ringer, the intracellular solution was K^+-free (Table I). The single suction pipette method for internal perfusion and voltage clamping.

current was recorded (Fig. 1*A*). The inward current was activated at membrane potentials ranging between −35 and −40 mV; it reached its maximum at about −15 mV for 2 ms and its reversal potential was 200 to 25 mV. As seen from current-voltage relationship shown in Fig. 1*B*, the outward current was activated early during the inward current increase at membrane potential levels between −25 and −30 mV. The outward current could be blocked by extracellular application of both TEA^+ (15 mmol/l) and Ba^{2+} (10 mmol/l).

The outward currents disappeared upon intracellular perfusion with K^+-free solutions (Fig. 2*A*), with the net inward currents being preserved only. Figure 2*B* illustrates the peak current-voltage relationship for the net inward current. The latter was activated at −35 to −40 mV, reached its maximum at −15 to −18 mV for about 1.5 ms and was inactivated at approximately 45 to 55 mV. The inward current could be eliminated by extracellular application of TTX (final concentration 10^{-5} mol/l; Fig. 3) or by withdrawal of Na^+ from the extracellular solution. Then, no residual inward current was recorded even upon increasing Ca^{2+} concentration (or that of Ba^{2+}) up to 10 mmol/l.

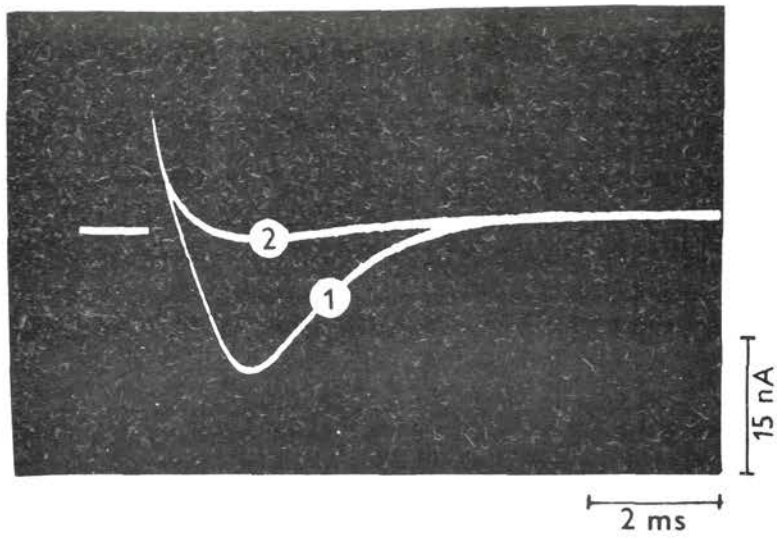

Fig. 3. TTX-block of the inward current in T-cells. 1-transient inward current at −50 mV holding potential and 30 mV depolarizing step in Ba^{2+}-enriched extracellular solution and K^+-free intracellular solution. 2-the same current as in 1 but 5 min after TTX (10^{-5} mol/l).

Kleinhaus and Prichard (1983) described Na^+-dependent spike potentials in the T-mechanosensory neurons. They observed that the maximum rate of somatic membrane depolarization followed extracellular Na^+-concentration in an approximately linear fashion, suggesting that other ions contributed little to the depolarization. TTX (5×10^{-5} mol/l) depressed the depolarization phase of the T-cell somatic action potential and raised the threshold for potential occurrence. The recorded fast inward current (Fig. 2) was carried by Na^+-ions. This suggestion is based on the elimination of this current in Na^+-free medium and its depression by TTX (10^{-5} mol/l). The current had a fast activation kinetics and reached its maximum for 1—2 ms; this corresponds to the development kinetics of the T-cell body action potential appearing after intrasomatic stimulation (Nicholls and Baylor 1968; Kristan 1982).

All somatic membranes investigated so far contained voltage-dependent Ca^{2+} channels (Hille 1984; Kostyuk 1984). After the removal of Na^+ from the extracellular solution, no residual inward current could be recorded in our experiments, even upon raising Ca^{2+} or Ba^{2+} concentration to 10 mmol/l. This suggests a total absence, or a presence of only a negligible voltage-dependent Ca^{2+}-conductance in the T-cell somatic membrane. This is supported by the finding that sustained action potentials carried by divalent ions (Johansen and Kleinhaus 1985) were not recorded in these cells in Na^+-free TEA^+-containing Ringer solution.

The outward current in the T-cells was blocked by extracellular TEA^+ or Ba^{2+} and disappeared in K^+-free intracellular solution (Fig. 2). It was carried by K^+ ions and resembled the delayed K^+ currents in axons (Hille 1984).

Kleinhaus and Prichard (1975; 1977) established that TEA^+ increases the spike duration of identified leech neurons. If a TEA^+-revealed somatic voltage-dependent Ca^{2+}-conductance resulting from K^+-conductance block (Johansen and Kleinhaus 1985) is the reason for the action potential prolongation, then Ca^{2+}-dependent inward currents should be recorded under voltage-clamp conditions after intracellular perfusion with K^+-free solutions or after TEA^+-application. This is the case with type N mechanosensory neurons of the leech (Valkanov and Boev 1986b). However, the net inward current in the T-cells shown in Fig. 3 was blocked by TTX (10^{-5} mol/l). Probably, the observed prolongation of the spike potential repolarization phase in the T-nerve cell body does not depend on the increase in membrane Ca^{2+}-conductance. A speculative explanation for this phenomenon might be a delayed inactivation kinetics of Na^+-channels and delayed activation kinetics of K^+-channels induced by TEA^+.

The characteristics of the ionic currents in the somata of T-mechanosensory neurons differ from those reported for P and N cells (Roberts and Almers 1984; Valkanov and Boev 1986b). These characteristics may be employed as an additional criterion for cell identification.

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