

Large Conductance Ca^{2+} -activated K^+ Channels in the Soma of Rat Motoneurons

B.V. Safronov, W. Vogel

Physiologisches Institut, Justus-Liebig-Universitaet Giessen, Aulweg 129, D-35392 Giessen, Germany

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Abstract. Properties of large conductance Ca^{2+} -activated K^+ channels were studied in the soma of motoneurons visually identified in thin slices of neonatal rat spinal cord. The channels had a conductance of 82 ± 5 pS in external Ringer solution (5.6 mM K_o^+ //155 mM K_i^+) and 231 ± 4 pS in external high- K_o solution (155 mM K_o^+ //155 mM K_i^+). The channels were activated by depolarization and by an increase in internal Ca^{2+} concentration. Potentials of half-maximum channel activation (E_{50}) were -13 , -34 , -64 and -85 mV in the presence of 10^{-6} , 10^{-5} , 10^{-4} and 10^{-3} M internal Ca^{2+} , respectively. Using an internal solution containing 10^{-4} M Ca^{2+} , averaged K_{Ca} currents showed fast activation within 2–3 msec after a voltage step to +50 mV. Averaged K_{Ca} currents did not inactivate during 400 msec voltage pulses. External TEA reduced the apparent single-channel amplitude with a 50% blocking concentration (IC_{50}) of 0.17 ± 0.02 mM. K_{Ca} channels were completely suppressed by externally applied 100 mM charybdotoxin. It is concluded that K_{Ca} channels activated by Ca^{2+} entry during the action potential play an important role in the excitability of motoneurons.

Key words: Potassium channel — Motoneurone — Spinal cord — Slice

Introduction

Potassium channels activated by Ca^{2+} ions entering the cell during an action potential (K_{Ca} channels) play an important role in cell excitability (Kaczorowski et al., 1996; Rudy, 1988). Different types of K_{Ca} channels were found in many neuronal and non-neuronal tissues (Barret, Magleby & Pallotta, 1982; Blatz & Magleby,

1986; Jonas et al., 1991). In motoneurons, K_{Ca} currents were shown to contribute to action potential repolarization and to the slow afterhyperpolarization (Walton & Fulton, 1986; Takahashi, 1990; Umemiya & Berger, 1994). It was suggested that the slow apamin-sensitive afterhyperpolarization (Zhang & Krnjevic, 1987; Viana, Bayliss & Berger, 1993) is caused by low conductance voltage-independent K_{Ca} channels (Blatz & Magleby, 1986). In contrast, large conductance TEA-sensitive K_{Ca} channels appear to contribute to action potential repolarization. Unfortunately, very little is known until now about the properties of single large conductance K_{Ca} channels in the soma of motoneurons. Single-channel and functional characteristics of K_{Ca} channels studied in some other preparations are not necessarily applicable to motoneurons, since many different isoforms of large conductance K_{Ca} channels are expressed in the central nervous system (Tseng-Crank et al., 1994).

The main purpose of this study was to describe the single-channel properties of large conductance K_{Ca} channels in rat motoneurons. In our preceding paper (Safronov & Vogel, 1995) we used thin slices of newborn rat spinal cord, in order to describe single voltage-gated Na^+ and K^+ channels in the soma of visually identified motoneurons. The main advantage of the slice preparation in comparison with neurones grown in cell culture is the possibility to study channels whose properties and distributions were not modified by enzymatic treatment nor changed during development in culture. The present investigation is a further step in characterizing ionic channels underlying the major voltage-gated and background conductances in the soma of motoneurons.

Materials and Methods

SLICE PREPARATION AND SOLUTIONS

The preparation of spinal cord slices and the methods of motoneurone identification have been previously described (Edwards et al., 1989;

Takahashi, 1990; Safronov & Vogel, 1995). In brief, 2- to 4-day-old rats were rapidly decapitated and the spinal cord was cut out in ice-cold Ringer solution. 200 μm thin slices were prepared from the lumbar enlargement (L3–6) of the spinal cord.

Ringer solution used for preparing and maintaining the slices contained (in mM): NaCl 115, KCl 5.6, CaCl_2 2, MgCl_2 1, glucose 11, NaH_2PO_4 1, NaHCO_3 25 (pH 7.4 when bubbled with a 95%–5% mixture of O_2 – CO_2). To reduce synaptic activity in motoneurons, the slices were perfused in the experimental chamber with a similar Ringer solution in which Ca^{2+} and Mg^{2+} concentrations were set to 0.1 and 5 mM, respectively (low- Ca^{2+} -high- Mg^{2+} -Ringer). These solutions were used in current-clamp experiments shown in Figs. 6 and 7. For single-channel recordings in inside-out and some outside-out patches, solutions in which the pH was buffered with HEPES-NaOH or HEPES-KOH were used. This Ringer solution contained (in mM): NaCl 136.4, KCl 5.6, CaCl_2 0.1, MgCl_2 5, glucose 11, Hepes 10. The pH was adjusted to 7.4 with 4.6 mM NaOH. External solution with 155 mM K^+ (high- K_o solution) contained (in mM): NaCl 5, KCl 152.5, CaCl_2 2, MgCl_2 1, Hepes 5 (pH was adjusted to 7.4 with 2.5 mM KOH).

0.05% bovine serum albumin was added to all solutions containing charybdotoxin and apamin (Latoxan, Rosans, France) to prevent nonspecific binding of the blocker molecules. TEA was directly added to external solutions.

The internal solution used for investigation of K_{Ca} channels contained (in mM): NaCl 5, KCl 150.6, MgCl_2 1 and Hepes 10 (pH was adjusted to 7.3 by 4.4 mM KOH). Different Ca_i^{2+} concentrations were prepared according to Barrett et al. (1982). Internal solutions containing 10^{-3} , 10^{-4} and 10^{-5} M Ca^{2+} were obtained by adding 1000, 100 and 10 μM CaCl_2 , respectively, directly to the solution (contamination by CaCl_2 from other salts was around 1.5 μM). 10^{-6} M concentration of free Ca^{2+} was obtained by addition of 80 μM EGTA and 74.4 μM CaCl_2 (Barrett et al., 1982). Internal solution used for the pipette filling in current-clamp experiments (Figs. 6 and 7) contained (in mM): NaCl 5, KCl 144.4, MgCl_2 1, EGTA 3 and Hepes 10 (pH was adjusted to 7.3 by 10.6 mM KOH). EGTA, known as a slow Ca^{2+} buffer, bound free intracellular Ca^{2+} in the resting neurones but had no influence on fast transient increase in internal Ca^{2+} concentration during single action potential.

Motoneurons were identified as the largest cells in the ventrolateral part of the ventral horn. The mean lengths of the long and short diameters of the cells investigated here were 25.5 ± 0.5 μm (34 cells) and the mean resting potential was -68.5 ± 2.0 mV (21 cells). In current-clamp recording mode the neurones fired spontaneous action potentials followed by typical prolonged afterhyperpolarizations. After an injection of sustained depolarizing currents, motoneurons always responded with trains of action potentials (Granit, Kernell & Shortess, 1963). Here and elsewhere the values are quoted as mean \pm SEM unless otherwise stated.

Inside-out patches were studied in an additional small (0.2 ml) chamber (Safronov & Vogel, 1995) which could be perfused with different internal solutions. A complete exchange of the solution in the small chamber required 6–10 sec. In these experiments, we employed perfusion capillaries made from glass instead of the normally used stainless steel needles, in order to prevent the interaction between EGTA and metal ions.

CURRENT RECORDINGS

Experiments were performed by means of the patch-clamp technique (Hamill et al., 1981). The patch pipettes were pulled from borosilicate glass tube (GC 150, Clark Electromedical Instruments, Pangbourne, UK). Some pipettes were coated with Sylgard 184 (Dow Corning, USA). The pipettes were fire-polished directly before the experiments

and had a final resistance of 4.5–22 M Ω . An EPC-7 patch-clamp amplifier (List, Darmstadt, Germany) was used for the channel recordings. The data were either filtered at 1 kHz and directly stored in a computer by using commercially available software (pCLAMP, Axon Instruments, Foster City, CA) or they were filtered at 10 kHz and first stored in a digital tape recorder (DTR-1202, Biologic, Claix, France). For further analysis, these data were replayed from tape, low-pass filtered at 1 kHz and stored on a disk. The frequency of digitization was at least two times higher than that of filtering. For calculation of the open probability (P_o), the channel was considered as open if its amplitude exceeded 50% of a control level. Capacity and leakage currents were digitally subtracted by using a divided pulse procedure. Offset potentials were nulled directly before formation of the seal. Liquid junction potentials were about 3 mV and were not corrected. All experiments were carried out at a room temperature of 21–24°C. The present study is based on recordings and measurements from 24 membrane patches and 29 whole cells.

Results

Single K_{Ca} channels in the soma of motoneurons were investigated in inside-out and outside-out patch configurations. Active K_{Ca} channels were observed in one of approximately three inside-out patches obtained with 5–22 M Ω pipettes. In outside-out patches the clamped membrane area was usually several times larger and almost all outside-out patches contained one to seven active K_{Ca} channels. In contrast to Na^+ -activated K^+ channels (Safronov & Vogel, 1996) which are mostly located in the vicinity of the axon or dendritic hillocks, K_{Ca} channels could be found in patches obtained from different regions over the soma membrane of motoneurons.

Figure 1A shows the activity of single K_{Ca} channels recorded from inside-out membrane patches. The pipettes were filled with external Ringer or high- K_o solutions. The bath solution in the small additional chamber (*see* Materials and Methods) facing the cytoplasmic side of the membrane contained 10^{-4} M Ca^{2+} . In these patches, only a few K_{Ca} channel openings were observed at -80 mV, but the channels could be activated by depolarizing voltage pulses. The amplitudes of the single-channel currents as a function of membrane potential (*i*-*E* curves) for external Ringer and high- K_o solutions are shown in Fig. 1B. Least squares fitting of the data points (from -60 to $+60$ mV) with a linear regression, under an assumption that the equilibrium potential for K^+ ions is -84 mV in Ringer solution and 0 mV in external high- K_o solution, gave the single-channel chord conductance of 82 ± 5 (SE) pS (4 patches) in Ringer solution and 231 ± 4 (SE) pS (3 patches) in external high- K_o solution (Fig. 1B). K_{Ca} channels showed pronounced outward rectification in external Ringer solution. The slope conductance of K_{Ca} channels at positive membrane potentials was up to 150 pS (3 patches).

As can be seen from the recordings in Fig. 1A, the opening of K_{Ca} channels took place within several

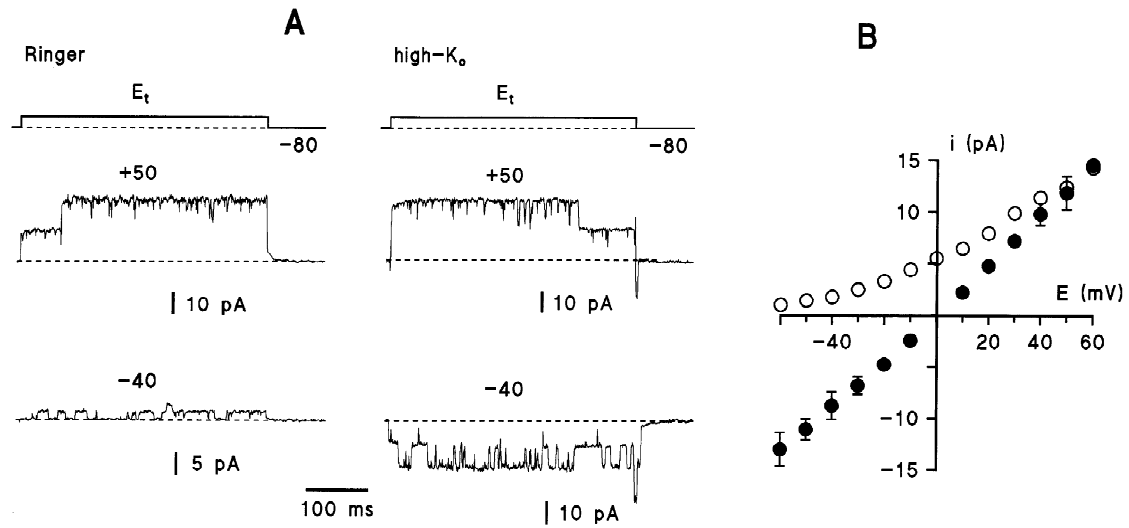


Fig. 1. Single K_{Ca} channels in the soma membrane of rat motoneurons. (A) Currents through K_{Ca} channels activated in Ringer and high- K_o solutions by voltage steps from -80 to -40 and $+50$ mV. Internal Ca^{2+} concentration was 10^{-4} M. Inside-out patches. (B), i - E relationships for K_{Ca} channels in Ringer (open symbols, 4 patches) and in high- K_o solutions (filled symbols, 3 patches). In all figures error bars denote \pm SEM if it exceeds the size of the symbols.

milliseconds after a potential step to $+50$ mV when internal Ca^{2+} concentration was 10^{-4} M. To investigate the kinetics of the channel activation by voltage at a constant internal Ca^{2+} concentration of 10^{-4} M, we constructed K_{Ca} channel currents (Fig. 2) by averaging the single-channel records similar to those shown in Fig. 1A. It could be reasonably assumed that these averaged currents were mostly K_{Ca} currents, since other voltage-gated K^+ channels, A- or delayed rectifier, present in this patch had much lower conductances (Safronov & Vogel, 1995). The half time of K_{Ca} channel activation ($\tau_{0.5}$) measured as the time needed to reach a half-maximum current amplitude for the patch shown in Fig. 2 (see insets) was 7.5 msec at -40 mV and 3.0 msec at $+50$ mV in Ringer solution. In high- K_o solutions $\tau_{0.5}$ was about 10 msec at -40 mV and 2.2 msec at a potential of $+50$ mV. Averaged K_{Ca} currents reached the steady-state level within less than 50–100 msec and did not show inactivation. K_{Ca} channel inactivation was also not observed in single-channel recordings lasting tens of seconds. After returning the potential to -80 mV, K_{Ca} currents deactivated during tens of milliseconds. Such a slow channel closing could be clearly seen in high- K_o solution (Figs. 1A and 2).

In further experiments, the dependence of K_{Ca} channel activation on internal Ca^{2+} concentration was investigated. Activity of K_{Ca} channels recorded in an inside-out patch at -60 mV in high- K_o solution in the presence of different internal Ca^{2+} concentrations are shown in Fig. 3A. Elevation of internal Ca^{2+} increased the channel open probability (P_o). Two- to six-second recordings were used for calculation of P_o values. Activation curves of K_{Ca} channels obtained at 10^{-6} , 10^{-5} , 10^{-4} and 10^{-3} M internal Ca^{2+} are shown in Fig. 3B. For each

Ca^{2+} concentration, the data points were first normalized to the maximum P_o value ($P_{o,max}$) obtained at positive potentials. Nonlinear least squares fitting of the data points with the Boltzmann equation gave E_{50} values of -13 ± 2 mV, -34 ± 1 mV, -64 ± 1 mV and -85 mV for 10^{-6} , 10^{-5} , 10^{-4} and 10^{-3} M Ca^{2+} , respectively (mean \pm SE, 3 inside-out patches). Thus, a tenfold increase in Ca^{2+} concentration produced a negative shift of the activation curve by approximately 20 mV.

K_{Ca} channels demonstrated a high sensitivity to externally applied TEA. The blocker reduced the amplitude of single-channel currents in a dose-dependent manner (Fig. 4A). The concentration of half-maximum block of K_{Ca} channels by TEA (IC_{50}) was 0.17 ± 0.02 (SE) mM (Fig. 4B, 2 outside-out patches).

Externally applied charybdotoxin at a concentration of 100 nM completely and reversibly suppressed K_{Ca} channel activity (3 outside-out patches). The kinetics of charybdotoxin block is illustrated in Fig. 5 with an outside-out membrane patch containing at least five K_{Ca} channels. The time constant of on-reaction was limited by the time of solution exchange under our experimental conditions (5–10 sec). The kinetics of the channel recovery was considerably slower. Full recovery from charybdotoxin usually required 0.5–2 min. The blocker reduced K_{Ca} channel P_o but did not affect the amplitude of the single-channel current (indicated by an asterisk in Fig. 5).

In current-clamp experiments, single action potentials became broader and the amplitude of slow afterhyperpolarization was considerably reduced after substitution of external Ringer solution with low- Ca^{2+} -high- Mg^{2+} -Ringer solution (Fig. 6, 5 cells). The action potential repolarization became also slower after addi-

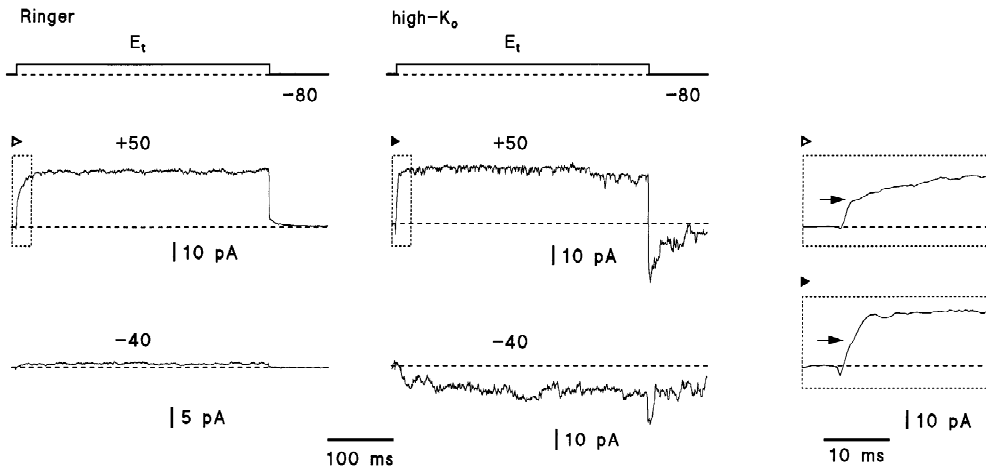


Fig. 2. Averaged K_{Ca} currents in external Ringer and high- K_o solutions. Each trace is a result of averaging 4 to 20 recordings similar to those shown in Fig. 1A. Holding potential was -80 mV. Voltage protocol is shown over the traces. Inside-out patches. Internal Ca^{2+} concentration was 10^{-4} M. The first 30 msec parts of both recordings at $+50$ mV are shown in insets at a higher time resolution. The arrows indicate the half-maximum current amplitudes.

tion of 100 nM charybdotoxin to Ringer solution (Fig. 7, 7 cells). In contrast, 1 μ M apamin selectively suppressed slow afterhyperpolarization (Fig. 7, 4 cells). Therefore, it could be concluded that charybdotoxin-sensitive large conductance K_{Ca} channels contribute to the repolarizing phase of action potential and to fast afterhyperpolarization in rat motoneurons, whereas Ca^{2+} -dependent slow afterhyperpolarization was produced by an apamin-sensitive K_{Ca} conductance (Morita & Barrett, 1990).

Discussion

K_{Ca} CHANNEL PROPERTIES

We recorded single large conductance K_{Ca} channels in the somatic membrane of neonatal rat motoneurons. Large conductance K_{Ca} channels were activated by both depolarization and an increase in internal Ca^{2+} concentration. The channel conductance of 231.1 pS in symmetrical high- K^+ solutions is close to the values reported for K_{Ca} channels in cultured rat skeletal muscle (Blatz & Magleby, 1986; Barrett et al., 1982), cultured spinal neurons of *Xenopus* (Blair & Dionne, 1985) and in cultured mouse motoneurons (McLarnon et al., 1995). In our preceding papers (Safronov, Kampe & Vogel, 1993; Safronov & Vogel, 1995) we have shown that somatic and axonal membranes of rat motoneurons are equipped with distinctly different types of voltage-gated K^+ channels. In contrast, large conductance K_{Ca} channels in the soma of motoneurons studied here appear to have similar unitary conductance and sensitivity to external TEA with K_{Ca} channels in enzymatically demyelinated amphibian axons (Jonas et al., 1991), if the differences in

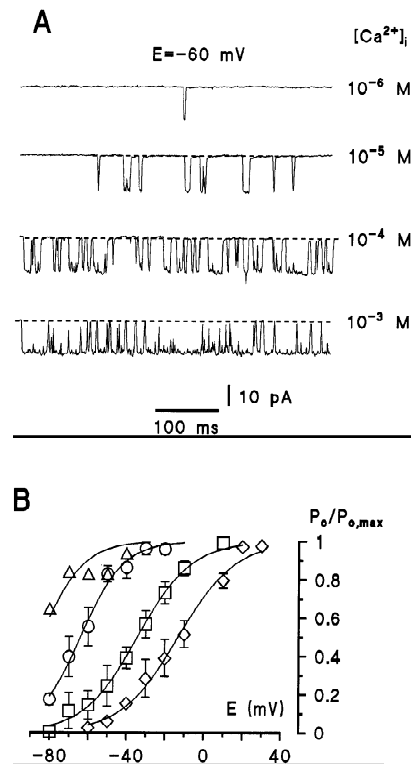


Fig. 3. Ca^{2+} - and voltage-dependent activation of K_{Ca} channels. (A) K_{Ca} channels at -60 mV in the presence of 10^{-6} , 10^{-5} , 10^{-4} and 10^{-3} M internal Ca^{2+} concentration. Inside-out patch. External high- K_o solution. (B) $P_o/P_{o,max}$ as a function of voltage at different internal Ca^{2+} concentrations. The points were fitted with the Boltzmann equation $1/[1 + \exp((E_{50} - E)/k)]$, where $E_{50} = -13 \pm 2$ (SE) mV and $k = 15$ mV for 10^{-6} M Ca^{2+} (diamonds), $E_{50} = -34 \pm 1$ (SE) mV and $k = 14$ mV for 10^{-5} M Ca^{2+} (squares), $E_{50} = -64 \pm 1$ (SE) mV and $k = 11$ mV for 10^{-4} M Ca^{2+} (circles) and $E_{50} = -85$ mV and $k = 11$ mV for 10^{-3} M Ca^{2+} (triangles). Data from 3 inside-out patches.

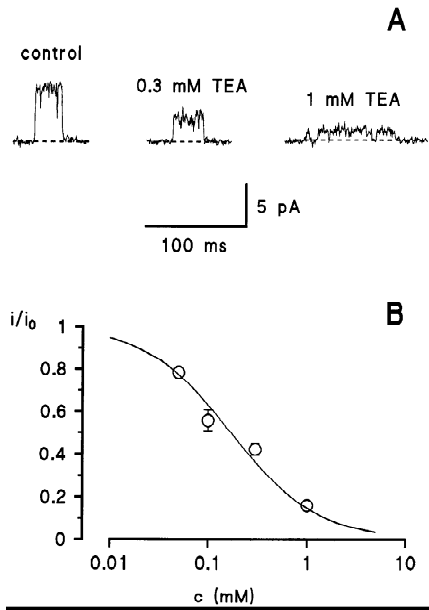


Fig. 4. Effect of external TEA on K_{Ca} channels. (A) Single K_{Ca} channel currents at 0 mV in external Ringer solution in the presence of 0, 0.3 and 1 mM TEA. Outside-out patch. (B) Concentration dependence of K_{Ca} channel block by TEA. The single-channel current amplitudes in the presence of TEA were determined as average currents during channel openings. The data points are fitted with the Hill equation: $IC_{50}/(IC_{50} + C)$, where IC_{50} was 0.17 ± 0.02 (SE) mM (2 patches).

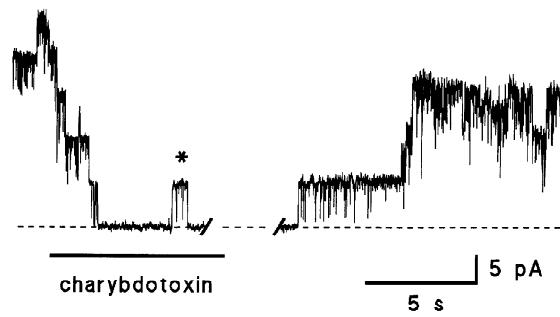


Fig. 5. Block of K_{Ca} channels by externally applied 100 nM charybdotoxin. Outside-out patch in Ringer solution at 0 mV. Charybdotoxin did not reduce the amplitude of the single-channel currents (indicated by asterisk).

temperature and K^+ concentration are taken into account. The channels described here were more sensitive to internal Ca^{2+} than K_{Ca} channels in myelinated amphibian axons (Jonas et al., 1991) and of those in cultured rat muscle (Barrett et al., 1982).

K_{Ca} channels studied here were suppressed by external TEA with IC_{50} of 0.17 mM. Such a sensitivity of K_{Ca} channels to TEA was fairly high in comparison with other types of K^+ channels described in somatic and axonal membranes of moto- and sensory neurones (Safronov & Vogel, 1995; Safronov, Bischoff & Vogel,

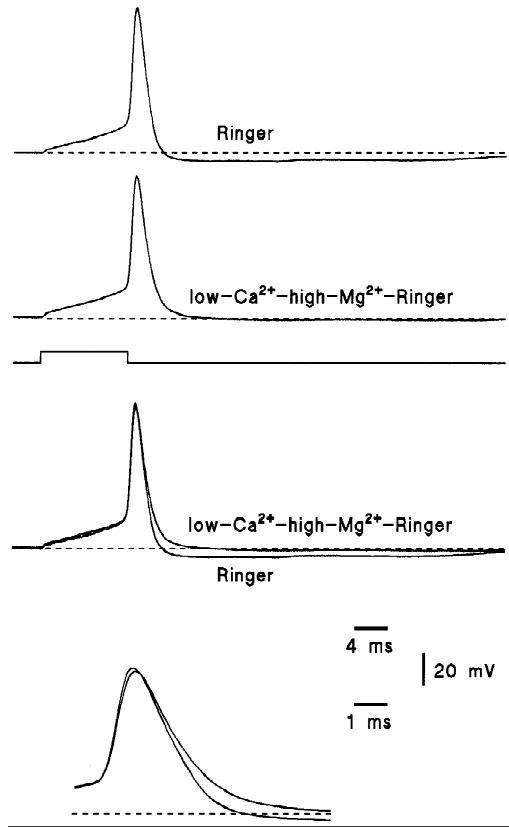


Fig. 6. Effect of external Ca^{2+} on the shape of action potential. The action potentials were recorded in external Ringer and low- Ca^{2+} -high- Mg^{2+} -Ringer solutions under current-clamp conditions. The resting potential of the neurone was kept at -65 mV by injection of steady-state current through the recording pipette. The action potentials were activated by depolarizing 10 msec current pulses of 60 pA amplitude. The third trace shows both action potentials superimposed. Lowermost trace, superimposed action potentials at higher time resolution.

1996; Vogel & Schwarz, 1995). TEA did not change the channel P_o but apparently reduced the amplitude of the single-channel currents as can be expected for a fast blocker (Hille, 1992). In contrast, charybdotoxin reduced K_{Ca} channel P_o without changing the single-channel currents. The time constant of recovery of K_{Ca} channels from charybdotoxin was in the range of minutes. Therefore, charybdotoxin demonstrated typical properties of a slow blocker (Hille, 1992).

FUNCTIONS OF K_{Ca} CHANNELS

K_{Ca} channels have multiple functions in different types of central neurones and provide a link between the level of intracellular Ca^{2+} and cell excitability. TEA-sensitive apamin-resistant K_{Ca} channels contribute to action potential repolarization and to afterhyperpolarization in rat medial vestibular nucleus neurones (Johnston, MacLeod

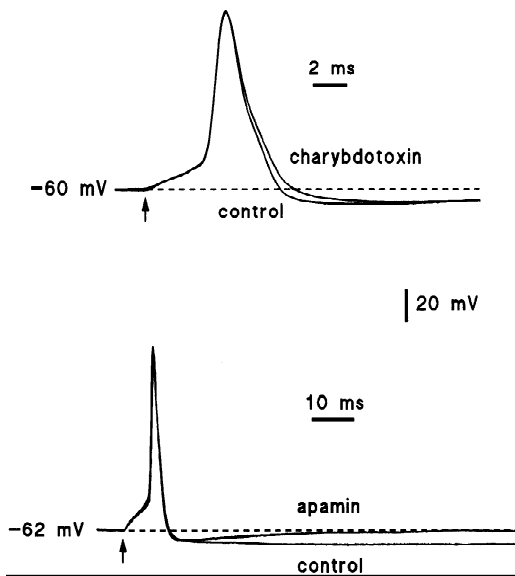


Fig. 7. Effects of charybdotoxin and apamin on action potentials. Upper traces, action potentials elicited by 5 msec current pulses of 110 pA in control (Ringer) solution and after addition of 100 nM charybdotoxin. Lower traces, action potentials recorded in another neurone in the presence and absence of 1 μ M apamin. Current pulses, 10 msec, 240 pA. Beginning of current injections are indicated by arrows.

& Dutia, 1994). TEA- and charybdotoxin-sensitive K_{Ca} currents generate fast afterhyperpolarization and have an influence on frequency of the first few action potentials during cell depolarization in rat hippocampal neurones (Lancaster & Nicoll, 1987). In motoneurons, K_{Ca} currents were supposed to contribute to the repolarizing phase of the action potential (Takahashi, 1990). In our current-clamp experiments we have observed a prolongation of action potentials in low- Ca^{2+} -high- Mg^{2+} -Ringer solutions or after addition of charybdotoxin to normal Ringer solution. Additional support of K_{Ca} channel involvement in the repolarizing phase of action potentials comes from analysis of open and closed time distributions studied in cultured mouse motoneurons (McLarnon et al., 1995). It was shown that K_{Ca} channels could be activated within 1–2 msec at positive potentials and they could be closed during several milliseconds at negative potentials. Furthermore, several millisecond long openings of large conductance K_{Ca} channels were directly seen in cell-attached patches at the moment when the action potential invaded the motoneurone (McLarnon, 1995), indicating that K_{Ca} channels contribute to membrane repolarization as well as to the fast afterhyperpolarization during action potentials in spinal motoneurons. In hypoglossal motoneurons K_{Ca} channels were shown to be activated by membrane depolarization and by Ca^{2+} ion influx through low-voltage-activated Ca^{2+} channels (Umehiya & Berger, 1994) which could be considered as the main source of Ca^{2+} supply for large conductance K_{Ca} channels.

Involvement of K_{Ca} channels in the repolarizing phase of action potentials could also be suggested from our experiments presented in Fig. 2. The half time of channel activation by voltage was 2–3 msec at +50 mV in internal solution containing 10^{-4} M Ca^{2+} (21–24°C). Similar fast activation kinetics of large conductance K_{Ca} channels was recently reported for cloned human *hsl* channels (DiChiara & Reinhart, 1995). Internal Ca^{2+} concentration used in our experiments was 10^{-4} M. Although it is much higher than the resting level of cytoplasmic Ca^{2+} in neuronal cells, such a high level of intracellular Ca^{2+} could be locally reached in the regions of a high density of voltage-activated Ca^{2+} channels (Augustine & Neher, 1992). It was shown for *Helix* neurones that only K_{Ca} channels clustered together with Ca^{2+} channels were activated under physiological conditions, whereas K_{Ca} channels in membrane areas free of Ca^{2+} channels remained silent (Gola & Crest, 1993). Thus, it could be suggested that the fast kinetics of K_{Ca} channel activation at positive potentials and clustering of K_{Ca} channels together with voltage-gated Ca^{2+} channels are important conditions for K_{Ca} channel involvement in the repolarization phase of the action potentials in the soma of motoneurons.

In conclusion, large conductance K_{Ca} channels together with voltage-gated Na^{+} and A- and delayed-rectifier K^{+} channels (Safronov & Vogel, 1995) form the basis of excitability in motoneurons. Further patch-clamp experiments with neurones in spinal cord slices could throw light on properties and functions of ionic channels, especially those underlying potential insensitive ionic currents which normally escape detection in classical voltage-clamp experiments but may nevertheless have tremendous impact on membrane resting potential and integrative behaviour of cells in central nervous system.

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References

- Augustine, G.J., Neher, E. 1992. Neuronal Ca^{2+} signaling takes the local route. *Current Opinion in Neurobiology* **2**:302–307
- Barrett, J.N., Magleby, K.L., Pallotta, B.S. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol.* **331**:211–230
- Blair, L.A.C., Dionne, V.E. 1985. Developmental acquisition of Ca^{2+} -sensitivity by K^{+} channels in spinal neurones. *Nature* **315**:329–331
- Blatz, A.L., Magleby, K.L. 1986. Single apamin-blocked Ca -activated K^{+} channels of small conductance in cultured rat skeletal muscle. *Nature* **323**:718–720
- DiChiara, T.J., Reinhart, P.H. 1995. Distinct effects of Ca^{2+} , voltage on the activation and deactivation of cloned Ca^{2+} -activated K^{+} channels. *J. Physiol.* **489**:403–418

- Edwards, F.A., Konnerth, A., Sakmann, B., Takahashi, T. 1989. A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pfluegers Arch.* **414**:600–612
- Gola, M., Crest, M. 1993. Colocalization of active K_{Ca} channels and Ca²⁺ channels within Ca²⁺ domains in Helix neurons. *Neuron* **10**:689–699
- Granit, R., Kernell, D., Shortess, G.K. 1963. Quantitative aspects of repetitive firing of mammalian motoneurons, caused by injected currents. *J. Physiol.* **168**:911–931
- Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391**:85–100
- Hille, B. 1992. Ionic channels of excitable membranes. Second edition. Sinauer, Sunderland, MA
- Johnston, A.R., MacLeod, N.K., Dutia, M.B. 1994. Ionic conductances contributing to spike repolarization and after-potentials in rat medial vestibular nucleus neurones. *J. Physiol.* **481**:61–77
- Jonas, P., Koh, D.-S., Kampe, K., Hermsteiner, M., Vogel, W. 1991. ATP-sensitive and Ca-activated K channels in vertebrate axons: novel links between metabolism and excitability. *Pfluegers Arch.* **418**:68–73
- Kaczorowski, G.J., Knaus, H.-G., Leonhard, R.J., McManus, O.B., Garcia, M.L. 1996. High conductance calcium activated potassium channels: Structure, pharmacology, and function. *J. Bioenerg. Biomembr.* **28**:255–267
- Lancaster, B., Nicoll, R.A. 1987. Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones. *J. Physiol.* **389**:187–203
- McLarnon, J.G. 1995. Potassium currents in motoneurons. *Prog. Neurobiol.* **47**:513–531
- McLarnon, J.G., Kim, S.U., Michikawa, M., Xu, R. 1995. Properties of inward and outward potassium currents in cultured mouse motoneurons. *Neuroscience* **64**:139–151
- Morita, K., Barrett, E.F. 1990. Evidence for two calcium-dependent potassium conductances in lizard motor nerve terminals. *J. Neurosci.* **10**:2614–2625
- Rudy, B. 1988. Diversity and ubiquity of K channels. *Neuroscience* **25**:729–749
- Safronov, B.V., Bischoff, U., Vogel, W. 1996. Single voltage-gated K⁺ channels and their functions in small dorsal root ganglion neurones of rat. *J. Physiol.* **493**:393–408
- Safronov, B.V., Kampe, K., Vogel, W. 1993. Single voltage-dependent potassium channels in rat peripheral nerve membrane. *J. Physiol.* **460**:675–691
- Safronov, B.V., Vogel, W. 1995. Single voltage-activated Na⁺ and K⁺ channels in the somata of rat motoneurons. *J. Physiol.* **487**:91–106
- Safronov, B.V., Vogel, W. 1996. Properties and functions of Na⁺-activated K⁺ channels in the soma of rat motoneurons. *J. Physiol.* **497**:727–734
- Takahashi, T. 1990. Membrane currents in visually identified motoneurons of neonatal rat spinal cord. *J. Physiol.* **423**:27–46
- Tseng-Crank, J., Foster, C.D., Krause, J.D., Mertz, R., Godinot, N., DiChiara, T.J., Reinhart, P.H. 1994. Cloning, expression, and distribution of functionally distinct Ca²⁺-activated K⁺ channel isoforms from human brain. *Neuron* **13**:1315–1330
- Umemiya, M., Berger, A.J. 1994. Properties and function of low- and high-voltage-activated Ca²⁺ channels in hypoglossal motoneurons. *J. Neurosci.* **14**:5652–5660
- Viana, F., Bayliss, D.A., Berger, A.J. 1993. Multiple potassium conductances and their role in action potential repolarization and repetitive firing behavior of neonatal rat hypoglossal motoneurons. *J. Neurophysiol.* **69**:2150–2163
- Vogel, W., Schwarz, J.R. 1995. Voltage-clamp studies in axons: macroscopic and single-channel currents. In: The Axon. S.G. Waxman, J.D. Kocsis, P.K. Stys, editors. pp. 257–280. Oxford University Press, Oxford
- Walton, K., Fulton, B.P. 1986. Ionic mechanisms underlying the firing properties of rat neonatal motoneurons studied in vitro. *Neuroscience* **19**:669–683
- Zhang, L., Krnjevic, K. 1987. Apamin depresses selectively the after-hyperpolarization of cat spinal motoneurons. *Neurosci. Lett.* **74**:58–62