Biochimica et Biophysica Acta, 773 (1984) 132–142
Elsevier

BBA 72106

THE POTENTIAL-DEPENDENT K^+ CHANNEL IN MOLLUSCAN NEURONES IS ORGANIZED IN A CLUSTER OF ELEMENTARY CHANNELS

V.N. KAZACHENKO * and V.I. GELETYUK

Laboratory of Nerve Cell Biophysics, Institute of Biological Physics of the U.S.S.R. Academy of Sciences, Pushchino, 142292 (U.S.S.R.)

(Received December 7th, 1983)

Key words: Patch-voltage clamp; K^+ channel; Elementary channel; Membrane potential; (Molluscan neurone)

Single potential-dependent K^+ channels were studied using the patch-voltage-clamp method. Two types of channel with identical, but oppositely directed, potential dependences were found. The channels of the first type (slow channels) are assumed to be responsible for the outward rectification. The properties of the channels of the second type (fast channels) are similar to those of the K^+ channels in neurone soma which create the fast transient currents. The kinetic characteristics of both types of channel are presented. The conductances of slow and fast K^+ channels are approx. 30 and 40 pS, respectively, at zero membrane potential and a K^+ concentration of 50 mmol/l at the inner side of the membrane. The following sequence of channel selectivity with respect to monovalent cations was found: $Tl^+ > K^+ > Rb^+ \gg Cs^+ \equiv Li^+ \equiv Na^+$. The probability of the channel open state monotonically decreases with free Ca^{2+} concentration at the inside membrane surface for both types of channel. It was found that the channels have discrete and multiple conductance substates. It is supposed that a unitary K^+ channel consists of approx. 16 elementary ones with conductances of approx. 2 pS (slow channels) and approx. 2.5 pS (fast channels) at zero potential. At +100 mV the elementary conductances are equal to approx. 4 and 5.5. pS, respectively. Thus, according to this assumption, the unitary channel is a cluster of elementary channels.

Introduction

Use of a new patch-voltage-clamp method for studying the biological membranes [1,2] allowed not only the direct determination of the characteristics of single ionic channels (conductance, lifetime, etc.), but also the study of some peculiarities of the channel operation. For example, it was found that practically all types of channel investigated exhibit burst-like activity (see, for instance, Refs. 3, 4). Recently it was shown that some types of ionic channels may have more than one conductive state [4–11]. So, this method permits study of those properties of the channels which (themselves) cannot be revealed using a traditional microelectrode technique. An additional advantage of the patch-clamp method is that ionic channels are studied in their natural membrane environment.

In this work, single potential-dependent K^+ channels in the molluscan neurones were studied. Two types of channel (fast and slow) were identified. Besides determination of their characteristics, we paid peculiar attention to the multiplicity of the channel conductance states.

Materials and Methods

Completely isolated neurones

The neurones were isolated after pronase pretreatment (0.35%, 0.1–0.5 h, 20–22°C) of fresh

* To whom correspondence should be addressed.

water mollusc *Lymnaea stagnalis* brain by the method described earlier [12]. Preliminary experiments showed that neither pronase digestion of the ganglia nor direct addition of the enzyme to the bath had appreciable effects on the channel currents studies. Experiments were performed on non-identified neurones (20–200 μm in diameter) from the left and right parietal and visceral ganglia. The characteristics of K^+ channels of a given type are identical for all cells.

Conditions of recording

The experiments were carried out using two variants of the patch-clamp method, 'cell-attached' and 'inside-out' ones [2] with pipette-membrane seals equal to 20–100 GΩ. In the first case the pipette was in contact with the neurone, and the potential on the patch membrane (V_m) was equal to the difference between the cell resting potential (assumed to be of about -50 mV) and the pipette potential. In the 'inside-out' experiments a patch of the membrane was isolated from the cell, and its internal (cytoplasmic) side was in contact with the bath solution. In this case, the membrane potential was determined as the bath potential with respect to the pipette one. The currents across the membrane are described with the usual sign convention, i.e., the currents flowing from the neurone (or from the experimental bath) to the external side of the membrane (to the pipette) were taken as positive and are displayed upwards. Liquid junction potential was 1–3 mV and is neglected.

Solutions

The pipette was filled with normal physiological solution containing (mmol/l): NaCl, 50; KCl, 1.5; $MgCl_2$, 1.5; and $CaCl_2$, 4; pH was adjusted to 7.5 by Tris-HCl (2.5 mmol/l). In the 'inside-out' experiments, the internal side of the membrane was perfused with the main bath solution (mmol/l): KCl, 50; Hepes-KOH, 5; at pH 7.1–7.2. In some experiments the internal solutions contained different concentrations of free Ca^{2+}. Ca^{2+} concentration was stabilized with Ca^{2+}-EGTA buffer (1 mmol/l) according to [13]. In studying the multiplicity of the conductance states of the channels the following modified solution in the pipettes was used (mmol/l): NaCl, 50; KCl, 1.5; $CaCl_2$, 4; $CdCl_2$, 2; tetrodotoxin, 0.1; ethacrynic acid, 1; pH 7.5 (Tris-HCl). Cd^{2+}, tetrodotoxin and ethacrynic acid were added to block channels other than K^+: Ca^{2+}, Na^+, and Cl^- ones, respectively. Modifications of the internal solution are mentioned for each set of the experiments.

Other conditions

The pipettes were fabricated from Pyrex. The tip diameters of the fire-polished pipettes were less than 1 μm. The resistance of the pipettes was 5–50 MΩ. The gigaseals were set up after application of negative hydrostatic pressure (10–50 cmH_2O) into the pipette. The experiments were performed using a special bath (0.1 ml) providing continuos exchange of the bath solutions (several bath volumes per s). The temperature was maintained nearly at 20–22°C. We analyzed only those data obtained in the experiments where a patch had one functionally active K^+ channel of any type.

Results

Identification of two types of K^+ channel

We have found two types of K^+ channel with different conductances and potential dependences of the kinetic characteristics. Fig. 1 represents the current recordings from the channels of both types at different levels of membrane potential. As seen, the currents display burst-like behaviour, i.e., comparatively short current pulses are combined in the impulse series (bursts).

The first striking difference between these channels is opposite direction of the potential dependences of the burst duration (t_b). For the first channel (Fig. 1A) the burst duration increases as the membrane potential is shifted towards positive values, whereas for the second channel the reverse situation is observed. In addition, at positive values of the membrane potential both the burst duration and duration of a single current impulse (τ_0) for the first channel are greater than those for the second channel. That is why we term the channel of the first type the slow K^+ channel (Fig. 1A), and the channel of the second type the fast K^+ channel (Fig. 1B).

Fig. 2A shows the current-voltage relationships for both types of channel. The curves extrapolated intersect the voltage axis at the same point which

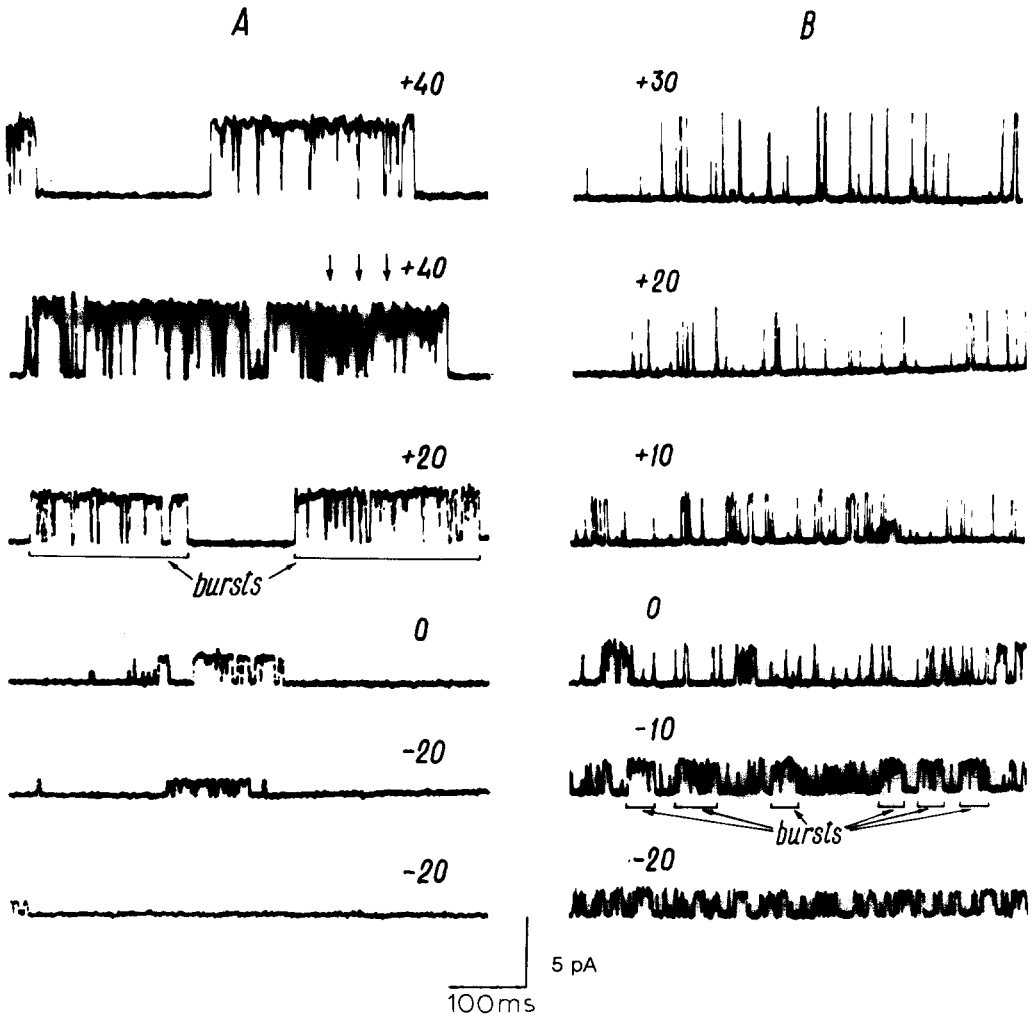

Fig. 1. Recordings of outward currents through the slow (A) and the fast (B) K^+ channels at different membrane potentials. Different 'inside-out' patches. The currents were recorded several seconds after shifting of the membrane potential from −50 mV to the potentials indicated (in mV) near the recordings. The pipettes contained normal physiological solution; the bath solution was as 50 mmol/l of KCl. Arrows indicate some intermediate sublevels of the current. Filtering, approx. 500 Hz.

denotes reversal potential (V_r). For the channels of both types, the reversal potentials obtained in the 'inside-out' experiments (50 mmol/l of internal KCl) range from −70 to −80 mV. In the 'cell-attached' experiments the reversal potentials are by nearly 10–15 mV more negative than the resting potential. A 4-fold decrease in the internal K^+ concentration shifts the reversal potential towards positive values by 30–35 mV (Fig. 2A) according to the Nernst equation for a potassium-sensitive electrode. The data confirm the potassium nature of the currents studied.

The current-voltage relationships (Fig. 2A) are non-linear. The calculations show that nonlinearity may be due to asymmetrical distribution of K^+ on both membrane sides (Goldman's rectification). The current-voltage curves give the chord channel conductance (g_k) as the ratio between the current at a given value of the membrane potential and the value of the electromotive force ($V_m - V_r$). The channel conductance increases with membrane depolarization. Table I gives avarage values of the conductances at three levels of the membrane potential and 50 mmol/l of internal KCl.

The conductances increase with K^+ concentration in the bath (Fig. 2B,C). This dependence is described by an empirical equation:

$$g_k = g_{max} \frac{[K^+]}{[K^+] + K_d} + g_0 \quad (1)$$

Here g_{max} is the maximal value of the channel conductance at saturating K^+ concentrations; K_d is the apparent dissociation constant; and g_0 is some residual conductance. For slow and fast channels, g_{max} equals 91 and 119 pS, and the dissosiation constants are 26 ± 4.0 (S.D., $n = 4$) and 51 ± 3.0 (S.D., $n = 4$) mmol/l, respectively. The residual conductance is 5–10 pS.

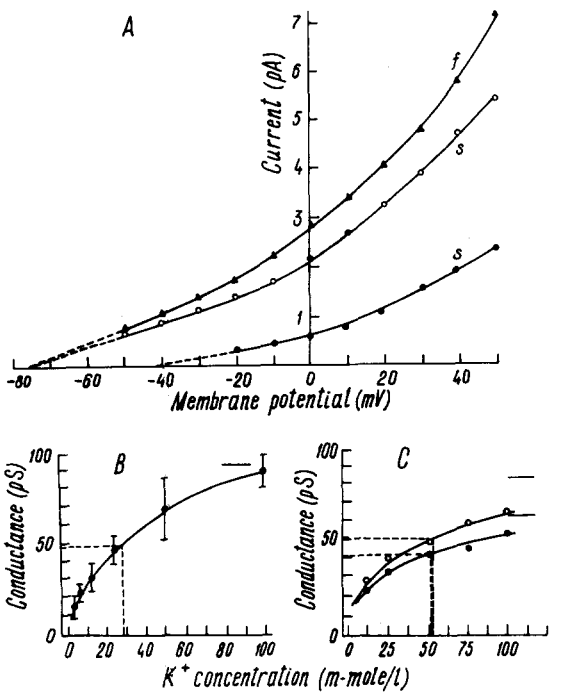

Fig. 2. (A) Current-voltage relationships of the fast (f) and slow (s) channels obtained when the bath solution contained 50 (▲———▲, O———O) or 12.5 (●———●) mmol/l KCl. (B) Dependence of the slow channel conductance on internal K^+ concentration. The conductance was measured at +100 mV membrane potential. The solid line is drawn according to Eqn. 1. Error bars give ±S.D. ($n = 4$). (C) Dependences of the fast channel conductance on internal K^+ concentration at zero (●———●) and +50 mV (O———O) membrane potential. The data of the same experiment. In (B) and (C) horizontal bars indicate the maximal channel conductances (obtained at 200 mmol/l of internal KCl). The procedure of obtaining the apparent dissociation constants is shown by dotted lines. 'Inside-out' patches. The pipettes were filled with normal physiological solution. Required concentrations of internal K^+ were obtained by dilution of 100 mmol/l KCl.

Ionic selectivity of the channels

To study the ionic selectivity of the channels with respect to monovalent cations (Li^+, Na^+, K^+, Rb^+ and Cs^+) the channel conductances were measured at +50 mV membrane potential several minutes after substitution of the bath KCl medium (100 mmol/l) by equimolar chloride solution with ions being studied. In the case of TlCl, a concentration of about 13 mmol/l of both KCl and TlCl was used due to poor solubility of TlCl. The following sequence of ionic selectivity was found:

$$Tl^+ > K^+ > Rb^+ \gg Cs^+ \cong Li^+ \cong Na^+ \quad (2)$$

The channels appeared to be practically impermeable to Li^+, Na^+ and Cs^+; the permeability for Rb^+ is 7–10-times less than that for K^+. The permeability for Tl^+ is about twice that for K^+. The sequence (2) is qualitatively similar to those found in other types of cell (see Ref. 14).

Kinetic characteristics of the K^+ channels

Fig. 3 shows the potential dependences of the kinetic characteristics of both types of channel. As seen, identical characteristics mainly have oppositively directed dependences on the membrane potential. For the slow K^+ channel, the duration of the bursts and probability of the channel open state increase with positivity of the membrane potential. The duration of single current impulses is practically unchanged. For the fast K^+ channel,

TABLE I

CONDUCTANCES OF THE SLOW AND FAST K^+ CHANNELS (pS)

The pipettes were filled with normal physiological solution. The bath solution was as 50 mmol/l of KCl. Figures in parentheses show the number of patches.

Channel	Conductance (pS); membrane potential (mV)		
	0	+50	+100
Slow	29 ± 2.5 (10)	38.5 ± 4.0 (6)	62.5 ± 5.5 (6)
Fast	41 ± 3.5 (5)	56 ± 4.0 (5)	83 ± 5.0 (5)

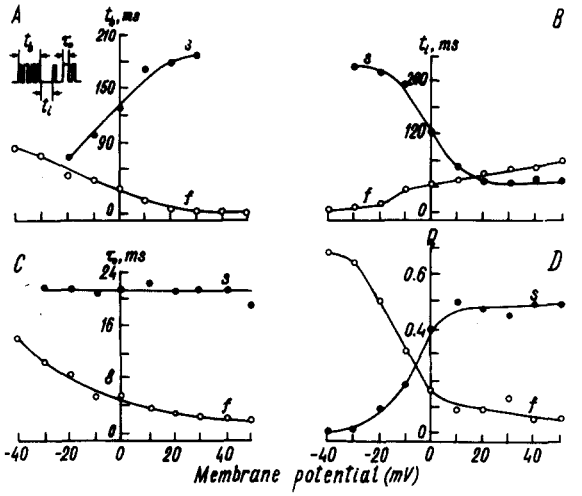

Fig. 3. Potential dependences of steady-state kinetic characteristics of the slow (s) and fast (f) channels. 'Inside-out' patches. The pipettes contained a normal physiological solution. The bath solution was as 50 mmol/l KCl. Insert; schematic representation of channel currents and their characteristics. Denotes: t_b, t_i, τ_0 and P_0 are the burst duration, interburst interval, duration of single current impulse, and probability of channel open state.

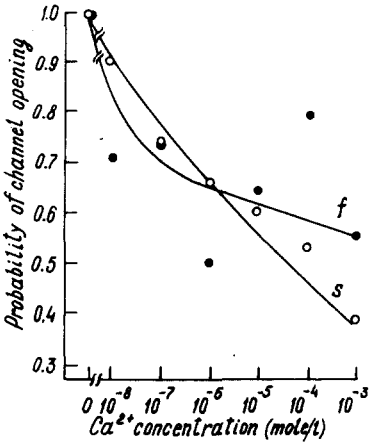

Fig. 4. Effect of internal Ca^{2+} on probability of channel opening for the slow (s) and fast (f) K^+ channels. Relative probability values, P_{Ca}/P_{EGTA}, we used. P_{Ca} is the probability of channel opening at a given Ca^{2+} concentration, P_{EGTA} is that for the solution without Ca^{2+} (1 mmol/l of EGTA). 'Inside-out' patches. The pipettes were filled with normal physiological solution. The bath solution consisted of 50 mmol/l KCl and the required Ca^{2+} concentration. Membrane potential, +50 mV. Each point of the plots represents an average value from 3–4 patches.

the reverse situation is observed, except for the duration of single current impulses, which is also potential-dependent and decreases with the membrane potential. Table II summarizes the values of the channel parameters.

Effect of internal Ca^{2+}

It is well known that many types of cell possess K^+ channels activated by intracellular Ca^{2+} [15]. In connection with this, a set of experiments with alteration of free Ca^{2+} at the internal membrane side was performed. For both types of K^+ channel, a monotonic decrease in probability of channel opening was found in a wide range of Ca^{2+} concentrations (from 0 to 1.0 mmol/l) (Fig. 4). The loss in such probability is accounted for mainly by shortening of the burst duration and increase of the intervals between the bursts (t_i).

Apparant dissociation constants for calcium action range from 0.1 to 1.0 mmol/l, indicating that the phenomenon observed could be considered as nonspecific.

Mg^{2+} inhibits the channels at higher concentrations than does Ca^{2+}. No special investigations were done on this.

Thus, an inhibitory effect of internal Ca^{2+} suggests that neither type of K^+ channel is Ca^{2+}-activated channels in the common sense.

TABLE II

THE CHARACTERISTICS OF THE SLOW AND FAST K^+ CHANNELS AT +50 mV

$P_{o\ max}$ is maximal value of probability of channel opening. It was reached at positive (for the slow channel) and negative (for the fast channel) values of the membrane potential.

Channel	Characteristics			
	t_b (ms)	t_i (ms)	τ_0 (ms)	$P_{0\ max}$
Slow	200–300	20– 50	10 –20	0.3–0.7
Fast	1– 5	100–150	0.5– 1.0	0.3–0.7

Blockers

The fast and slow K^+ channels differ from each other by their sensitivity to various blockers (Ba^{2+}, Cs^+, tetraethylammonium and 4-aminopyridine).

The mechanisms of action of a blocker are the same for both types of channel and are as follows.

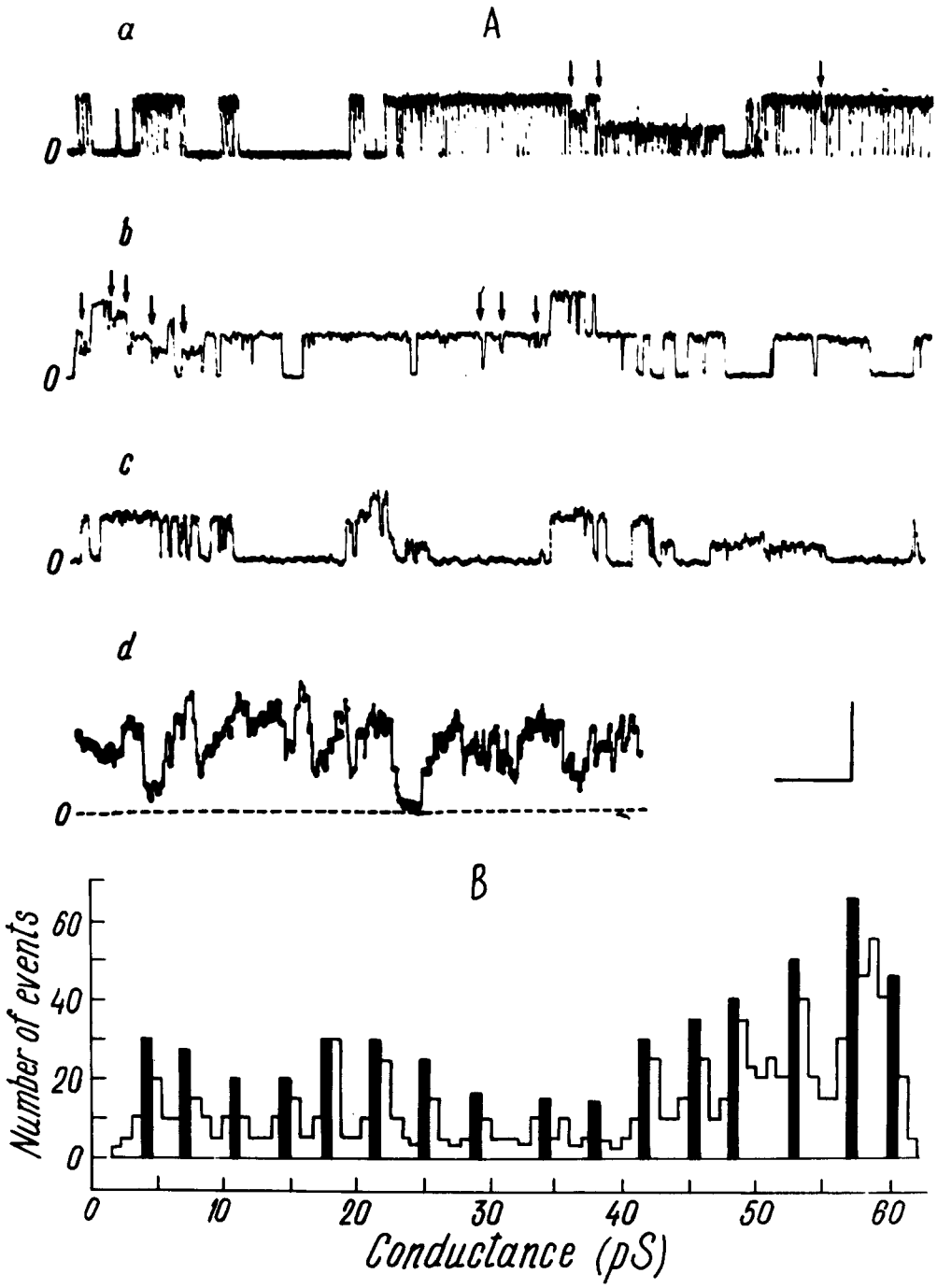

Fig. 5. (A) Outward currents through the slow K^+ channels. The recordings were made in the 'cell-attached' experiments (a, b) and in 'inside-out' ones (c, d), '0' indicates shut states of the channels. In (a,b) the membrane potential was kept at +50 mV (assuming the resting potential −50 mV). Fixed membrane potential in (c,d) +40 mV. The arrows in (a,b) indicate some intermediate current levels. In (c,d) the bath solution contained 50 mmol/l KCl. The pipettes contained normal physiological solution with blockers added (see Materials and Methods). Approx. 1 kHz pass filter. Calibrations: 5.5, 7.0, 10.5 and 3.5 pA and 150, 30, 30 and 10 ms for (a), (b), (c) and (d), respectively. (B) Distribution of observed conductance sublevels for the slow K^+ channel. 'Inside-out' patch. Membrane potential, +100 mV. The pipette was filled with physiological solution containing bloking agents: tetrodotoxin, Cd^{2+}, and ethacrynic acid. The bath solution was 50 mmol/l KCl. Approximately central columns of the distribution peaks are shaded black. Sampling time, approx. 0.5 s.

Ba^{2+} (in concentrations from 0.01 μmol/l to 1.0 mmol/l) shortens the burst duration and lifetime of single impulses.

Cs^+ and 4-aminopyridine decrease the amplitudes of the currents, and tetraethylammonium produces both effects.

The slow K^+ channel is effectively blocked by Ba^{2+} with two apparent constants of blocking, K_b (0.1 μmol/l and 20 μmol/l), and by tetraethylammonium ($K_b \approx 5$ mmol/l).

The fast K^+ channel is blocked by Ba^{2+} and Cs^+ with the constants 20 μmol/l and 0.25 mmol/l, respectively. The blocking constant for 4-aminopyridine is about 10 mmol/l.

In detail, the effects of the blockers will be described in special papers (Ref. 16, and Kazachenko and Geletyuk, unpublished data).

Multiplicity of the conductance states

The slow K^+ channel. It was found that the channel conductance has a number (approx. 16) of multi-states. This phenomenon was first registered while analysing the current oscillations within the current bursts. The flickering channel transits rather more often between intermediate substates than between completely open and completely closed states. Some of the current substates are shown in Fig. 1A and Fig. 5A.

In the 'cell-attached' experiments, the intermediate substates appear comparatively rarely (Fig. 5A a,b) However, after excision of a patch, the frequency of appearance of the substates may sharply increase (Fig. 5A, c). Fig. 5B demonstrates distribution of the conductance substates obtained in one of the 'inside-out' experiments. The distribution has approx. 16 equidistant peaks differing from each other by approx. 4 pS (+100 mV).This value characterizes, probably, an elementary conductance step. At membrane potential of zero the elementary conductance equals 1.5–2.0 pS, and at +50 mV approx. 2.8 pS. Thus, the potential dependence of the elementary conductance is similar to that of the whole-channel conductance (see Fig. 2A and Table I).

In some 'inside-out' experiments the rectangular current impulses might be converted with time into 'current noise' (Fig. 5A,d). In these cases the repair of the seal was tested by substitution of internal KCl for NaCl when only leakage currents were registered. It was found that K^+ 'current noise' is not due to disturbance of the seal. The 'current noise' has discrete sublevels. The elementary conductance step of the 'noisy' channel coincides with that obtained from the distribution similar to that shown in Fig. 5B.

The conversion of the current impulses into the 'current noise' reflects peculiar degradation of the channel conductance. The degradation proceeds as spontaneous and irreversible process under the experimental conditions used. Breakup of the channel conductance into several substates can be induced by internal Cd^{2+} (0.1–10 μmol/l) (see Fig. 6). First the channel forms ordinary current

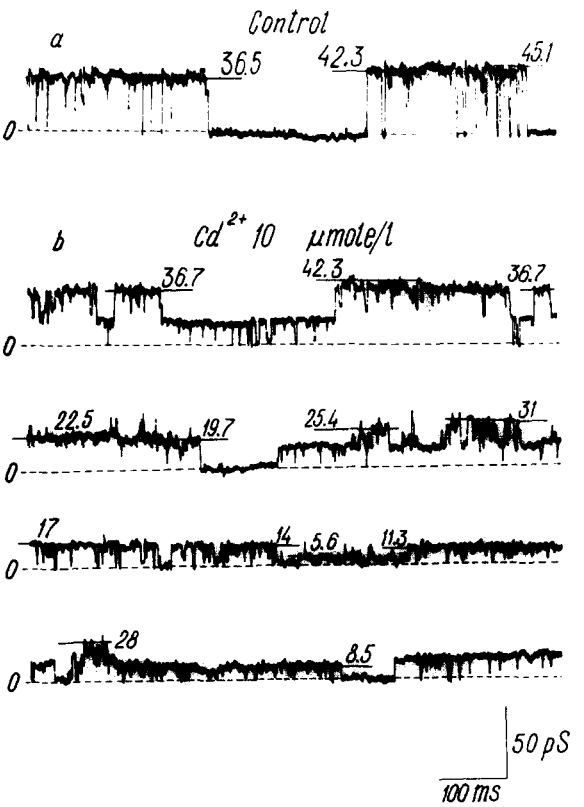

Fig. 6. Effect of internal Cd^{2+} on the slow channel. (a) The channel activity before application of Cd^{2+}. (b) Selected recordings of the channel activity several minutes after application of Cd^{2+} ($1 \cdot 10^{-5}$ mol/l). Figures near the recordings indicate conductance levels (pS). 'Inside-out' experiments. The pipette contained physiological solutions with blockers (see Materials and Methods). The bath solution was 50 mmol/l of KCl in (a) and that $+Cd^{2+}$ in (b). The membrane potential, +50 mV. Low pass filtering, approx. 500 Hz.

bursts (Fig. 6,a). Several seconds after substitution of initial bath solution (50 mmol/l KCl) by the solution with Cd^{2+} (10 μmol/l) the burst-like behaviour of the current is disrupted and practically all 16 substates of the channel conductance can be revealed (Fig. 6,b). The substates are multiple to approx. 2.8 pS (+50 mV). In this experiment, the original conductance of approx. 45 pS is cleaved into two main independent substates (approx. 15 pS and approx. 30 pS). All the remaining substates are interrelated intermediate levels of these two main conductances. The effect of Cd^{2+} is irreversible, at least in the course of the experiment (1–2 h).

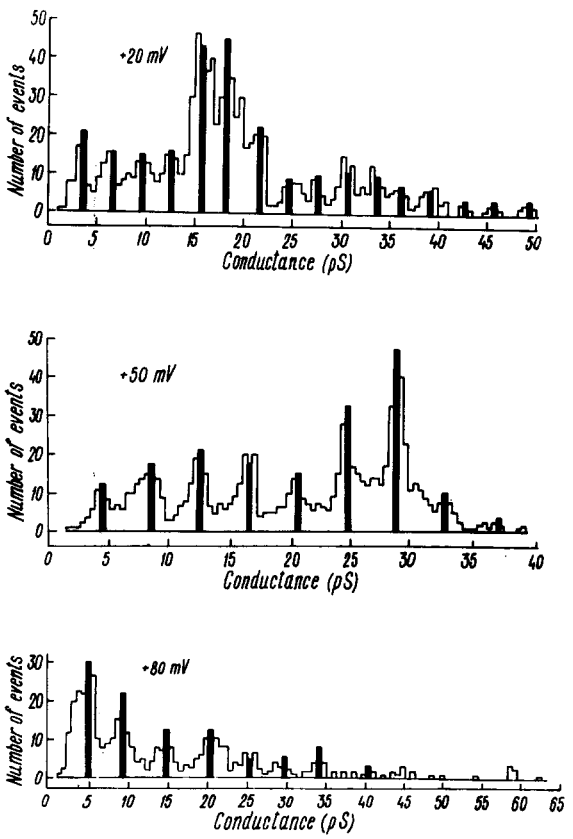

Fig. 7. Distributions of amplitude values of single conductance impulses for the fast channel at +20, +50, and +80 mV of the membrane potential. 'Inside-out' patch. The pipette was filled with physiological solution tetrodotoxin, Cd^{2+} and ethacrinic acid. The bath solution contained 50 mmol/l of KCl. Approximately central columns of the peaks in the distributions are shaded black. At +50 and +80 mV the channel comparatively rarely displayed the highest values of conductance (40–60 and 65–80 pS at +50 and +80 mV, respectively), so they are not shown in distributions. Low pass filtering, approx. 500 Hz.

Thus, we beleive that spontaneous reversible current oscillations, spontaneous irreversible degradation of the channel conductance, and splitting of the channel conductance by Cd^{2+} suggest that the slow K^+ channel may acquire a number (approx. 16) of discrete and multiple values of conductance.

The fast K^+ channel. At positive values of the membrane potential the fast K^+ channel forms primary single current impulses (Fig. 1B), and, as a result, comparatively rarely displays intermediate conductive substates similar to those of the slow K^+ channel (Fig. 1A, Fig. 5A). However, it was observed that sometimes the channel creates a substantial portion of the current impulses of various decreased amplitudes. It was supposed that this phenomenon reflects the conductance sublevels of the fast K^+ channel. Fig. 7 shows the amplitude-frequency distribution of the current impulses at three values of the membrane potential. Each distribution has a number of equidistant peaks. The intervals between neighbouring peaks are approx. 3.0, 4.1 and 5.0 pS for +20, +50 and +80 mV, respectively.

One may think that the variety of the current amplitudes is due to low resolution of the technique. Indeed, in our case (Fig. 7, low-pass filtering approx. 500 Hz) the current impulses with duration of less than approx. 0.3 ms must experience great loss of amplitude. However, if original current impuses are of the same amplitude, the amplitude distribution for impulses registered (at one-exponential distribution of the impulse durations) would be monotonically increased due to low passing ability of the technique. Real distributions (Fig. 7) have a number of peaks. That is why we believe that these peaks characterize intermediate substates of the fast K^+ channel. As in the case of the slow K^+ channel, the number of substates of the fast K^+ channel is about 16.

In four experiments, spontaneous and irreversible degradation of the channel conductance was observed. Fig. 8 shows four stages of this process. After excision of the patch the channel forms the usual currents corresponding to the conductance of approx. 60 pS (+50 mV) during 15–20 min (Fig. 8a). Later the current impulses become bi-

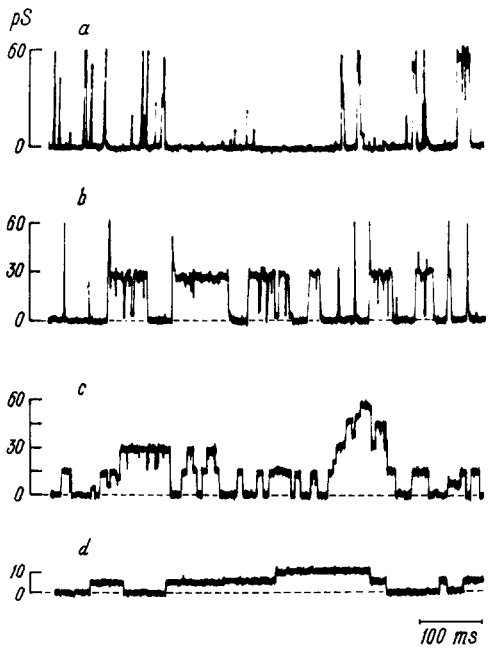

Fig. 8. Degradation of the fast channel conductance. (a) Initial conductance impulses (60 pS) registered at the beginning of the experiment. (b) 15–20 min later the conductance broke up into two main substates, approx. 60 and 30 pS. (c) The conductance broke up into four slightly interrelated substates each of approx. 16 pS (after next 30 min). The conductance impulses at the end of the experiment (1.5 h after it began). Registering conditions: 'inside-out' patch; +50 mV (a, b, c) and +100 mV (d). The pipette was filled with physiological solution containing tetrodotoxin, Cd^{2+}, and ethacrinic acid. The bath solution was as 50 mmol/l of KCl.

phasic. The first phase is transient, and the second one prolonged (Fig. 8b). After the next approx. 30 min the original conductance subdivided into four practically independent sublevels of approx. 16 pS each (Fig. 8c). Finally, about 1.5 h after the start of the experiment, the channel conductance breaks into smallest, elementary, independent subunits similar to those shown in Fig. 8d (about 4 pS at +50 mV and 5.5 pS at +100 mV). Thus the potential dependence of the elementary conductance is similar to that of the whole channel conductance (see Fig. 2A, Table I). As seen from Fig. 8, the conductance splitting prolonges duration of the current impulses.

The intermediate conductance substates arising in the course of the experiment are, most likely, derivatives of one and the same original conductance, for the following reasons. (1) The appearance of the substates of reduced conductances is accompanied by a vanishing of original substates of large conductance. (2) The total conductance of cleaved substates does not exceed the conductance of an original channel. (3) The values of the elementary conductances arising due to the split (Fig. 8d) coincide with those obtained from distribution of reversible substates (Fig. 7). Average values of the elementary conductances obtained by both means are as follows: 2.5 ± 0.4 pS (zero potential), 3.8 ± 0.4 pS (+50 mV), and 5.5 ± 0.5 pS (+100 mV) (S.D., $n = 5$).

The data considered show that both the fast and slow K^+ channels have about 16 multiple intermediate substates of the conductance. The transitions between the substates may be spontaneous (reversible or irreversible) or induced by outer agents (for example, by Cd^{2+}).

Discussion

Two types of K^+ channel

Three types of potential-dependent K^+ channel have been identified in the somatic membrane of the molluscan neurones [17,18]. (1) The channels of the outward rectification activated presumably at the membrane potentials positive to −30 to −20 mV. These channels are blocked by externally and internally applied tetraethylammonium. (2) channels responsible for the transient currents. Steady-state inactivation of these channels is completed near the resting and more positive potentials and is completely removed at about −100 mV. The fast currents are slightly sensitive to tetraethylammonium, but can be blocked by 4-aminopyridine ($K_b = 1–2$ mmol/l). (3) The channels activated by increase in ionized calcium concentration in the cytosol either due to Ca^{2+} influx through voltage-dependent Ca^{2+} channels or intracellular injection of Ca^{2+} salts. Activation of the channels can be prevented when the voltage-dependent Ca^{2+} influx is blocked, for example, by external application of blocking ions (Ni^{2+}, Cd^{2+}, etc.).

We describe some properties of two type of single K^+ channel, fast and slow, with kinetically identical but oppositely directed potential dependences (Fig. 4). The activity of the channels is

blocked by internal Ca^{2+} over a wide concentrational range, so the channels differ from classical Ca^{2+}-activated K^+ channels.

The slow K^+ channels are, most likely, responsible for the outward rectification. The following facts support this assumption. (1) The channels open predominantly at the potentials more positive than -30 to -20 mV. (2) The channel conductance is blocked by internally applied tetraethylammonium, an apparent dissosiation constant being of approx. 5 mmol/l (Kazachenko and Geletyuk, unpublished data). (3) The channels are insensitive to aminopyridine. (4) The sequence of the channel selectivity (2) with respect to monovalent ions is similar to that of the channels of the outward rectification in other types of cell (see Ref. 14).

The fast K^+ channel discussed in the present paper with respect to its potential dependence is similar to that responsible for the fast transient currents in molluscan soma [17,18]. The main discrepancies between the fast channels described here and those studied by other authors by microelectrode methods are as follows. First, in our case the fast channels are inactivated at positive values of the membrane potential (Fig. 4D) rather than at -50 mV as found in microelectrode investigations. Second, the channels are slightly sensitive to 4-aminopyridine ($K_b \approx 10$ mmol/l, cf. Ref. 18).

The potential dependence of the probability of the channel open state for the fast K^+ channel (Fig. 3D) characterizes steady-state inactivation of the channel. Recently we have shown that the inactivation mechanism is disturbed by excision of a patch [11]. So, we believe that a shift of the activation curve towards positive values of the membrane potential may result from partial or complete disturbance of the inactivation mechanism.

Slight sensitivity to 4-aminopyridine is characteristic not only for the single fast K^+ channel but also for integral K^+ currents measured in the whole neurones (Chemeris N.K., personal communication).

The fast K^+ channels are known to be responsible for repetitive firing [19]. In this connection it is of interest that we have observed the fast K^+ channels presumably in pacemaker neurones, which may be an additional argument in favour of our assumption of the nature of the fast K^+ channels studied.

Multiplicity of the conductance states

The data presented in this paper suggest that the fast and slow K^+ channels in molluscan neurones have multi-states of the conductance. The following observations are in favour of the suggestion. (1) Discreteness of both spontaneous reversible oscillations within the bursts (Fig. 5, the slow K^+ channel), and amplitudes of single impulses (Fig. 7, the fast K^+ channel). (2) Spontaneous irreversible splitting of the channel conductance (conversion of rectungular current bursts into multiple independent substates, Fig. 8). (3) Splitting of the slow channel conductance induced by Cd^{2+} (Fig. 6). Similar phenomena are characteristic of Cl^- channels in the molluscan neurones [10], K^+ channels in the glial cells and K^+ channels of different kinds in some other types of cell (molluscan and rat heart muscle cells, salivary gland cells) (Geletyuk and Kazachenko, unpublished data).

In the norm, all conductance substates of a channel are associated in such a way that their activities are almost synchronous, and due to that rectangular current impulses are created. Under unknown conditions (or due to Cd^{2+}) the interrelation between substates is either partially reduced or completely destroyed. As a result, either reversible transitions at intermediate levels or irreversible degradation of the channel conductance are observed. These phenomena are believed to characterize the unitary ionic channel as a 'functional' cluster combining about 16 functional substates.

What is structural basis of the conductance multistates observed? There may exist, at least, two principal modes of channel organization. (1) A channel has a single ionic pore. In this case intermediate substates of the conductance may arise due to fluctuations of an effective diameter of the pore. The latter may alter because of fluctuations of either residual chains of polypeptides forming the pore, or a number of the channel subunits (as in the case of alamethicin [20] or melittin [21] pores incorporated into lipid bilayers). (2) Unitary channel represents an aggregate (cluster) of small identical ionic pores (channel subunits). These elemetary channels are associ-

ated with each other and operate as a single channel of large conductance. In such a model, disturbance of the synchronism in operation of the elementary channels leads to appearance either of reversible fluctuations of the channel conductance or irreversible splitting of the conductance.

So far, the experimental data are too incomplete to permit a final conclusion concerning the cannel structure. But we believe that both the discrete multiple character and the irreversible splitting of the channel conductance are in favour of the organization of the channels in clusters rather than a single ionic pore with an oscillating effective diameter.

Recently, several reports have appeared about two [4–6] or three [7–9] conductance substates in the ionic channels in biological membranes. A great number of conductance substates has been found while studying the Ca^{2+} channels [22] and Cl^- channels [23] isolated respectively from *Characean* cells or the 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 has been reported by Manella et al. [24].

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

References

1 Neher, E. and Sakmann, B. (1976) Nature 260, 799–802
2 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100
3 Fenwick, E.M., Marty, A. and Neher, E. (1982) J. Physiol. (Lond.) 331, 599–635
4 Barret, J.N., Magleby, K.L. and Pallotta, B.S. (1982) J. Physiol. (Lond.) 331, 211–230
5 Hamill, O.P. and Sakmann, B. (1981) Nature 294, 462–464
6 Siegelbaum, S.A., Camardo, J.S. and Kandel, E.R. (1982) Nature 299, 413–417
7 Benham, C.D. and Bolton, T.B. (1983) J. Physiol. (Lond.) 340, 469–486
8 Sauvé, R., Roy, G. and Payet, D. (1983) J. Membrane Biol. 74, 41–50
9 Blatz, A.L. and Magleby, K.L. (1983) Biophys. J. 43, 237–241
10 Geletyuk, V.I. and Kazachenko, V.N. (1983) Dokl. Acad. Nauk. U.S.S.R. 268, 1245–1247 (in Russian)
11 Geletyuk, V.I. and Kazachenko, V.N. (1983) Biofizika 28, 994–998 (in Russian)
12 Kostenko, M.A., Geleyuk, V.I. and Veprintsev, B.N. (1974) Comp. Biochem. Physiol. 49A, 89–100
13 Portzehl, H. Caldwell, P.C. and Rüegg, J.C. (1964) Biochim. Biophys. Acta 79, 581–591
14 Adams, D.J., Smith, S.J. and Thompson, S.H. (1980) Annu. Rev. Neurosci. 3, 141–167
15 Meech, R.W. (1978) Annu. Rev. Biophys. Bioeng. 7, 1–8.
16 Kazachenko, V.N. and Geletyuk, V.I. (1984) Biol. Membranes 2, in the press (in Russian)
17 Thompson, S.H. (1977) J. Physiol. (Lond.) 265, 465–488
18 Thomas, S.H. and Aldrich, R.W. (1980) in The Cell Surface and Neuronal Function (Cotman, C.W., Poste, G. and Nicolson, G.L., eds.), pp. 49–85, Elsevier/North-Holland Biomedical Press, Amsterdam
19 Connor, J.A. and Stevens, C.F. (1971) J. Physiol. (Lond.) 213, 21–30
20 Boheim, G. (1974) J. Membrane Biol. 19, 277–303
21 Hanke, W., Methfessel, C., Wilmsen, H.-U., Katz, E., Jung, G. and Boheim, G. (1983) Biochim. Biophys. Acta 727, 108–114
22 Volkova, S.P., Lunevsky, V.Z., Spiridonov, N.A. and Vinokurov, M.G. (1980) Biofizika, 25, 537–541 (in Russian)
23 Miller, C. and White, M.M. (1979) J. Biol. Chem. 254, 10161–10166
24 Manella, C.A., Colombini, M. and Frank, J. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 2243–2247