J. Membrane Biol. 86, 9–15 (1985)

The Journal of
Membrane Biology

Single Cl^- Channels in Molluscan Neurones: Multiplicity of the Conductance States

V.I. Geletyuk and V.N. Kazachenko
Institute of Biological Physics, USSR Academy of Sciences, Pushchino, Moscow Region, 142292, USSR

Summary. Properties of the single Cl channels were studied in excised patches of surface membrane from molluscan neurones using single-channel recording technique. These channels are controlled by Ca^{2+} and K^+ acting on cytoplasmic and outer membrane surfaces, respectively, and by the membrane potential. The channels display about 16 intermediate conductance sublevels, each of them being multiples of ~12.5 pS. The upper level of the channel conductance is about 200 pS. The channel behavior is consistent with an aggregation of channel-forming subunits into a cluster.

Key Words molluscan neurone · patch voltage-clamp technique · single Cl channel · calcium · potassium · multiplicity of the condutance states

Introduction

Employment of the patch voltage-clamp method for studying the biological membranes (Hamill et al., 1981) allows not only to determine directly the characteristics of single ionic channels (conductance, life time, etc.), but to study some peculiarities of the channel operation. For example, it was found that practically all types of single channels investigated exhibit burst-like activity (*see*, for instance, Barrett, Magleby & Pallotta, 1982; Fenwick, Marty & Neher, 1982). Recently it was shown that some kinds of ionic channels may have more than one conductive state (Hamill & Sakmann, 1981; Siegelbaum, Camardo & Kandel, 1982; Benham & Bolton, 1983; Blatz & Magleby, 1983; Geletyuk & Kazachenko, 1983*a*,*b*; Sauvé, Roy & Payet, 1983; Sakmann & Trube, 1984).

So, the method suggests studying those properties of the channels which cannot be revealed by traditional microelectrode technique. An additional advantage of the patch-clamp method is that ionic channels are studied in their natural membrane environment.

We used the patch voltage-clamp method to study anion-selective ionic channels in the neurones of fresh water mollusc *Lymnaea stagnalis*. The neurones have two classes of Cl^- channels: the channels activated by acetylcholine (Chemeris et al., 1982), and those activated by alkali metal ions (K^+, Rb^+ and Cs^+) applied externally (Kislov & Kazachenko, 1975). In this paper we describe single K^+-sensitive Cl^- channels. It has been found that the channels are sensitive not only to external K^+, but also to internal Ca^{2+}. Particular attention is concentrated here on the multiplicity of the channel conductance states.

Materials and Methods

Single-channel currents were recorded from "inside-out" membrane patches (Hamill et al., 1981) excised from completely isolated neurones of fresh water mollusc *Lymnaea stagnalis*. The neurones were isolated from the right and left parietal ganglia pretreated with pronase (0.35%, 0.1 to 0.5 hr, 20 to 22°C) (Kostenko, Geletyuk & Veprintsev, 1974). The main results were tested on the neurones mechanically isolated from nontreated brain. No essential differences were observed between the two cases. The tip diameter of fire-polished micropipettes fabricated from molybdenum glass or Pyrex® was <1 μm, and the resistance of the pipettes filled with the solutions was 10 to 50 MΩ.

In the experiments different bathing and pipette solutions were used (Table 1). The main solutions were A (pipette solution) and G (bathing solution). Other modified solutions are specified in the text. The experiments were performed at 20 to 22°C. In the paper the following abbreviations and notations are used. EGTA, ethylene glycol-*bis*(2-aminoethylether)N,N'-tetraacetic acid; TTX, tetrodotoxin; TEA, tetraethyl ammonium chloride; $[\ ]_p$, ionic or salt concentration in the pipette; $[\ ]_b$, ionic or salt concentration in the bath; V_r, reversal potential of the currents; V_m, patch membrane potential determined as the bath potential with respect to the pipette potential; g_{Ch}, channel conductance; P_o, probability of the channel open state determined as the ratio of the time that the channel was open to the sampling time; n, number of investigated patches.

Table 1. Composition of solutions (in mM)

	NaCl	KCl	Total $CaCl_2$	EGTA	Free Ca^{2+}	$MgCl_2$	CsCl	TEA-Cl	TTX	Tris-HCl	HEPES-NaOH	pH
Pipette solutions												
A	50	1.5	4	0	4	1.5	0	0	0	2.5	0	7.5
B	50	15	4	0	4	1.5	0	0	0	2.5	0	7.5
C	50	0	4	0	4	1.5	0	0	0	2.5	0	7.5
D	50	0	0.01	0	0.01	0	0	0	0.1	2.5	0	7.5
E	0	0	0.01	0	0.01	0	50	0	0	2.5	0	7.5
Bathing solutions												
F	50	0	0	1	0	0	0	0	0	0	2.5	7.2
G	50	0	0.93	1	0.001	0	0	0	0	0	2.5	7.2
H	0	0	0.93	1	0.001	0	0	0	0	50	2.5	7.2
I	0	0	0.93	1	0.001	0	0	50	0	0	2.5	7.2

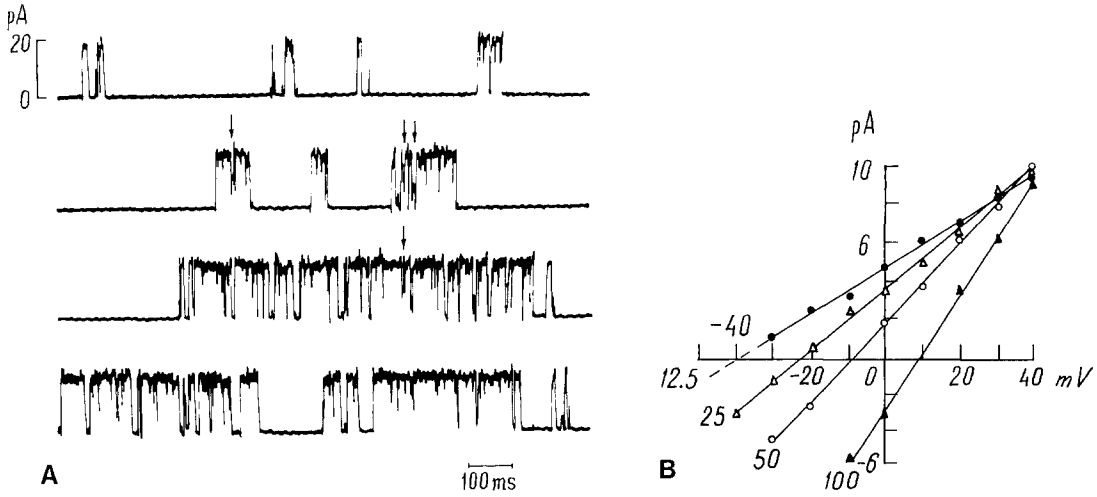

Fig. 1. Channel currents and *I–V* relations. (*A*) Records of the Cl^- channel currents arising in "inside-out" patch at V_m = 100 mV. The currents correspond to moving of Cl^- ions through the channel from the pipette to the bath (outward currents). A micropipette was filled with normal physiological solution (solution A). Arrows indicate some intermediate states of the channel currents. 500 Hz filtering. (*B*) *I–V* relations of the channel for different values of $[NaCl]_b$. The pipette was filled with solution B ($[K^+]_p$ = 15 mM). The bath solutions contained various NaCl concentrations (in mM): 12.5, 25, 50 and 100 as indicated near the lines. Free $[Ca^{2+}]_b$ was about 1 μM. The solutions with decreased amounts of NaCl were prepared by diluting that with 100 mM NaCl. As the bath solution was replaced by that with reduced NaCl, V_r was shifted in negative direction almost in accordance with the Nernst equation for a Cl^--sensitive electrode (15 to 17 mV) (V_{Na} in these cases was shifted theoretically toward positive direction)

Results

Identification of the Cl^- Channels

In approximately 30% of the patches, rectangular current impulses of a large amplitude (corresponding to g_{Ch} = 200 pS) were observed after formation of the "inside-out" patch (Fig. 1*A*). The currents were identified as those passing through individual ionic channels. Usually a patch contained one active channel, rarely, two independent channels, but never three or more. *I–V* relations of the channel were linear and gave the value of g_{Ch} = 201 ± 6.2 pS (SD, n = 22) at $[NaCl]_b$ = 50 mM (Fig. 1*B*). The currents had V_r of near to 0 mV (±3 mV, n = 22) for the bath and pipette solutions symmetrical in respect to $[Cl^-]$.

To study the ionic nature of the currents the influence of the ionic composition of the bathing solution on V_r was tested. It was found that V_r is sensitive to Cl ions only (Fig. 1*B*). Twofold decrease in $[Cl^-]_b$ shifts V_r by 15 to 17 mV towards

negative values, i.e., practically in accordance with the Nernst equation for a Cl^--sensitive electrode. Thus, we conclude that the currents investigated are created by the Cl^- channels. In this set of the experiments different values of $[Cl^-]_b$ were obtained by dilution of 100 mM solution of NaCl; thereby, the ionic strength at the inner side of the membrane was changed. We refused to replace chloride by large impermeable ions such as proprionate, citrate, etc., because these ions destroy the Cl^- channels (the amplitude of the channel current became nonstable).

Regulation of the Cl^- Channel Activity by External K^+ and Internal Ca^{2+}

It was found that the channel activity is governed by ionic conditions at both inside and outside membrane surfaces. The following conclusions were made from the experiments. (1) The channel opens rarely when the pipette solution is K^+ free (solution C), and the bathing solution is Ca^{2+} free (solution F) ($P_o < 0.01$, $n = 7$, $V_m = +50$ mV). (2) Probability of the channel open state rises to ~0.1 ($V_m = +50$ mV) at free $[Ca^{2+}]_b = 1$ μM even when $[K^+]_p = 0$ (solutions C, D, G). Under these conditions the channels open predominantly at positive values of the membrane potential. (3) Probability of the channel open state increases at enhanced values of $[K^+]_p$ and even at $[Ca^{2+}]_b = 0$ (solutions B, F). In this case the channel opens presumably at negative values of the membrane potential ($P_o = 0.1$ to 0.3 at -50 mV). (4) The most favorable conditions for the channel activity are created at $[Ca^{2+}]_b \geq 1$ μM and $[K^+]_p \geq 5$ mM.

Figure 2 shows the potential dependence of probability of the channel open state at $[Ca^{2+}]_b = 1$ μM (solution G) and two values of $[K^+]_p$ (1.5 and 10 mM, solutions A and B, respectively). Generally, probability of the channel open state increases with positive values of the membrane potential at all concentrations of ionized Ca^{2+} in the bath when $[K^+]_p$ is less than ~5 mM. At higher $[K^+]_p$ values the situation is reversed: probability of the channel open state increases with negative values of the membrane potential.

Thus, we have dealt with the Cl^- channels controlled by Ca^{2+} and K^+ acting on cytoplasmic and outer membrane surfaces, respectively, and by the membrane potential. The Cl^- channels investigated may have some relationship to the Ca^{2+}-activated Cl^- channels observed in immature *Xenopus* oocytes (Barish, 1983) and in *Characeae* cells (Lunevsky et al., 1983), and the K^+-sensitive Cl^- currents in squid giant axon (Strickholm, 1981).

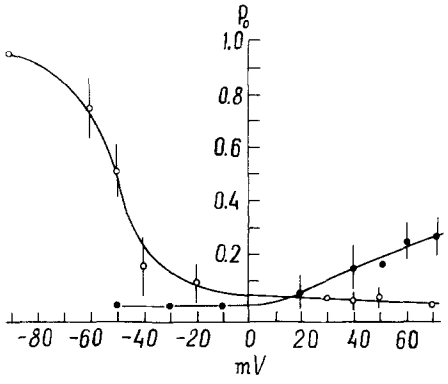

Fig. 2. Potential dependences of probability of the channel open state. Pipettes contained either normal physiological solution A (●) or that with increased K^+ concentration (15 mM) (○). The bath solution was the same as solution G. The data were taken for four (●) and five (○) patches. Error bars give ±SD

The channel operation has some peculiarities. (1) Often the channel opens extremely irregularly: the periods of its activity alternate with prolonged shut periods (up to several minutes). (2) Independently of the presence of Ca^{2+} in the bath and K^+ in the pipette the channel activity decreases with time (tens of minutes). (3) The channel opening can be stimulated by prolonged positive prepulses ($\geq +50$ mV, to several minutes; *cf.* Blatz & Magleby, 1983). Such irregularity of the channel operation presents some difficulties in studying the time and concentration dependences of the channel characteristics.

Multiplicity of the Conductance States

The records in Fig. 1*A* show that the Cl^- channel may turn on and off several times before it closes completely for a long time. This burst-like behavior is observed for most types of single ionic channels in biological membranes. Frequently during the burst activity the channel exhibits partial closing or opening at intermediate substates. In Fig. 1*A* some intermediate substates are shown by arrows.

One may think that the conductance substates observed are due to summation of the outward Cl^- currents with any inward currents of other nature. This seems unlikely due to the following reasons. (1) In usual experiments when the pipette is filled with normal physiological solution (A), and the bath contains solution G we do not observe independent inward currents during the interburst intervals for the Cl^- channel. As a rule, we rarely register inward currents which must be the Ca^{2+} or Na^+ currents. These currents disappear in a few minutes after formation of isolated "inside-out" patch and then the

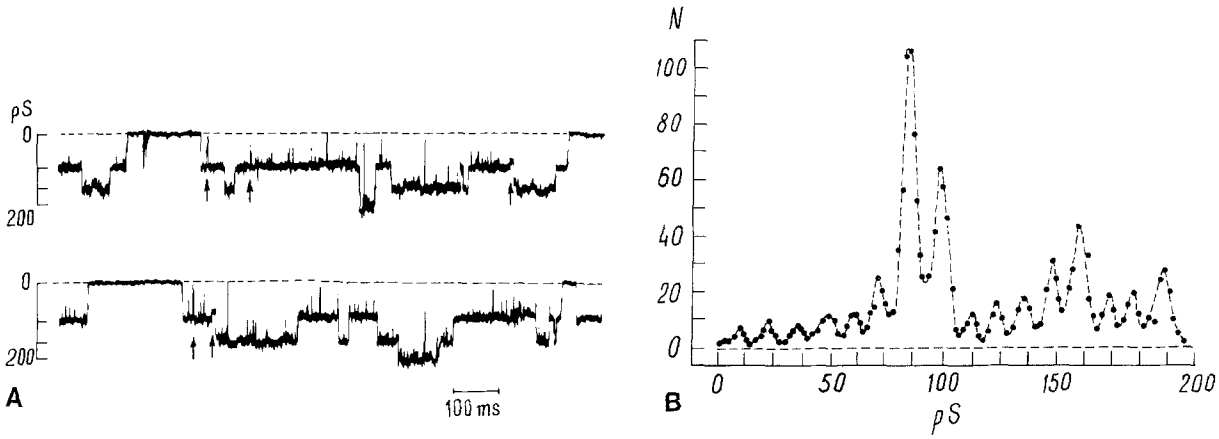

Fig. 3. Multiplicity of the Cl channel substates. (*A*) Records of the channel activity at −60 mV. The pipette contained solution B (15 mM KCl). Total time of the record is equal to 2.5 min. The probability of a completely closed state, 0.4. Arrows indicate some intermediate short-living substates. 800 Hz filtering. (*B*) Histogram of different conductance substates at −60 mV for the patch described in (*A*). The histogram was constructed for the channel bursts only. The time spent is equal to ~1.5 min. About 16 conductance peaks can be distinguished in the histogram. *N*, number of events

baseline is clear. It is possible that the Ca^{2+} and Na^+ channels are either irreversibly inactivated or destroyed due to isolation of a patch (*see also* Fenwick et al., 1982; Cavalié et al., 1983). (2) Applications of different solutions preventing cationic transport through the membrane (solutions D, E, G–I) does not eliminate the intermediate substates of the Cl^- channel conductance. For different combinations of the pipette and bathing solutions mentioned only Cl^- currents can be expected due to the following reasons: (i) K^+ and Mg^{2+} are excluded from the solutions; (ii) Na^+ transport is prevented either by blocking of the Na^+ channels with TTX or by replacing Na^+ by impermeable ions, $Tris^+$, TEA^+, or Cs^+; (iii) Ca^{2+} is used in rather small concentrations at both sides of the path membrane, so the Ca^{2+} currents (if any exist, in principle) are negligible. In all cases the Cl^- channels with $g_{Ch} = 200$ pS ± 20 pS and $V_r = 0 \pm 10$ mV are registered. The deviations of V_r are in accordance with the Cl^- activity in the solutions.

Thus, we believe that the fluctuations of the conductance observed in the boundaries of the burst result rather from changes of the conductance states of the Cl^- channel activity itself than from summation of the Cl^- currents with any currents of other nature.

The records in Fig. 1*A* were obtained when the pipette was filled with normal physiological solution (A). In this case the channel opens mainly at positive potentials and comparatively rarely displays prolonged intermediate states. When the pipette contains a high amount of K^+ (solution B) or low Ca^{2+} concentration (solutions D, E) the Cl^- channel opens frequently at negative levels of the membrane potential, and intermediate conductance substates are clearly distinguished. Figure 3*A* shows an example of such an experiment. The records were made at −60 mV. The channel activity looks as if three independent channels with the conductances of 87.5, 62.5 and 50 pS open. Actually all three conductance sublevels are interrelated and represent the most probable substates of a single channel with conductance of 200 pS. The interrelation of the channel subunits follows from their related openings. The first subunit (87.5 pS) may open inadvertently (26 events). The opening of the second subunit always follows that of the first subunit (110 events); and the opening of the second subunit is followed by that of the third one (42 events). In turn each of these three main substates has many intermediate short-living sublevels. Figure 3*B* demonstrates a correponding distribution of all conductance substates. The distribution displays about 16 equidistant peaks with intervals between the neighboring ones being of 12 to 14 pS. The diagram shows that in this case three intermediate substates are the most plausible (87.5, 150 and 200 pS).

At positive values of the membrane potential the maximum of probability of the channel open state lies in the range of several upper sublevels. Figure 4 shows a typical recording of the currents through the Cl^- channel at +50 mV and high time resolution. As seen, the channel displays a great number of intermediate conductance states. To a first approximation, the conductance substates are grouped near 16 equidistant levels. The intervals between neighboring sublevels are of about 12 to 13

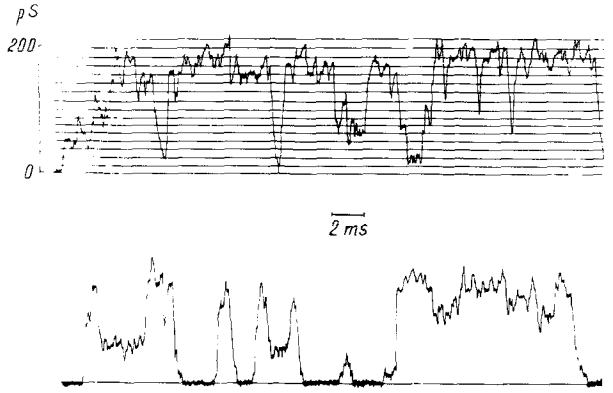

Fig. 4. Records of single-channel activity at high time resolution. At usual time scale the channel activity was similar to that shown in Fig. 1*A*. As seen the channel displays about 16 intermediate conductance sublevels. V_m = +50 mV. Micropipette was filled with solution A. The bath solution was like solution G. Filtering at approx. 2 kHz

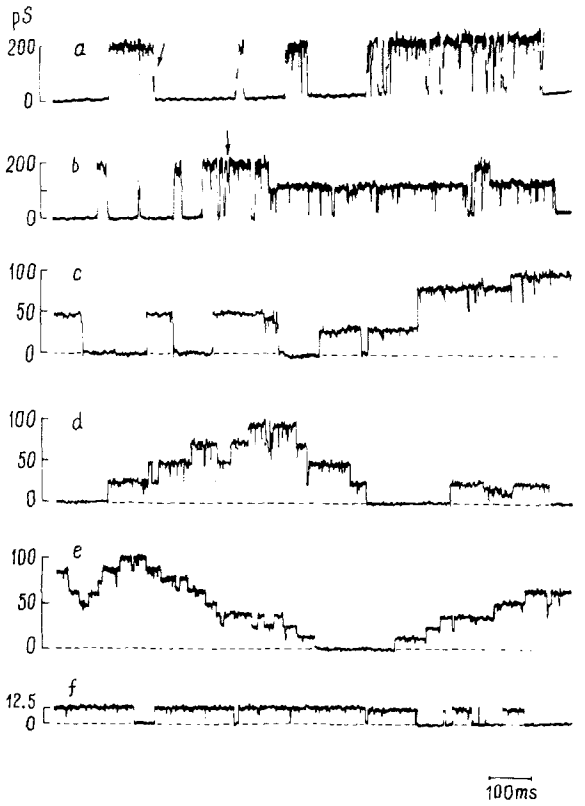

Fig. 5. Degradation of the channel conductance. The phenomenon appears in the following manner. Firstly, original channel conductance (200 pS) is registered (*A*). Sometimes reversible transitions of the conductance between intermediate levels are observed (indicated by arrows in (*A*) and (*B*)). Five to 30 min later these intermediate levels elongate (as in *B*) and stepwise rising and falling phases of the current impulses appear as if the original channel conductance is converted into several (2, 3, etc.) interrelated portions (*C*). In time, this interrelation may disappear and further splitting occur (*D–F*) down to elementary conductance, 12.5 pS. In parallel, disappearance of some substates is observed. The pipette contained solution D, and the bath, solution G. V_m = +100 mV. Bandwidth filtering, 500 Hz (*A–D*) and 200 Hz (*E*, *F*)

pS. So, this observation confirms the multistate behavior of the channel illustrated in Figs. 1 and 3. In addition, the following two conclusions can be derived from Fig. 4. (1) Generally, all substates are short-living, an average life-time of any substate being of about 0.2 msec. Even prolonged substates represent fast fluctuations of the conductance in the vicinity of any averaged sublevel. (2) Frequently, openings and closings of the channel are not instantaneous processes but stepwise ones; because of that the rising and falling phases of the current are extended up to several milliseconds (*cf.* Colquhoun & Sakmann, 1981).

Thus, based on the phenomena described we believe that the Cl^- channels have about 16 multiple conductance substates.

The Channel Degradation

From our viewpoint the unusual phenomenon, irreversible degradation of the channel conductance, supports the existence of 16 multiple conductance states of the Cl^- channels.

The phenomenon looks as if the initial channel (200 pS in conductance) breaks down into the channel fragments with different conductances up to elementary conductance of about 12.5 pS. In time some fragments can irreversibly disappear from the field of observation, so that sometimes no channel activity is registered. Frequently, a channel breaks down into a pair of channels of the same conductance (200 pS → 2 × 100 pS; 100 pS → 2 × 50 pS, etc.). In fact, different variations of the channel degradation are possible. An example of the channel degradation is shown in Fig. 5. In all cases it was found that split channels of lowered conductance are Cl^- channels.

The distribution in Fig. 3 and the recordings of the channel activity in Fig. 4 show that the Cl^- channel has 16 multiple conductance substates. Then one should expect that all kinds of channel conductances multiple to an elementary one might be observed in the experiments as a result of the channel degradation. Table 2 provides an example of such a case. The data were obtained while measuring the conductances of the split channels. Based on these observations, the conclusion can be made that the single channel with the conductance of ~200 pS consists of 16 elementary channels with

Table 2. Expected and observed conductance values of split Cl^- channels (pS)[a]

12.5	25	37.5	50
12.5 ± 1.7(6)	25 ± 1.5(5)	36.6 ± 2.5(3)	51.8 ± 4.4(5)
62.5	75	87.5	100
62.5 ± 3.3(2)	74 ± 5(4)	87.5 ± 5(2)	100 ± 5.1(12)
112.5	125	137.5	150
—	126.3 ± 4.8(6)	—	150.1 ± 6.2(6)
162.5	175	187.5	200
—	177 ± (1)	185 ± 7.7(2)	201.3 ± 6.2(22)

[a] The upper figures in the pairs indicate the expected conductance values of split channels on the assumption that an elementary conductance equals 12.5 pS, and the bottom ones are the experimental conductance values ($[Cl^-]_p$, $[Cl^-]_b \approx 50$ mM). Numbers in parentheses represent the number of patches.

the conductance of 12.5 pS. The data are in agreement with those from the analysis of the results illustrated in Figs. 3 and 4.

Discussion

Two main results presented in the paper are: (1) activation of single Cl^- channels by Ca^{2+} and K^+ acting on cytoplasmic and outer membrane surfaces, respectively; and (2) existence of the number (16) of intermediate multiple substates available for the whole channel conductance (200 pS).

Intracellular Ca^{2+} is known to activate specific K^+ channels in many types of cells (Thomas & Aldrich, 1980). It is interesting that in none of the experiments on the neurones studied were the Ca^{2+}-activated K^+ channels registered, although the channels of this type are often observed in the cells of other types of the same species of mollusc (heart, salivary glands; *our own observations*). This fact may indicate that the Ca^{2+}-activated K^+ channels are either destroyed due to isolation of a patch or absent in the neurones studied. If this is the latter case, the Ca^{2+}-dependent Cl^- channels may play the same role as the Ca^{2+}-activated K^+ channels since the Cl^- reversal potential in the neurones lies near the resting potential (Chemeris et al., 1982). At least, participation of Cl ions in the overshoot of the action potential in the neurones was found earlier (Krasts, 1978). No special investigations on the existence of the Ca^{2+}-dependent K^+ channels in the neurones of a given molluscan species were done.

Recently, Ca^{2+} was shown to activate nonselective cation channels in neuroblastoma (Yellen, 1982) and cultured muscle cells (Colquhoun et al., 1981). Participation of intracellular Ca^{2+} in activation of anion membrane conductance was reported so far for *Characeae* cells (Lunevsky et al., 1983) and immature *Xenopus* oocytes (Barish, 1983). Activation of Cl^- channels in the muscle cells occurs without intracellular Ca^{2+} (Blatz & Magleby, 1983).

Studies of the influence of extracellular K^+ on Cl^- permeability are not extensive either. Earlier we demonstrated that at elevated K^+ concentrations (as well as Rb^+ and Cs^+) in the outer solutions, the Cl^- conductance in isolated *Lymnaea stagnalis* neurones greatly increases (Kislov & Kazachenko, 1975). Lesser effects of extracellular K^+ were observed in muscle (Hutter & Warner, 1967) and squid axon (Strickholm, 1981).

Two types of observations suggest that the Cl^- channel described has a number of multiple conductance substates: (1) spontaneous reversible transitions of the channel conductance at intermediate levels during the impulse bursts; and (2) spontaneous irreversible disintegration of whole channel conductance into different and independent substates down to elementary ones. In both cases an elementary conductance step is one and the same: 12.5 pS. This value is close to the step size of multistate Cl^- channel in spinal neurones activated by glycine (Hamill, Bormann & Sakmann, 1983).

The phenomena observed may suggest that the Cl^- channel consists of a number (16) of subunits. In the norm the subunits are probably associated with each other and synchronously open or close the channel creating rectangular current impulses of a large amplitude. Under unknown conditions the interrelation of the subunits is either partially reduced or completely destroyed and reversible or irreversible transitions of the channel at intermediate substates are observed. The question to be solved is whether the subunits form a single large pore with variable effective diameter or an aggregate (cluster) of identical pores (channel-subunits) of small conductance. The data on splitting of the channel conductance into independent sublevels may support the clustary organization of the Cl^- channel. Then the degradative processes described above can be treated from the point of breaking of the cluster into independent elementary channel subunits.

Availability of multi-states is not a unique property of the Cl^- channel. Up to now two (Hamill & Sakmann, 1981; Barrett et al., 1982; Siegelbaum et al., 1982; Hamill et al., 1983), three (Benham & Bolton, 1983; Blatz & Magleby, 1983; Hamill et al., 1983; Sauvé et al., 1983) or four (Sakmann & Trube, 1984) conductive substates of the ionic channels in biological membranes have registered. Recently we have examined several kinds of potential-dependent K^+ channels (including Ca^{2+}-activated K^+ channels) in the molluscan neurones, glial and cardiac cells and the rat neurones (Kazachenko & Gele-

tyuk, 1983, 1984). The multiple conductance substates (4–16) and the channel degradation were revealed in all cases. Great numbers of the conductance substates were found while studying the Ca^{2+} channels (Volkova et al., 1980) and Cl^- channels (White & Miller, 1979) isolated, respectively, from the *Characeae* cells or electric organ of *Torpedo californica* and incorporated into lipid bilayers. Structural and functional evidence for multiple complexes of the anion-selective channels in the outer membrane of mitochondria was reported by Manella, Colombini and Frank (1983).

Thus, it is quite possible that the ability to aggregate into complexes (clusters) is an intrinsic property of channel-forming materials in different types of biological membranes.

References

Barish, M.E. 1983. A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol. (London)* **342:**309–325

Barrett, J.N., Magleby, K.L., Pallotta, B.S. 1982. Properties of single calcium-activated potassium channels in cultured rat muscles. *J. Physiol. (London)* **331:**211–230

Benham, C.D., Bolton, T.B. 1983. Patch-clamp studies of slow potential-sensitive potassium channels in longitudinal smooth muscle cells of rabbit jejunum. *J. Physiol. (London)* **340:**469–486

Blatz, A.L., Magleby, K.L. 1983. Single voltage-dependent chloride-sensitive channels of large conductance in cultured rat muscle. *Biophys. J.* **43:**237–241

Cavalié, A., Ochi, R., Pelzer, D., Trautwein, W. 1983. Elementary currents through Ca^{2+} channels in guinea pig myocytes. *Pfluegers Arch.* **398:**284–297

Chemeris, N.K., Kazachenko, V.N., Kislov, A.N., Kurchikov, A.L. 1982. Inhibition of acetylcholine responses by intracellular calcium in *Lymnaea stagnalis* neurones. *J. Physiol. (London)* **323:**1–19

Colquhoun, D., Neher, E., Reuter, H., Stevens, C.F. 1981. Inward current channels activated by intracellular Ca in cultured muscle cells. *Nature (London)* **294:**752–754

Colquhoun, D., Sackmann, B. 1981. Fluctuations in microsecond range of the current through single acetylcholine receptor ion channels. *Nature (London)* **294:**464–466

Fenwick, E.M., Marty, A., Neher, E. 1982. Sodium and calcium channels in bovine chromaffin cells. *J. Physiol. (London)* **331:**599–635

Geletyuk, V.I., Kazachenko, V.N. 1983*a*. Single K^+-dependent Cl^- channel in the molluscan neurones: Multiplicity of the conductance states. *Dokl. Acad. Nauk SSSR* **268:**1245–1247 *(in Russian)*

Geletyuk, V.I., Kazachenko, V.N. 1983*b*. Discreteness of the conductance of single potential-dependent K^+ channel in the molluscan neurones. *Biofizika* **28:**994–998 *(in Russian)*

Hamill, O.P., Bormann, J., Sakmann, B. 1983. Activation of multiple-conductance state chloride channels in spinal neurones by glycine and GABA. *Nature (London)* **305:**805–808

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp technique for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hamill, O.P., Sakmann, B., 1981. Multiple conductance states of single acetylcholine receptor channels in embryonic muscle cells. *Nature (London)* **294:**462–464

Hutter, O.F., Warner, A.E. 1967. Action of some foreign cations and anions on the chloride permeability of frog muscle. *J. Physiol. (London)* **189:**445–460

Kazachenko, V.N., Geletyuk, V.I. 1983. Clustary organization of ionic channels. *In:* Biological Membranes and Function. *(Abstr.)* p. 183. III Soviet-Swiss Symposium. USSR Academy of Sciences, M.M. Shemyakin Institute of Bioorganic Chemistry. Tashkent

Kazachenko, V.N., Geletyuk, V.I. 1984. The potential-dependent K^+ channel in molluscan neurones is organized in a cluster of elementary channels. *Biochim. Biophys. Acta* **773:**132–142

Kislov, A.N., Kazachenko, V.N. 1975. Potassium activation of the chloride conductance in the isolated snail neurones. *Stud. Biophys.* **48:**151–153

Kostenko, M.A., Geletyuk, V.I., Veprintsev, B.N. 1974. Completely isolated neurones in the mollusc *Lymnaea stagnalis*. A new objective for nerve cell biology investigation. *Comp. Biochem. Physiol.* **49A:**89–100

Krasts, I.V. 1978. The amplitude of action potential and calcium ion gradient on the membrane of mollusc neurone. *Comp. Biochem. Physiol.* **60A:**195–197

Lunevsky, V.Z., Zherelova, O.M., Vostrikov, I.Y., Berestovsky, G.N. 1983. Excitation of *Characeae* cell membrane as a result of activation of calcium and chloride channels. *J. Membrane Biol.* **72:**43–58

Manella, C.A., Colombini, M., Frank, J. 1983. Structural and functional evidence for multiple channel complexes in the outer membrane of *Neurospora crassa* mitochondria. *Proc. Natl. Acad. Sci. USA* **80:**2243–2247

Sauvé, R., Roy, G., Payet, D. 1983. Single channel currents from *Hela* cells. *J. Membrane Biol.* **74:**41–50

Siegelbaum, S.A., Camarado, J.S., Kandel, E.R. 1982. Serotonin and cyclic AMP close single K^+ channels in *Aplysia* sensory neurones. *Nature (London)* **299:**413–417

Strickholm, A. 1981. Ionic permeability of K^+, Na^+ and Cl^- in potassium depolarized nerve. *Biophys. J.* **35:**677–697

Thomas, S.H., Aldrich, R.W. 1980. Membrane potassium channels. *In:* The Cell Surface and Neuronal Function. C.W. Cotman, G. Poste, and G.L. Nicolson, editors. pp. 49–85. Elseveier/North-Holland Biomedical, Amsterdam

Volkova, S.P., Lunevsky, V.Z., Spiridonov, N.A., Vinokurov, M.G., Berestovsky, G.N. 1980. About chemical composition of the Ca^{2+} channels in *Characeae* cells. *Biofizika* **25:**537–541 *(in Russian)*

White, M.M., Miller, C. 1979. A voltage-gated anion channel from the electric organ of *Torpedo californica*. *J. Biol. Chem.* **254:**10161–10166

Yellen, G. 1982. Single Ca^{2+} activated nonselective cation channels in neuroblastoma. *Nature (London)* **296:**357–359

Received 23 March 1984; revised 10 October 1984