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Single K^+ Channels in Embryonic Leech Ganglion Cells

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Abstract. We investigated the properties of single K^+ channels in the soma membrane of embryonic leech ganglion cells using the patch-clamp technique. We compared these K^+ channels with the K^+ channels found previously in Retzius neurons of the adult leech.

In ganglion cells of 9- to 15-day-old embryos we characterized eight different types of K^+ channels with mean conductances of 21, 55, 84, 111, 122, 132, 149 and 223 pS.

The 55 pS and 84 pS channels showed flickering and were active for less than 2 min after excising the patch. The 111 pS channel was an outward rectifier, and the open state probability (p_o) decreased in the inside-out configuration when the Ca^{2+} concentration was raised from pCa 7 to pCa 3.

The 122 pS channel also showed outward rectification. This type of channel was activated after changing from the cell-attached to the inside-out configuration and it did not inactivate during more than 30 min. The p_o was Ca^{2+}- and voltage-insensitive. One hundred μM glibenclamide reversibly reduced p_o.

The 132 pS channel was an outward rectifier and was Ca^{2+}-insensitive.

The 149 pS channel inactivated in the inside-out configuration. The 149- and the 223 pS channel showed inward rectification.

The 111 pS channel had similar properties to the Ca^{2+}-dependent K^+ channel and the 122 pS channel resembled the ATP-inhibited K^+ channel found previously in Retzius neurons of the adult leech.

Key words: Patch clamp — Central nervous system — ATP — Ca^{2+} — Glibenclamide

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Introduction

K^+ currents have been characterized during early development in the central nervous system (CNS) of several invertebrate and vertebrate preparations. It was demonstrated that K^+ outward currents appeared earlier than Na^+ and Ca^{2+} inward currents during the ontogenesis of annelids (Schirrmacher, 1990), birds (Bader et al., 1983; Valverde et al., 1992) and mammals (Ahmed et al., 1986).

At the single-channel level, however, K^+ currents have been analyzed in only a few embryonic vertebrate tissues. Stretch-activated K^+ channels, for example, were found in the membrane of the blastomere of the loach (Medina & Bregestovski, 1991), Na^+- and Ca^{2+}-activated K^+ channels in embryonic chick neurons (Dryer et al., 1989; Dryer et al., 1991) and Ca^{2+}-activated K^+ channels in the skeletal muscle of the rat (Ferguson, 1991). In avian fibroblasts six different types of K^+ channels were identified (French & Stockbridge, 1988).

In some preparations, different types of K^+ currents occur at different times during the development. The sequence of occurrence seems to be specific for the type of cell (Nerbonne et al., 1986; Yool et al., 1988; Schirrmacher, 1990; Surmeier et al., 1991; Valverde et al., 1992).

Furthermore, some types of K^+ channels change their electrophysiological properties during development. In *Xenopus* neurons the Ca^{2+}-sensitivity of one type of K^+ channel increased during development whereas conductance and voltage sensitivity remained unaffected (Blair & Dionne, 1985). The mean open times of a K^+ channel of Purkinje neurons increased tenfold during development (Yool et al., 1988). In smooth muscle cells of the human aorta an increase of the open times of a Ca^{2+}-dependent K^+ channel was also observed (Bregestovski et al., 1988).

In embryonic ganglion cells of the leech *Hirudo me-*

dicinalis K^+ currents were investigated only macroscopically by means of the whole-cell configuration of the patch-clamp technique. Such currents were identified as early as the 6th day of development (Meis & Deitmer, 1993).

In the present study we have characterized different types of K^+ channels in the soma membrane of embryonic leech ganglion cells for the first time on the single-channel level. We were able to compare their properties with those of adult leech Retzius neurons characterized in a previous study (Frey, Hanke & Schlue, 1993).

Materials and Methods

Patch-clamp experiments were performed on embryonic ganglion cells of the medicinal leech, *Hirudo medicinalis*, in primary culture. Maintenance and breeding of the leeches have been described by Schirrmacher and Deitmer (1989). Ganglion cells from 9- to 15-day-old embryos were isolated as follows: After removal from the leech, ganglion chains were enzyme treated with collagenase/dispase (Boehringer Mannheim, 0.5 mg/ml culture medium, room temperature) for 30 to 90 min depending on the age of the embryos. The ganglion chains were transferred to culture dishes (Nunc) with sterile culture medium and the ganglion cells were dissociated mechanically by means of a fire polished glass pipette (Schirrmacher & Deitmer, 1991). The ganglion cells were kept at 20°C for up to three days, but most of the experiments were performed on cells after one day in primary culture.

The culture medium consisted of: 100 ml Leibovitz-15 medium with glutamine (cations in mM: 144.7 Na^+, 5.8 K^+, 1.4 Ca^{2+}, 1.8 Mg^{2+}; Gibco), 2 ml heat-inactivated fetal calf serum (Gibco), 10 mM HEPES (Roth) adjusted to pH 7.4 with NaOH. The standard bathing solution (K^+ solution) and the pipette solution contained (in mM): 120 KCl, 1 $CaCl_2$, 10 HEPES adjusted to pH 7.4 with KOH. In ion selectivity measurements, 115 mM KCl was replaced by 115 mM NH_4Cl or 115 mM NaCl (NH_4^+ or Na^+ solution). K^+ solution of pCa 7 was obtained by reducing the Ca^{2+}-concentration to 0.01 mM and adding 123.2 μM EGTA (Sigma) (Pershadsingh & Mc Donald, 1980). TEA (Merck-Schuchardt) was added to the K^+ solution shortly before use. A 2-mM-stock-solution of glibenclamide (Hoechst) was prepared in 100 mM KOH and added to the K^+ solution to provide the desired final concentration.

Patch-clamp recordings were performed on the soma membrane of embryonic ganglion cells according to the method of Neher and Sakmann (*cf.* Hamill et al., 1981). Patch pipettes were pulled from borosilicate glass capillaries (Clark Electromedical Instruments GC150F-10) using a two-stage horizontal microelectrode puller (Mecanex BB-CH-PC; pipette resistance 30–50 MΩ). Single channel currents were recorded in the cell-attached and the inside-out configuration. During experiments in the inside-out configuration, the bathing solution was exchanged by means of a single-barreled perfusion pipette (up to 12 solutions could be handled by a valve; the exchanging time was less than 15 sec). All experiments were carried out at room temperature.

The electrical signal was amplified by a L/M-EPC7 List amplifier and filtered at 2 kHz by an 8-pole low-pass filter (Rockland). Data were digitized (50,000 data points at a 4-kHz rate) and analyzed on a computer using programs written in the institute and the pCLAMP 5.5 program (Axon Instruments). The open state probabilities (p_o) were determined as follows. First, integral amplitude histograms were constructed from the recordings. The peaks belonging to the open and closed states were defined and the area beneath each peak was integrated. The ratio of the area of each open state and of the total area was defined as p_o. The single-channel conductances were calculated from the linear portion of the current/voltage-relationships with K^+ solution on both sides of the membrane patch if not otherwise indicated. The potentials are denoted according to the physiological definition. In all recordings cation fluxes from pipette to bath are shown as upward deflections and negative in sign. The closed states are always marked by bars.

Results

We analyzed K^+ channels in the soma membrane of ganglion cells from 9-to-15-day-old leech embryos in primary culture using the cell-attached and the inside-out configuration of the patch-clamp technique. The various K^+ channels differed mainly in their mean conductance and their gating. In the Table, the electrophysiological properties of eight K^+ channels — the 21, 55, 84, 111, 122, 132, 149, and 223 pS channels — are summarized.

Table 1. Summary of the electrophysiological properties of 8 different types of K^+ channels in the soma membrane of leech embryonic ganglion cells in the inside-out configuration (i.-o. conf.)

Conductance	Characteristics	Inactivation	Rectification	Pharmacology
21 ± 2 pS		−		
55 ± 3 pS	Transient activation in the i.-o. conf.; flickering, bursting	++		
84 ± 4 pS	Transient activation in the i.-o. conf.; flickering, bursting	++		
111 ± 2 pS	Ca^{2+} sensitivity of p_o	−	Outward	TEA sensitivity of the single-channel current amplitude
122 ± 4 pS	Activation in the i.-o. conf.; bursting	−	Outward	Glibenclamide sensitivity of p_o
132 ± 3 pS	Not observed at 9–10-day-old embryos	−	Outward	TEA sensitivity of the single-channel current amplitude
149 ± 4 pS	Flickering, bursting	+	Inward	
223 ± 3 pS	Not observed at 9–10-day-old embryos	+	Inward	

++: fast inactivation; +: inactivation; −: no inactivation.

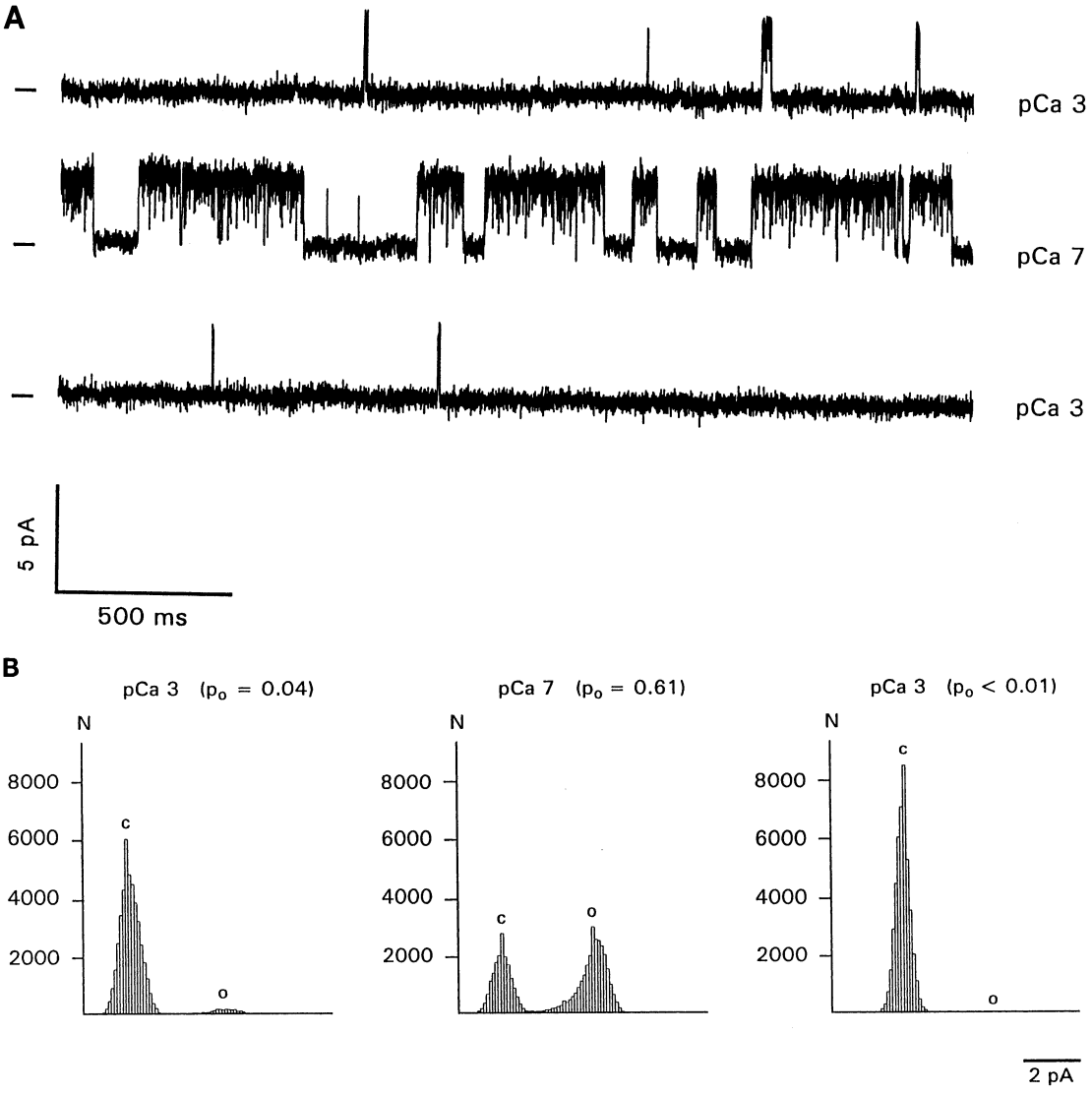


Fig. 1. Ca^{2+} dependence of the 111 pS channel: recording (*A*) and amplitude histograms (*B*) in the inside-out configuration (bath: K^+ solution/pCa 3 or pCa 7; holding potential: −40 mV; age: 15 days). The reduction of the Ca^{2+} concentration at the cytoplasmatic face of the membrane from pCa 3 to pCa 7 induced a prolongation of the opening events and a decrease in the intervals between the opening events (*A*) p_o increased from 0.04 (pCa 3) to 0.61 (pCa 7) (*B*). This effect was reversible. *c, o:* closed (open) state; *N:* counts; band width: 0.1 pA.

These channels were all selective for K^+ over Na^+ and according to the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin & Katz, 1949) a permeability quotient for K^+ over Na^+ of at least eight was calculated. The permeability quotient for K^+ over NH_4^+ was determined for the 111, 122, 132, and 223 pS channels, and the value was at least 6. Because of the instability of the seals at positive potentials and a poor signal-to-noise ratio, it was not possible to determine exact reversal potentials. Thus, only minimum values for the selectivities are given.

The results obtained from 9- to 10- and from 11-to-15-day-old embryos were united. This division into two age groups should give information as to which types of K^+ channels are expressed before the segmentation of the germinal plate is terminated on the 11th day of development (Fernandez & Stent, 1982). The 21, 55, 84, 122, and 149 pS channels were found in both groups whereas the 132 pS and the 223 pS channels were only observed in the group of 11-to-15-day-old embryos.

Since eight different types of K^+ channels are present in embryonic leech ganglion cells, we were unable to investigate all of them in detail. First, we will describe the properties of two channels, the 111 pS and the 122 pS channel, which have similar properties to the Ca^{2+}-dependent and the ATP-inhibited K^+ channels in adult leech Retzius neurons which we analyzed recently (Frey et al., 1993). Afterwards we describe five types of K^+ channels which have not been found in adult leech Retzius neurons.

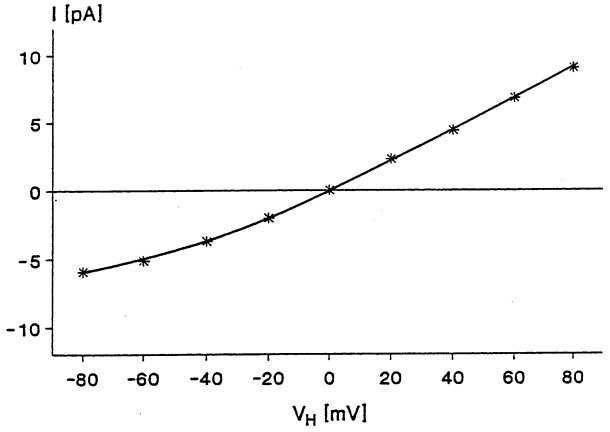


Fig. 2. Current/voltage-relationship of the 111 pS channel in the inside-out configuration (bath: K^+ solution). The mean values of the single-channel amplitudes (I) are plotted *vs.* the corresponding holding potentials (V_H) ($n = 3$). The deflection at negative holding potentials reveals the outward rectification of this type of channel.

K^+ Channels Observed in the Embryonic and in the Adult Leech CNS

111 pS Channel

We analyzed the Ca^{2+} dependence, the rectifying characteristics, and the TEA sensitivity of the 111 pS channel in the inside-out configuration.

The 111 pS channel exhibited a Ca^{2+} dependence of p_o. Figure 1 shows a typical recording together with the corresponding amplitude histograms. Upon a reduction of the Ca^{2+} concentration from pCa 3 to pCa 7 at the cytoplasmatic face of the patch p_o increased from 0.04 to 0.61. The Ca^{2+} dependence was reversible.

The current/voltage-relationship in symmetrical K^+ solution (Fig. 2) demonstrates that the 111 pS channel is an outward rectifier. The slope conductance of 111 ± 2 pS (measured over the linear portion of the plot, $n = 3$) decreased by 23% at −60 mV holding potential.

The effect of the K^+ channel blocker TEA was investigated at the cytoplasmatic face of inside-out patches. Ten mM TEA reduced the single-channel amplitude by 28 ± 2% at −40 mV and by 34 ± 7% at +40 mV holding potential ($n = 3$).

122 pS Channel

The activity of the 122 pS channel increased during the first 2 min after changing from the cell-attached to the inside-out configuration. The p_o, the Ca^{2+} and voltage dependence, the rectifying characteristics, and the glibenclamide sensitivity of the 122 pS channel were investigated in the inside-out configuration.

Following excision, the 122 pS channel did not inactivate, even when the duration of the recording exceeded 30 min (Figs. 3 and 7). The enhancement of the channel activity was observed as well in culture medium as in Na^+ and K^+ solution. The p_o increased from 0.01 to 0.9 in the experiment of Fig. 3. The opening events were prolonged and the intervals between the opening events were shortened. The opening events appeared in bursts.

The p_o was not influenced by a change of the Ca^{2+} concentration from pCa 3 to pCa 7. The p_o was voltage insensitive in the range of −80 to +80 mV with a mean value of 0.84 ± 0.07 ($n = 32$) (Fig. 4).

The current/voltage-relationship in symmetrical K^+ solution is shown in Fig. 5. The conductance of 122 ± 4 pS ($n = 8$) at positive holding potentials diminished by 30% at −60 mV holding potential. The 122 pS channel thus is an outward rectifier.

The enhancement of channel activity after the excision of the patch is a property typical of ATP-inhibited K^+ channels (Frey et al., 1993). This enhancement can be explained by a decrease of the ATP-concentration at the cytoplasmatic face of the membrane. The sulfonylurea glibenclamide specifically blocks ATP-inhibited K^+ channels (Ashcroft & Ashcroft, 1990). In a previous study, we demonstrated that glibenclamide also blocks the ATP-inhibited K^+ channel in Retzius neurons of the adult leech CNS (Frey et al., 1993). In the CNS of the rat glibenclamide was used as a probe for this type of channel (Mourre, Widmann & Lazdunski, 1990). We now investigated the effect of glibenclamide on the 122 pS channel at the cytoplasmatic face of inside-out patches, and the result is shown in Fig. 6. One hundred μM glibenclamide reduced p_o significantly whereas the single-channel amplitude was unaffected ($n = 4$). In the experiment in Fig. 6 p_o was reduced by 60%. The effect of glibenclamide was reversible.

K^+ Channels only Observed in the Embryonic Leech CNS

Five of the embryonic K^+ channels — the 55, 84, 132, 149, and the 223 pS channels — have not been observed previously in adult leech ganglion cells. Their properties are described below.

55 pS and 84 pS Channel

The 55 pS and the 84 pS channel were active for less than 1 or 2 min after the excision of the patch. They showed flickering and bursting events, both in culture medium and in K^+ solution.

Figure 7 shows a recording of the 55 pS channel shortly after the formation of the inside-out configuration and after 7 min of recording. Because of the rapid inactivation of the 55 pS and the 84 pS channel it was impossible to record complete current/voltage relationships

Fig. 3. Recording of the 122 pS channel in the cell-attached configuration (upper trace) and in the inside-out configuration (lower trace) (bath: culture medium; holding potential: −40 mV; age: 9 days). Changing from the cell-attached to the inside-out configuration was accompanied by a prolongation of the opening events and a shortening of the intervals between the opening events. p_o increased from 0.01 to 0.9.

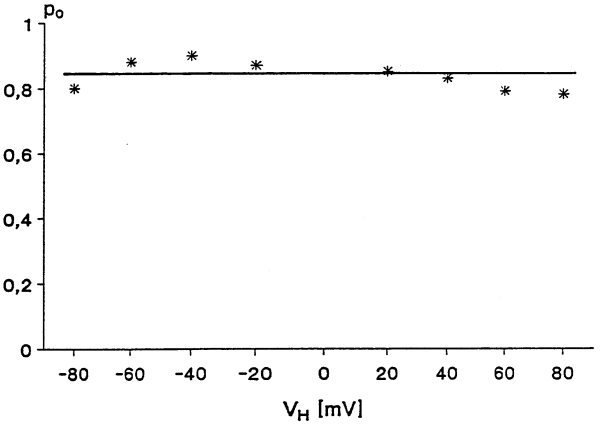


Fig. 4. Voltage-sensitivity of the 122 pS channel in the inside-out configuration: the mean values of p_o are plotted *vs.* the corresponding holding potentials (V_H) (bath: K^+ solution; $n = 5$). p_o was not significantly voltage dependent in the range of −80 to +80 mV. The mean value of p_o was 0.84 ± 0.07 ($n = 32$).

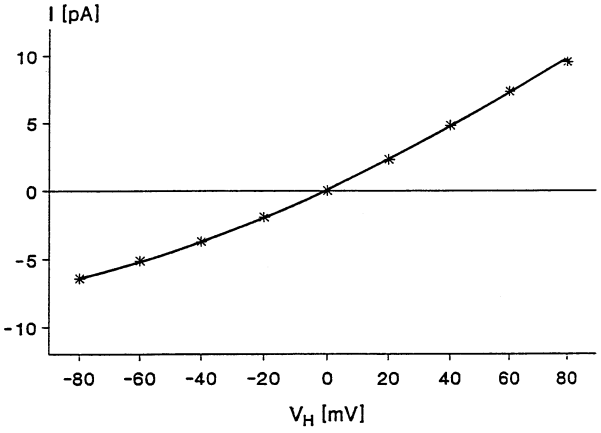


Fig. 5. Current/voltage relationship of the 122 pS channel in the inside-out configuration (bath: K^+ solution; $n = 8$). Note outward rectification.

in the inside-out configuration with K^+ solution as bathing solution. Thus, the single-channel conductances of 55 ± 3 pS ($n = 8$) and of 84 ± 4 pS ($n = 4$) were not defined from the slope of the current/voltage relationships. They were determined as the quotient from current amplitude and potential at one point, namely in the inside-out configuration at −40 mV, the resting membrane potential of leech neurons *in situ* (Schlue & Deitmer, 1984; Frey & Schlue, 1993), with culture medium as bathing solution.

132 pS Channel

The 132 pS channel was only observed in the group of 11-to-15-day-old embryos. Figure 8 shows a single-channel recording at holding potentials between −80 and +80 mV in the inside-out configuration with symmetrical K^+ solution. The p_o was not significantly voltage-dependent and was unaffected by a reduction of the Ca^{2+} concentration from pCa 3 to pCa 7.

Figure 9 demonstrates the outward rectifying characteristics of this type of channel. The conductance of 132 ± 3 pS ($n = 3$) was reduced by 28% at a holding potential of −60 mV.

The TEA sensitivity was investigated in the inside-out configuration. The application of 10 mM TEA reversibly reduced the single-channel amplitude by 24 ± 3% at −40 mV and by 45 ± 13% at +40 mV holding potential ($n = 3$).

149 pS Channel

Figure 10*A* shows a recording of the 149 pS channel in the inside-out configuration at −40 mV holding potential. The 149 pS channel showed flickering and bursting and inactivated in the inside-out configuration after a few

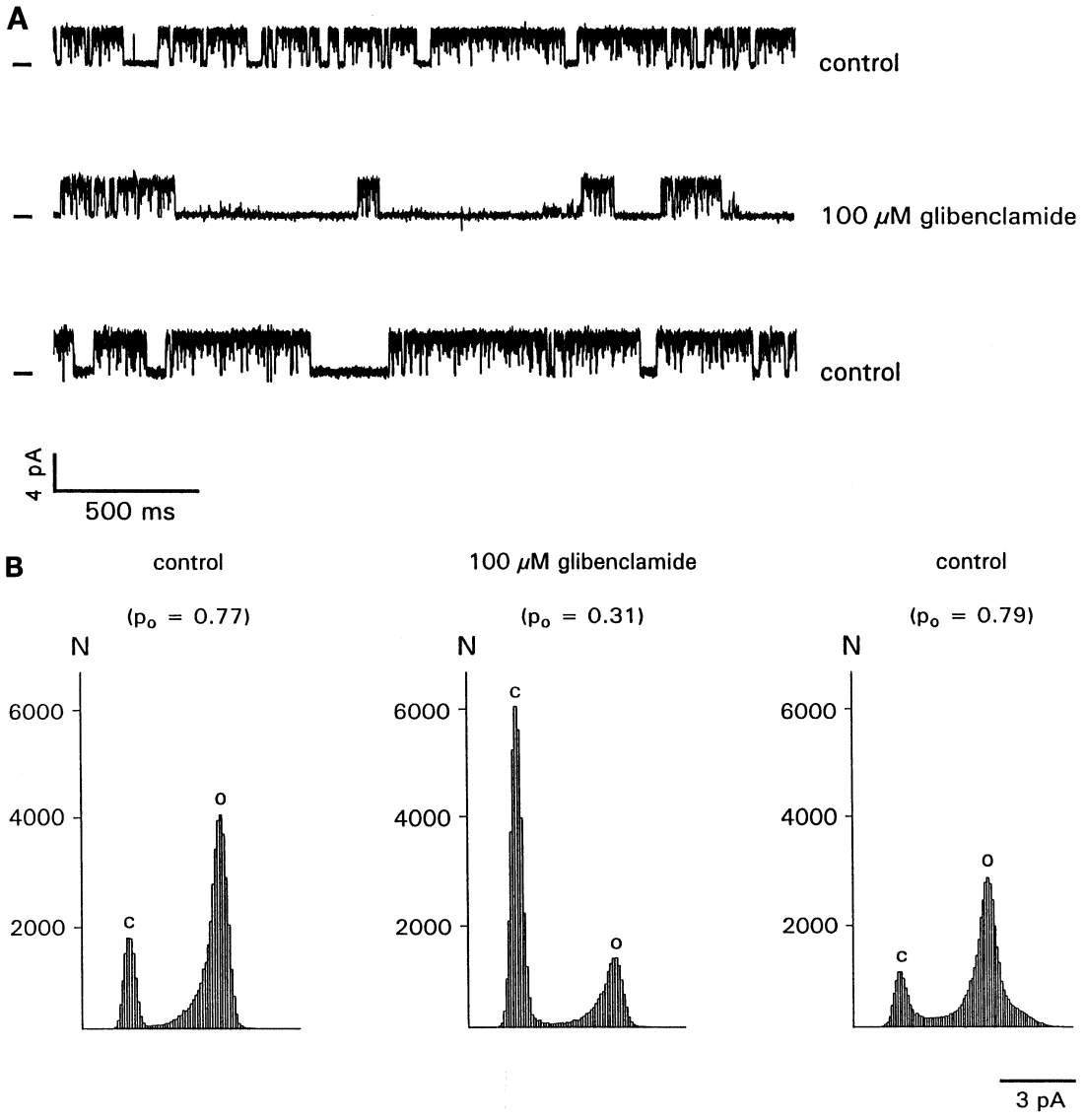


Fig. 6. Glibenclamide sensitivity of the 122 pS channel: recording (*A*) and amplitude histograms (*B*) (bath: K^+ solution without (control) and with 100 μM glibenclamide; holding potential: −40 mV; age: 10 days). The addition of 100 μM glibenclamide induced a shortening of the opening events and a prolongation of the intervals between the opening events (*A*). p_o decreased from 0.77 to 0.31 in the presence of glibenclamide (*B*). The glibenclamide-effect was reversible. *c, o:* closed (open) state; *N:* counts; band width: 0.1 pA.

minutes, although not as rapidly as the 55 pS and the 84 pS channels.

The current/voltage relationship in Fig. 10*B* indicates that the single-channel conductance was reduced at positive holding potentials. The conductance of 149 ± 4 pS ($n = 6$) decreased by 33% at +60 mV holding potential, thus, this type of channel is an inward rectifier, in contrast to the 111, 122 and the 132 pS channels.

223 pS Channel

The 223 pS channel, like the 132 pS channel, was only found in the group of 11-to-15-day-old embryos. Fig. 11*A* shows a single-channel recording in the inside-out configuration. This type of channel also inactivated after several minutes in the inside-out configuration. The current/voltage relationship in Fig. 11*B* reveals the inward rectification of the 223 pS channel; the conductance of 223 ± 3 pS ($n = 3$) was reduced by 37% at +60 mV and by 50% at +100 mV holding potential.

Discussion

In a previous study, we analyzed the electrophysiological properties of two different K^+ channels in the soma membrane of Retzius neurons of the adult leech CNS

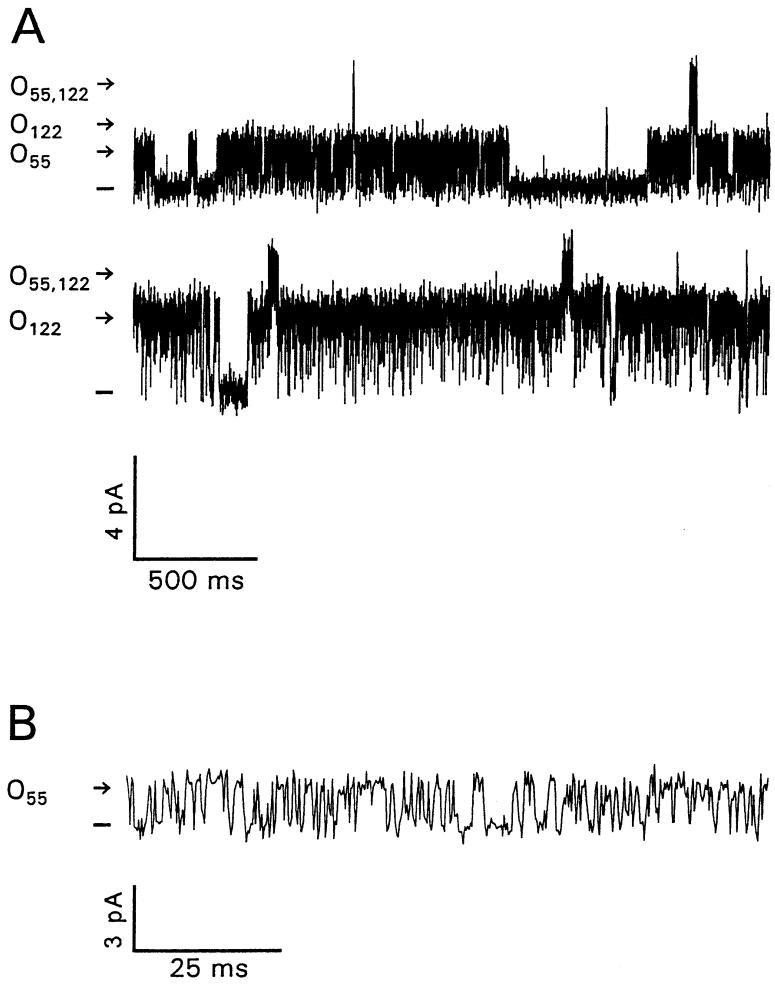


Fig. 7. Recording of a 55 pS and a 122 pS channel shortly after changing from the cell-attached to the inside-out configuration at two different time scales (*A*, upper trace; *B;* bath: culture medium) and after 7 min (*A*, lower trace; bath: K^+ solution) at −40 mV holding potential (age: 13 days). Shortly after the excision of the patch the 55 pS channel showed bursting and flickering. After 7 min the 55 pS channel was almost completely inactivated whereas the 122 pS channel was activated. o_{55}: open state of the 55 pS channel; o_{122}: open state of the 122 pS channel; $o_{55,122}$: open state of both channels.

(Frey et al., 1993). In order to determine which types of K^+ channels are present during the early stages of development, we carried out a similar study using embryonic leech ganglion cells. We found evidence for the presence of five embryonic K^+ channels not previously identified in the adult leech. Two of the embryonic K^+ channels, however, share many properties with the adult channels described in our earlier work.

K^+ Channels Observed in the Embryonic and in the Adult Leech CNS

K^+ channels with a conductance of about 20 pS were found in both embryonic (21 pS channel) and in adult ganglion cells of the leech. Adult Retzius neurons possess K^+ channels of about 20 pS in the soma membrane (Frey, 1994), in the axonal stump (Bookman & Dagan, 1987) and in growth cones (Kolb et al., 1993). K^+ channels of about 20 pS have also been described in the soma membrane of *anterior-pagoda* neurons (Pellegrini, Simoni & Pellegrino, 1989) and pressure neurons (Goldermann, Hanke & Schlue, 1994).

Two of the embryonic channel types, namely the 111 and the 122 pS channel, have similar properties as the Ca^{2+} dependent and the ATP-inhibited K^+ channel in adult leech Retzius neurons.

The 111 pS channel is similar to the Ca^{2+}-dependent K^+ channel in Retzius neurons of the adult leech in the following aspects (Frey et al., 1993):

(i) *conductance:* the conductances were similar (embryonic: 111 ± 2 pS, adult: 114 ± 5 pS)

(ii) *outward rectification*

(iii) *Ca^{2+} dependence:* p_o decreased when the Ca^{2+} concentration in the inside-out configuration was elevated from pCa 7 to pCa 3.

These similarities suggest that the embryonic 111 pS channel and the adult Ca^{2+}-dependent K^+ channel are the same type of K^+ channel.

The embryonic 122 pS channel and the adult ATP-inhibited K^+ channel had the following properties in common (Frey et al., 1993):

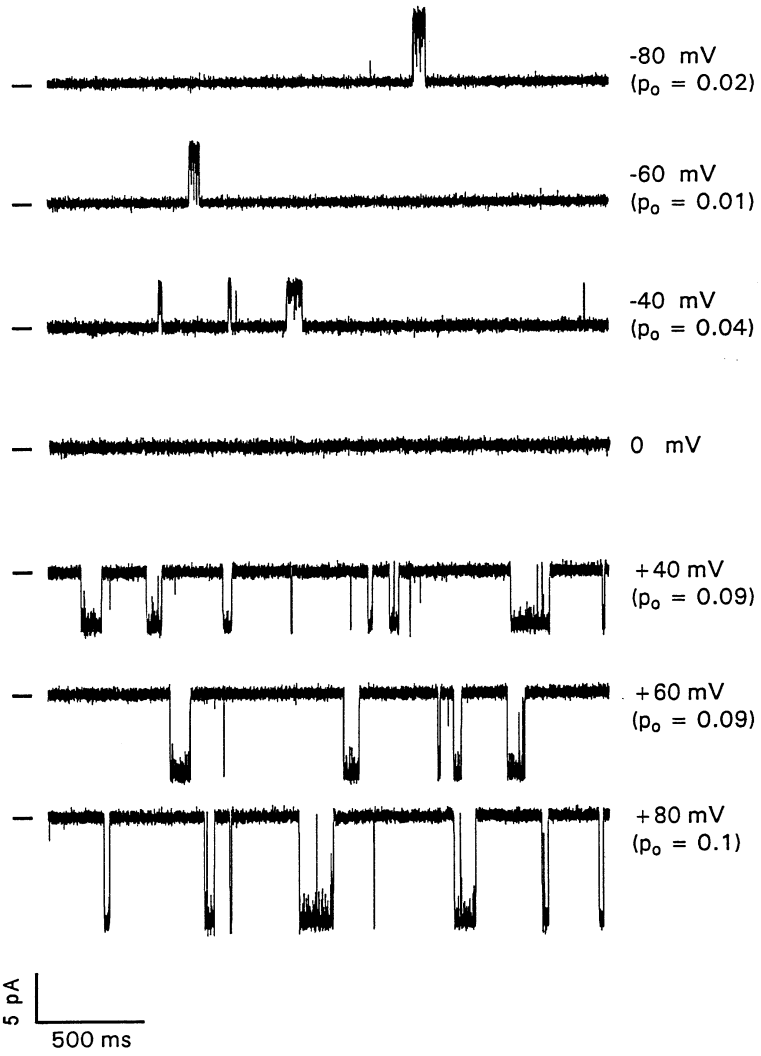


Fig. 8. Recording of the 132 pS channel in the inside-out configuration at holding potentials in the range of −80 to +80 mV (bath: K^+ solution; age: 15 days). The corresponding values of p_o are indicated.

(i) *conductance:* the conductances were in the range of the standard deviation (embryonic: 122 ± 4 pS, adult: 112 ± 6 pS)

(ii) *outward rectification*

(iii) *open state probability:* p_o increased after changing from the cell-attached to the inside-out configuration. p_o was voltage and Ca^{2+} insensitive.

(iv) *pharmacology:* sensitivity to glibenclamide, a specific blocker of ATP-inhibited K^+ channels.

These similarities indicate that the two K^+ channels are of the same type. The glibenclamide sensitivity of adult Retzius neurons seems to be higher than that of embryonic ganglion cells. As the embryonic cells could not be identified it cannot be decided if the glibenclamide sensitivity changes during development or if the glibenclamide sensitivity of the ATP-inhibited K^+ channel varies between different cell types. Autoradiographic experiments showed for example that the density of the glibenclamide-binding sites in the rat brain changes during development (Mourre et al., 1990; Jiang, Xia & Haddad, 1992).

K^+ Channels Observed Only in the Embryonic Leech CNS

Five of the K^+ channels in embryonic ganglion cells have not been observed in adult leech ganglion cells. There are two possible explanations:

(i) A leech ganglion consists of more than 400 cells (Macagno, 1980). Only a few identified ganglion cells of adult leeches are the target for neurobiological studies. It might be possible that the experiments were performed on other embryonic ganglion cells which express the 55, 84, 132, 149, and the 223 pS channel.

(ii) The K^+ channel repertoire might change during development. The 55, 84, 132, 149, and the 223 pS channel might be expressed only during embryonic stages of development or the density of these channels might decrease. The patch-clamp technique allows only the investigation of those channels on the single-channel level which are in the patch under the pipette. It is thus possible that these five types of K^+ channels have not yet been identified in adult neurons because of a low density.

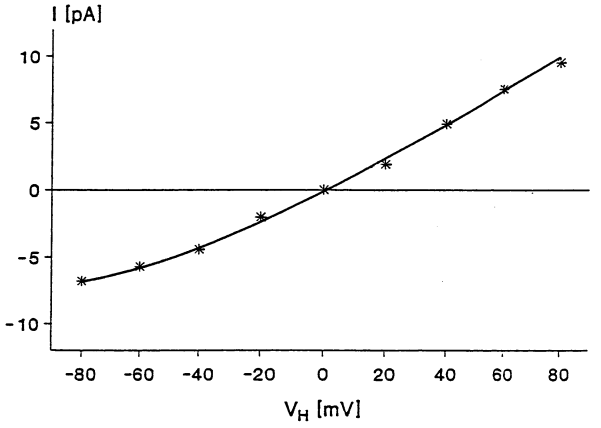
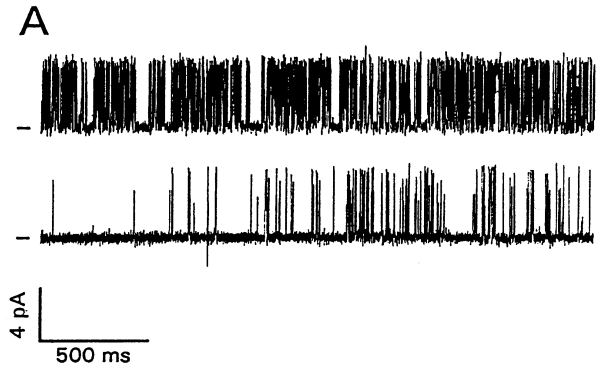


Fig. 9. Current/voltage relationship of the 132 pS channel in the inside-out configuration (bath: K^+ solution; $n = 3$). The channel showed outward rectification.



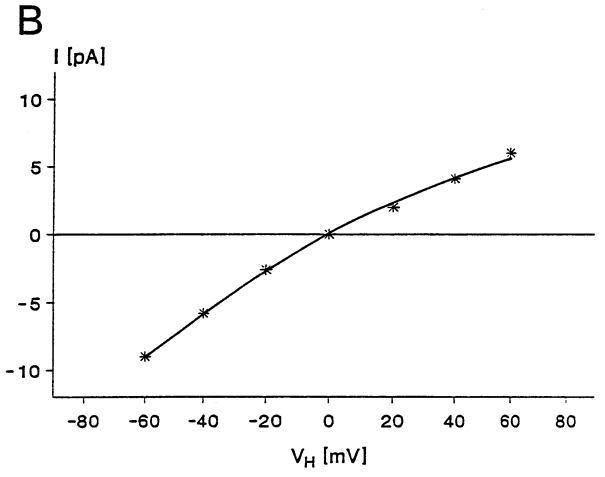


Fig. 10. Recording (*A*) and current/voltage relationship (*B*) of the 149 pS channel in the inside-out configuration. (*A*) Immediately after the excision of the patch the channel showed flickering (upper trace). The channel inactivated after 3 min (lower trace) (bath: Na^+ solution (upper trace), K^+ solution (lower trace); holding potential: −40 mV; age: 10 days). (*B*) The deflection at positive potentials reveals inward rectification (bath: K^+ solution; $n = 2$).

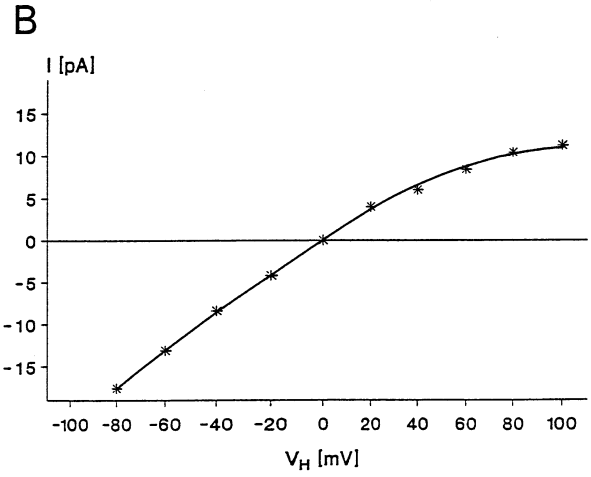


Fig. 11. Recording (*A*) and current/voltage relationship (*B*) of the 223 pS channel in the inside-out configuration (bath: K^+ solution. (*A*) holding potential: −40 mV; age: 14 days. (*B*) $n = 3$). The channel showed inward rectification.

The 132 and the 223 pS channel were only observed in the group of 11-to-15-day-old leech embryos. These K^+ channels might not be expressed during early development or a low number of these channels might be present on days 9 and 10 of development such that they were not detected with the patch-clamp technique. It might also be possible that different cells have different survival rates at different ages so that even between embryonic ages the experiments might be performed on quite different populations of neurons.

A stretch-activated K^+ channel with a conductance of 218 pS has been described in the soma membrane of *anterior-pagoda*, noxious, touch, and Retzius neurons of the adult leech (Pellegrino et al., 1990). However, this stretch-activated K^+ channel showed outward rectification in contrast to the embryonic 223 pS channel. Thus, they seem to be two different types of K^+ channels.

A physiological role of the different types of K^+ channels might so far only be proposed for the 111 pS and the 122 pS channel. The 111 pS channel (Ca^{2+}-dependent K^+ channel) may play a role in generating the resting membrane potential because its p_o is Ca^{2+} dependent and its p_o is high at the intracellular pCa value of

leech neurons (Deitmer & Schlue, 1983; Hochstrate, Piel & Schlue, 1995). The 122 pS channel (ATP-inhibited K^+ channel) may play a role in maintaining the membrane potential constant — independently from the energy state of the cell.

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