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A chloride channel in rat and human axons

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Current recordings from single chloride channels were obtained from excised and cell-attached patches of rat and human axons. In rat axons the channels showed an outwardly rectifying current–voltage relationship with a slope conductance of 33 pS at negative membrane potentials and 65 pS at positive potentials (symmetrical 150 mM CsCl). They were measurably permeable for cations ($P_{Na}/P_{Cs}/P_{Cl}=0.1/0.2/1$). Channel currents were independent of cytoplasmatic calcium concentration. Inactivation was not observed and gating was weakly voltage dependent. Cl^- channels in human axons showed similar gating behavior but had a lower conductance.

Enzymatic dissociation and demyelination of myelinated axons enables single channel current recordings from the nodal and paranodal region [11]. Using this method one type of Na^+ channel and several types of K^+ channel have been described [11, 12]. Another axonal conductance known from macroscopic current recordings in squid axons [10], non-myelinated rat axons [3] and myelinated rabbit axons [4] is Cl^- dependent. To our knowledge, single chloride channels have not been described previously in mammalian axons.

A certain type of Cl^- channel with distinctive properties has been found in a variety of other cells, e.g. cultured hippocampal neurons [5, 16], *Drosophila* neurons [18], crustacean axons [13] or tracheal epithelial cells [17]. This channel has an intermediate conductance (10–100 pS), is significantly permeable to cations (permeability ratio in the range of 0.1–0.35) and shows weak voltage-dependent gating. This type of channel has been designated as a 'background' Cl^- channel by Franciolini and Petris [6]. A Cl^- channel with similar electrophysiological characteristics was observed in about 5% of all gigaseal recordings from axonal patches obtained in the present study.

Experiments were performed on ventral rat spinal roots and specimens of human sural nerves. Male Wistar rats (250–350 g) were anaesthetized with urethane (1.25 mg/kg, i.p.). After laminectomy 5 – 10 ventral roots were removed and transferred into a culture dish. Enzymatic dissociation and paranodal demyelination were performed in two steps according to the procedures described by Jonas and coworkers [11] with some modifications. Spinal roots were first treated at 37°C with collagenase ('Worthington' type CLS II, 135 U/mg; Biochrom, Berlin; 3 mg/ml, diluted in normal Ringer solution) for 80 min and subsequently incubated for 30 min in protease (type XXIV; 8 U/mg; Sigma, Deisenhofen, F.R.G.; 1 mg/ml, diluted in calcium-free Ringer solution). After this procedure the spinal roots were cut into 3 mm segments and single fibers were dissociated by gentle shaking. Finally, they were transferred into dishes precoated with poly-L-lysine (1 mg/ml; Sigma). Sural nerves were obtained from patients who required nerve biopsy for clinical diagnosis at the Department of Neurology, Technical University of Munich. Human nerves were treated as described above. However, in the first step, collagenase plus protease (type X, 62 U/mg; Sigma; 0.03 mg/ml) were used for 120 min.

Single channel recordings were performed in solutions of the following composition (in mM): (a) 'CsCl': 150 CsCl, 0.464 $CaCl_2$, 1.18 $MgCl_2$, 1 EGTA, 10 HEPES; (b) 'NaCl': 150 NaCl, 0.464 $CaCl_2$, 1.18 $MgCl_2$, 1 EGTA, 10 HEPES and (c) 'NMDG-Cl': 150 *N*-methyl-D-glucamine chloride, 0.464 $CaCl_2$, 1.18 $MgCl_2$, 1 EGTA, 10 HEPES; pH 7.4 (adjusted with NaOH). Wherever solutions did not have one of the compositions given above, it is explicitly mentioned in the text. Experiments were done at room temperature, approximately 22°C. Recordings were performed by standard patch-clamp technique [8]. Patch pipettes were drawn (DMZ puller; Zeitz, Augsburg, F.R.G.) from borosilicate glass tubings

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(GC 150 TF - 10, Clark Electromedical Instruments, Pangbourne, U.K.), coated with Sylgard and fire-polished immediately before the experiment. The pipettes had a resistance of 10–20 MΩ (150 mM CsCl). Recordings were made with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA, U.S.A.). The current signals were low pass filtered at 1.2 kHz and digitized at a sampling rate of 10 kHz. They were stored and analyzed using a Tandon 386/33 computer and pClamp 5.5 software (Axon Instruments). Voltages given indicate membrane potential.

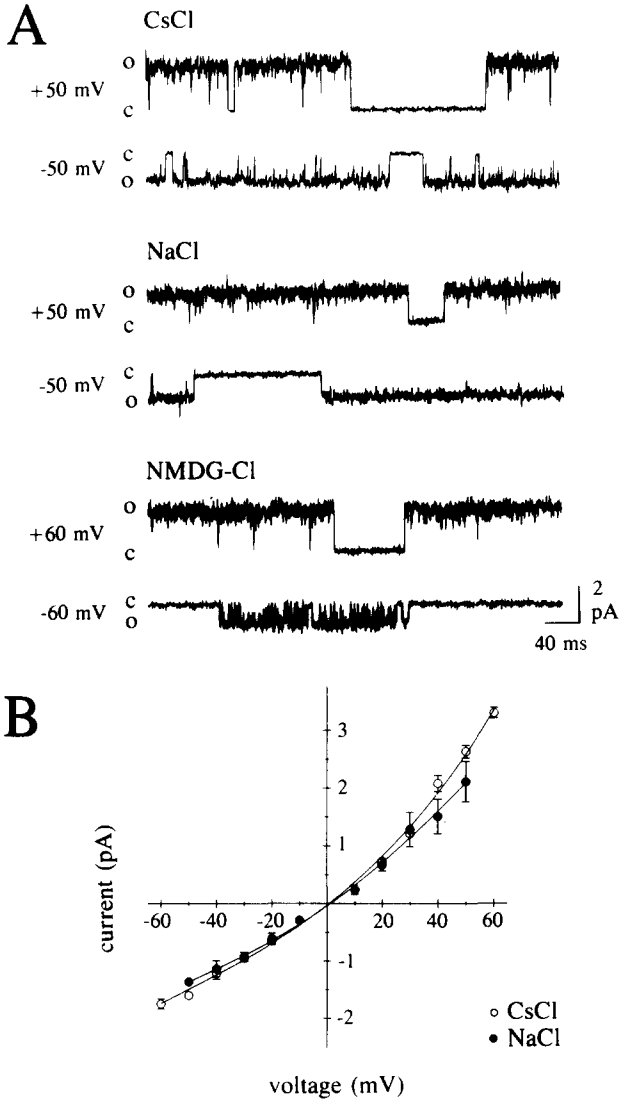

Fig. 1. A: single channel recordings of the Cl^- channel from rat axons in symmetrical 150 mM CsCl solution (excised, outside-out configuration), in symmetrical 150 mM NaCl solution (excised, inside-out configuration) and in symmetrical 150 mM *N*-methyl-D-glucamine Cl^- (NMDG-Cl) solution (cell-attached configuration). Voltages given are membrane potentials. Closed and open states are indicated by c and o, respectively. B: single channel current–voltage relationship of the Cl^- channel from rat spinal roots. Illustrated data are averaged (mean ± S.D.) from 4 recordings in symmetrical CsCl (○) and from 4 recordings in symmetrical NaCl (●) and each current value is the average of 5 amplitude measurements from these 4 channels. In CsCl solutions the slope conductance ranged from 33 ± 3 pS (mean ± S.D.) at negative membrane potentials to 65 ± 2 pS at positive membrane potentials with a reversal potential close to 0 mV. The slope conductance in NaCl was 24 ± 1 pS for inward currents and 46 ± 6 pS for outward currents. The curves were fitted by a polynomial function.

The recordings illustrated in Fig. 1A were obtained from patches of the (presumed) paranodal area in different symmetrical solutions: CsCl, NaCl and NMDG-Cl. Channel activity was observed in the cell-attached as well as in the excised configuration. Current–voltage relationships shown in Fig. 1B reveal an outward rectification, which is typical for most of the non-transmitter gated Cl^- channels [6, 7]. In 150 mM CsCl solutions slope conductance ranged from 33 ± 3 pS (mean ± S.D., $n=4$) at negative membrane potentials (−50 to −20 mV) to 65 ± 2 pS at positive membrane potentials (+ 20 to + 50 mV) with a reversal potential close to 0 mV. In symmetrical NaCl solutions the slope conductance was 24 ± 1 pS ($n=4$) for inward currents and 46 ± 6 pS for outward currents. Channel activity was also found in solutions with NMDG-Cl.

We have observed different patterns of gating. Single channel gating was mainly characterized by long lasting open and short closed states (see Fig. 1A) and no in-activation. In < 10% the channel spontaneously shifted to a rapid 'flickering' behavior during the recordings. In addition, long lasting closed states were found. Similar gating behavior was described for a Cl^- channel from lobster walking leg nerves [13]. Open probability (P_o) was calculated from the all point amplitude histogram of patches with only one channel. For each voltage a minimal time of 15 s was analyzed. In most recordings P_o was high (0.80–0.95; not shown). P_o showed a weak voltage dependence; in some patches it increased with depolarization. Since a Ca-dependent Cl^- conductance has been reported, e.g. in cultured mouse spinal neurones [14], we tested the effects of different Ca concentrations (ranged from 2.2 mM to nominally Ca-free solutions). In our experiments single channel currents were not influenced by cytoplasmatic calcium (not shown).

Further experiments were performed in asymmetrical solutions to estimate permeability ratios for cations versus Cl^-. Therefore, the shift of the reversal potential of the current–voltage relationship was measured. By means of the Goldman-Hodgkin-Katz equation (using ion activities as given by Robinson and Stokes [15]) permeability ratios were calculated after subtraction of junction potentials. In all experiments, unilateral changes in the Cl^- concentration shifted the reversal potential in the direction expected for a Cl^- channel (see Fig. 2). In one set of experiments the bath contained 150 mM NaCl and the pipette was filled with 75 mM NaCl (50% of NaCl was replaced isoosmotically by sucrose).

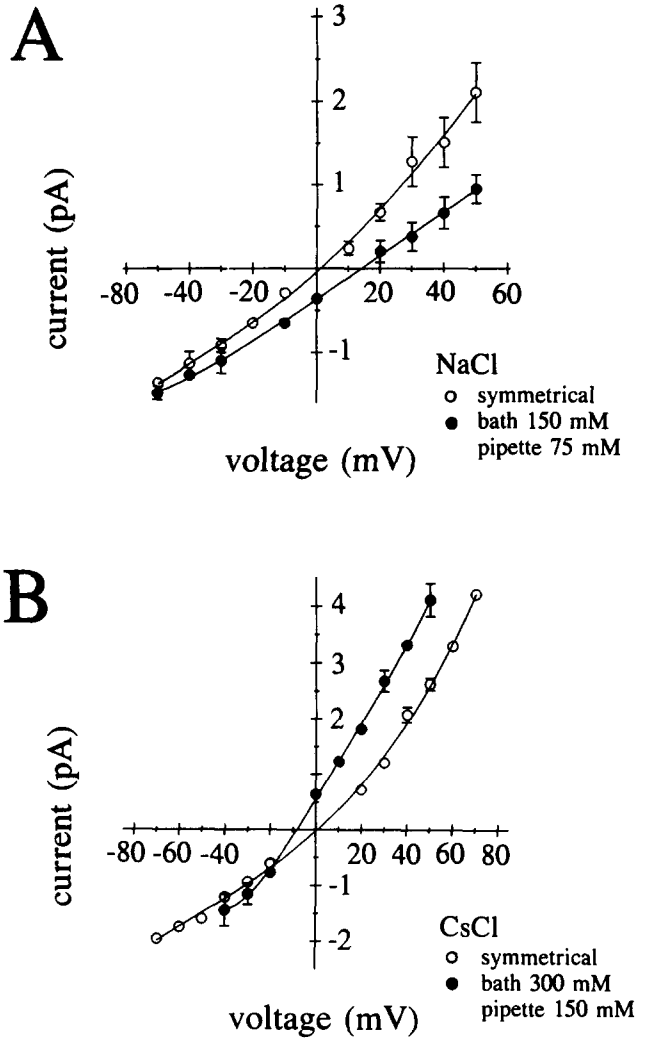

Fig. 2. Single channel current–voltage relationship of the Cl^- channel from rat axons in symmetrical and asymmetrical solutions. Illustrated data are averaged (mean ± S.D.) and determined as described in Fig. 1. A: data from recordings in the inside-out configuration. Unfilled circles (○) show the current–voltage relationship under conditions of symmetrical NaCl ($n=4$). The reversal potential was +1.5 mV. Filled circles (●) represent data ($n=4$) with 150 mM NaCl in the bath and 75 mM NaCl in the patch pipette (50% of NaCl substituted isoomotically by sucrose). Note the reversal potential of +14 mV, i.e. there was a shift of +12.5 mV. B: data from recordings in the outside-out configuration. Unfilled circles (○) show data under conditions of symmetrical 150 mM CsCl ($n=4$). The reversal potential was +0.5 mV. Filled circles (●) show the current–voltage relationship with 150 mM CsCl in the pipette and 300 mM CsCl in the bath ($n=3$). The reversal potential was −9.5 mV, i.e. the shift was −10 mV. For calculation of permeability ratios see text. The curves were fitted by a polynomial function.

As illustrated in Fig. 2A, this caused a shift of the reversal potential of ≈ +12.5 mV, i.e. the permeabitity ratio P_{Na}/P_{Cl} was ≈0.1. In a second set of experiments performed in the outside-out configuration the pipette contained 150 mM CsCl solution and the bath solution consisted of 300 mM CsCl (Fig. 2B). The shift of the reversal potential of ≈ −10 mV measured under these conditions revealed a permeability ratio of P_{Cs}/P_{Cl} ≈0.2. Experiments with potassium were not performed because there is a high density of K^+ channels in axons, which makes it difficult to identify Cl^- channels. The observed significant permeability for cations was not unexpected, because in serveral tissues Cl^- channels with such characteristics have been described [2, 5, 17].

Fig. 3A shows single channel recordings from a human axon performed in symmetrical NaCl. In human axons, we also observed a Cl^- channel, which, however, had a lower conductance. The current–voltage relationship revealed outward rectification (see Fig. 3B). Slope conductance was 14 ± 1 pS at negative membrane potentials (−50 to −20 mV) and 20 ± 1 pS ($n=3$) at positive potentials. Gating characteristics of human and rat Cl^- channels were similar (see above).

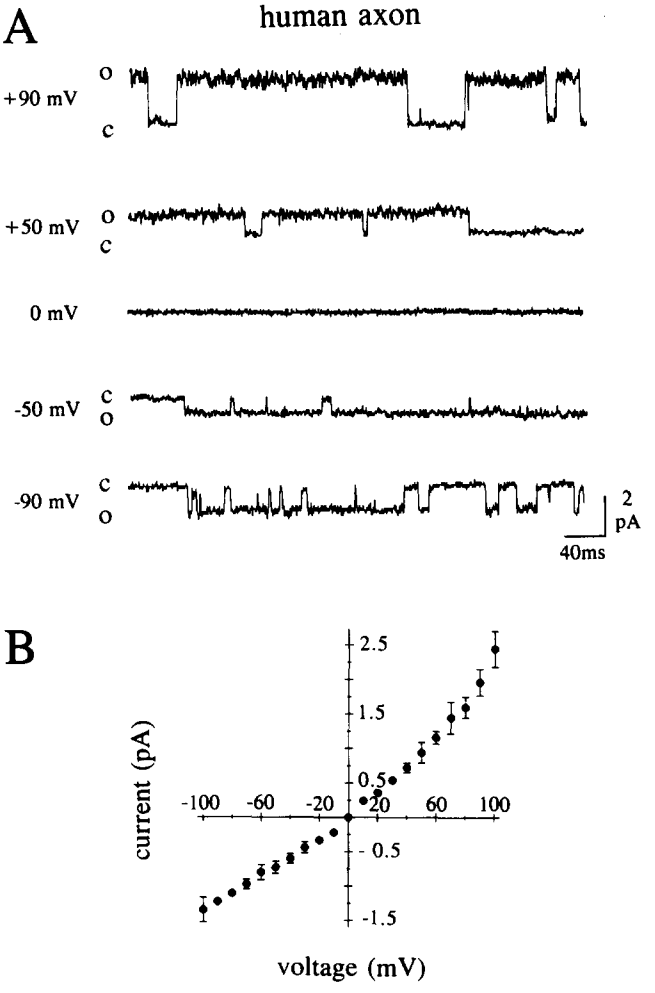

Fig. 3. A: single channel recordings of the Cl^- channel from specimens of human sural nerve in symmetrical 150 mM NaCl solution (excised, inside-out configuration). Closed and open states are indicated by c and o, respectively. B: single channel current–voltage relationship of the Cl^- channel. Illustrated data are averaged (mean ± S.D.) from 3 recordings in symmetrical NaCl and each current value is the average of 5 amplitude determinations from these 3 channels. At potentials from −50 to −20 mV the slope conductance was 14 ± 1 pS and at potentials from 20 to 50 mV it was 20 ± 1 pS.

In contrast to sodium and potassium currents, there are very few reports of macroscopic Cl^- currents in axons. Since most Cl^- channels show weak voltage- and time-dependent behavior [6], they may have been dismissed as leak current. The Cl^- channel found in our experiments may thus contribute to leakage current. Chiu and Schwartz [3] described a presumed Cl^- current in rabbit axons, which accounts for about 5% of total current. In squid axon, Inoue [10] recorded a Cl^- current, which showed strong outward rectification and no time-dependent properties. At resting potential this current contributed $< 10\%$ of the total conductance. These macroscopic currents have many similarities with the Cl^- channel described in this study.

In summary, we observed a Cl^- channel on rat and human axons which is (a) open within a wide voltage range, (b) does not inactivate and (c) shows outward rectification. Functionally, this channel may contribute to the background conductance found in axons (see for discussion refs. 1, 9), which stabilizes membrane potential and consequently maintains normal cell excitability.

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