Voltage-insensitive Na Channels of Different Selectivity in Human Leukemic Cells

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Abstract. Patch clamp method was used to search for, and characterize ion channel activity which may participate in cation influx in human myeloid leukemia K562 cells In cell-attached, outside-out and whole-cell experiments two types of voltage-insensitive Na-permeable channels were identified with different selectivities for monovalent cations, referred to as channels of high (HS) and low (LS) selectivity The unitary conductance was similar for both channel types being 12 pS (145 mmol/l Na, 23 °C) The relative permeability P_{Na}/P_K estimated from the extrapolated reversal potential values were 10 and 3 for HS and LS channels, respectively Both HS and LS channels were found to be impermeable to bivalent cations (Ca^{2+} or Ba^{2+}) The activity of HS and LS channels displayed a tendency to increase with depolarization Both channel types were not blocked by tetrodotoxin and were insensitive to amiloride in the concentration range of up to 100 μmol/l At higher concentrations (0 1–2 mmol/l), amiloride reversibly inhibited HS channels only The results obtained lead us to conclude that, under physiological conditions, both types of Na-permeable channels may provide sodium influx in leukemic cells Our data imply the existence of a novel family of Na channels in blood cells

Key words: Patch clamp — Leukemia cell — Plasma membrane — Na channel — Ion selectivity

Introduction

Intracellular Na^+ plays an important role in the control of many cell functions including volume regulation and transport processes such as Na/H exchange, Na/Ca exchange, Na-coupled uptake of nutrients Data have appeared indicating that the

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regulation of cytosolic free Ca^{2+} is mainly an Na-dependent mechanism (Liu and Kao 1990, Battle et al 1991, Lipp and Niggli 1994) Several lines of evidence have suggested that cytosolic free Na^+ concentration affected different intracellular reactions (Motulsky and Insel 1983, Basudev et al 1995) and, in particular, modulated the activity of K^+, Cl^- and Ca^{2+} channels in plasma membrane (Kameyama et al 1984, Harvey et al 1991, Balke and Wier 1992) Well-defined voltage-gated channels (Hille 1992) provide fast passive Na^+ entry underlying the action potential generation in nerve cells Numerous works have been devoted to the description of the known superfamily of amiloride-sensitive Na channels in the reabsorbing epithelial tissues (Benos et al 1995) Membrane mechanisms providing passive Na^+ influx in other nonexcitable cells, particularly blood cells, are less certain Our recent study on isolated macrophages first presented single current data showing the activity of voltage-insensitive Na-selective channels (Negulyaev and Vedernikova 1994) Amiloride sensitivity of these channels was extremely low as compared to epithelial channels (Benos et al 1995) On the other hand, amiloride-blockable Na conductance was recorded in B lymphoid cells in whole-cell experiments (Bubien and Warnock 1993)

In this paper, background activity of ion channels mediating cation influx in the plasma membrane of human myeloid leukemia K562 cells was examined Two types of voltage-insensitive Na-conducting channels were found differing by their selectivities for monovalent cations

Materials and Methods

Cells

Human myeloid leukemia K562 cells obtained from Cell Culture Collection (Institute of Cytology, St Petersburg, Russia) were maintained at 37 °C in glass flasks in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO) and antibiotics (100 μg/ml streptomycin and 100 units/ml penicillin) Cells were plated on coverslips (0 4 × 0 4 cm) 1 3 days before experiment

Electrophysiology

Patch clamp method in the cell-attached, outside-out and whole-cell modes (Hamill et al 1981) was used Ionic current measurements were performed at 22–23 °C essentially as described earlier (Negulyaev and Vedernikova 1994) Briefly, patch pipettes were fabricated with Pirex capillary tubing, coated with Sylgard 184, polished immediately before use, they had a resistance of 10–15 MΩ Current signals recorded on magnetic tape were low-pass filtered with Bessel 4-pole filter using a cutoff frequency ranging from 200 to 1 000 Hz The signals transferred to the computer were digitized at 1 ms/pt with 12 bit accuracy and analysed off-line Averaged data are

given as mean ± S E M (number of experiments) Channel activity was estimated using the probability for single channel being open (P_o) P_o was calculated as total open time divided by total length of the record where there is essentially only one open level To measure integral currents in response to voltage step in whole-cell configuration, the resistance of patch electrode ranged from 3 to 5 MΩ and the slow component of capacity current was compensated Current signal was stored at 0 1–10 ms/pt sampling intervals

Solutions

Normal Na external solution for outside-out and whole-cell recordings contained (in mmol/l) 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/Tris OH (pH 7 3), the same solution was in the pipette for cell-attached measurements The bath solution for cell-attached recordings contained 145 mmol/l KCl instead of NaCl to nullify the resting membrane potential Pipette solution for outside-out and whole-cell recordings contained (in mmol/l) 10 KCl, 63 K_2SO_4, 20 HEPES-KOH (pH 7 3), 1 $MgCl_2$, 2 EGTA and an appropriate quantity of $CaCl_2$ to establish the final free calcium concentration at the level of 0 1 or 0 01 μmol/l The latter was used in most of the whole-cell experiments to minimize the activity of other channel types In ion-substitution experiments, all Na^+ in the external solution was replaced by equimolar quantity of a monovalent cation (Li^+, Cs^+) or by 100 mmol/l of bivalents (Ca^{2+}, Ba^{2+}) To record tetrodotoxin-sensitive voltage-gated Na currents, pipette solution contained fluoride as the main anion, and the level of free calcium did not exceed 0 01 μmol/l HEPES, EGTA, amiloride, tetrodotoxin were from Sigma (St Louis, Mo, USA)

Results

Background activity of Na-conducting channels in cell-attached patches

Cell-attached experiments were performed to examine single channel activity in intact myeloid leukemia cells Inward unitary currents were observed in 23 out of 99 stable patches which appeared to represent Na^+ influx through single ionic channels in plasma membrane Cell-attached recordings revealed two types of cation Na-permeable channels in K562 cells (Figs 1, 2) Representative current records suggest that these channels were active at different levels of holding membrane voltage (Fig 1) The level of background channel activity was rather low, mean open state probability (P_o) did not exceed 0 10–0 15 Fig 2 represents summarized current-voltage relation (10 patches) which could be adequately described by two regression lines shifted along the voltage line from each other This indicates two populations of cation channels differing considerably by their Na/K selectivities Current-voltage curves were linear at least within the range of negative potentials The slopes of both regression lines were very close and corresponded to unitary con-

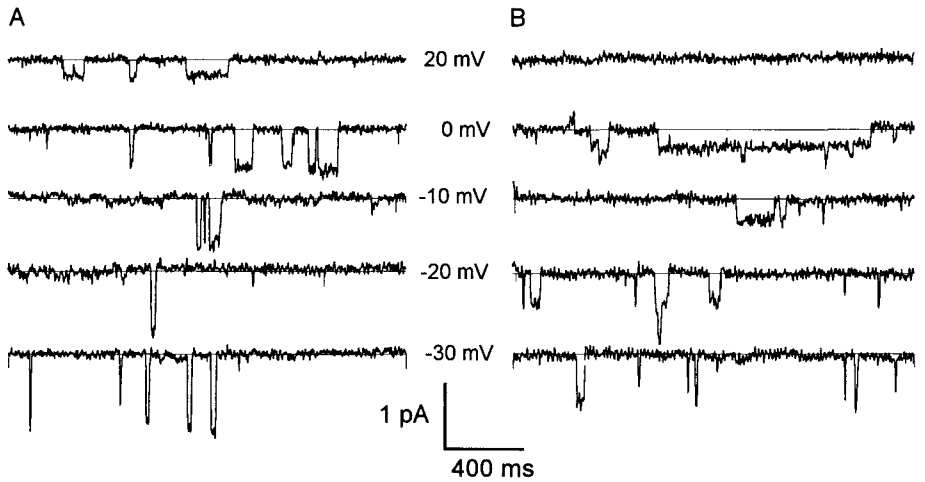

Figure 1. Representative cell-attached recordings of single channel currents in myeloid leukemia cells Holding membrane potentials are indicated near traces The currents were filtered at 100 Hz (*A*) Activity of Na channels of high selectivity (HS) Unitary conductance was 13 2 pS in this patch extrapolated reversal potential was 49 8 mV (*B*) The activity of Na channels of low selectivity (LS) Unitary conductance was 12 7 pS reversal potential was 15 7 mV

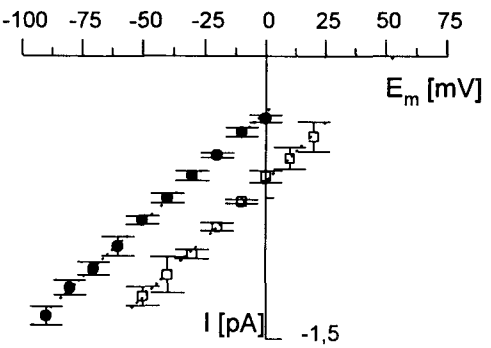

Figure 2. Current-voltage relationship collected from 10 cell-attached experiments indicates two types of voltage insensitive Na permeable channels in K562 cell membrane Data shown are means ± S E M for Na channels of high (HS open squares) and low (LS, filled circles) selectivity Unitary conductance and extrapolated reversal potential values are presented in Table 1

ductance of about 12 pS The parameters of current-voltage curves obtained from cell-attached (Fig 2) and outside-out (Fig 3, see below) measurements are shown in Table 1 including mean conductance values and extrapolated reversal potentials The first type (current records in Fig 1*A*) referred to as channels of high selectivity (HS) were observed in 7 cell-attached experiments The second type (Fig 1*B*) referred to as channels of low selectivity (LS) were observed in 18 experiments In 2 patches, the activities of both channels – LS and HS – were recorded Further

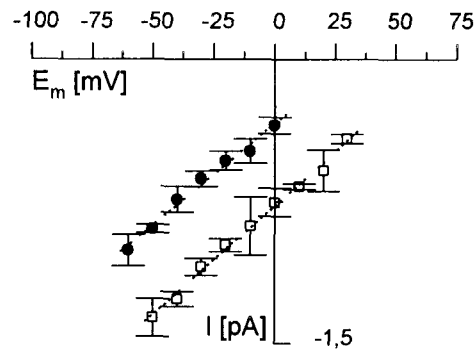

Figure 3. Current-voltage relationships of HS (open squares) and LS (filled circles) Na-permeable channels obtained in 7 outside-out experiments The slope of both regression lines is very close showing unitary conductance about 12 pS The parameters of current-voltage curves are included in Table 1

Table 1. Characteristics of HS and LS Na-permeable channels in K562 cells obtained in cell-attached and outside-out experiments

Recording	Channel type	Unitary conductance [pS]	Extrapolated reversal potential, [mV]	Number of patches
cell-attached	HS	12 2 ± 1 0	+54 ± 5	5
cell-attached	LS	11 9 ± 0 5	+25 ± 2	5
outside-out	HS	11 5 ± 1 5	+60 ± 10	4
outside-out	LS	11 2 ± 1 0	+31 ± 4	3

experiments on excised outside-out fragments, with known composition of internal and external solutions allowed us to perform direct measurements of conductance characteristics of the channels

Permeation properties of single sodium channels

Being a suspension cell line, K562 cells but weakly adhere to the surface of coverslips We obtained outside-out preparations by careful excising membrane fragments from the cell In 9 out of 32 stable patches, activity of Na-conducting channels was observed similar to those recorded in cell-attached experiments (see Figs 1, 2) Outside-out recordings also revealed two types of Na-permeable channels (HS and LS) in K562 cells Fig 3 presents current-voltage relation data collected from 7 outside-out patches in the control external solution Parameters of current-voltage curves are presented in Table 1 showing unitary conductance of 11–12 pS for HS and LS channels Relative permeability P_{Na}/P_K estimated from extrapolated values of reversal potential using Goldman-Hodgkin-Katz equation (Hille 1992) was 10 and 3 for HS and LS channels, respectively This implies that these two channel types having very similar conductances are characterized by different Na/K selec-

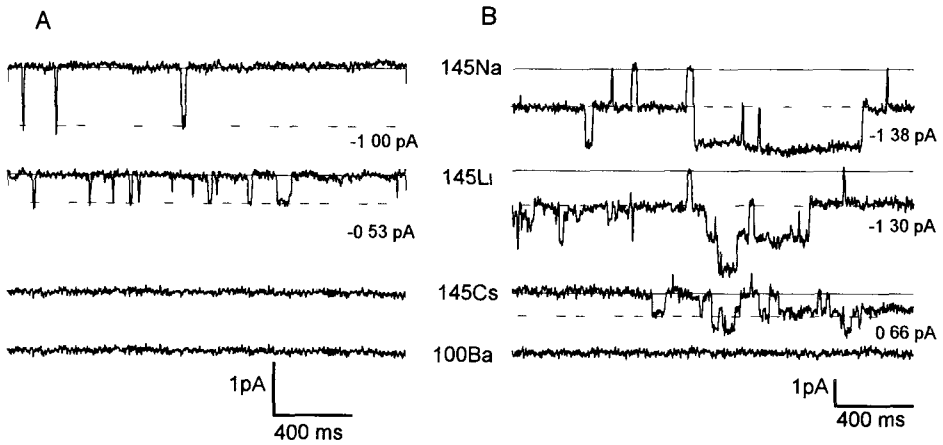

Figure 4. Selective properties of voltage-insensitive Na-permeable channels examined in ion-substitution experiments Representative current records in the control Na-containing external solution (top) and after equimolar replacement of all Na^+ with Li^+, Cs^+ or with 100 mmol/l Ba^{2+} as indicated (*A*) Outside-out recordings of currents through HS channels Membrane potential was −10 mV (*B*) Whole-cell recordings of single currents through LS channels Membrane potential was −80 mV Few active channels in the cell membrane allowed to resolve single openings in whole-cell measurements

tivities The results of outside-out measurements (Fig 3) fully confirmed our data obtained from cell-attached experiments (Figs 1,2)

To estimate directly selective properties of single Na channels in K562 cells, ion-substitution measurements were carried out in outside-out and whole-cell experiments (Fig 4) Recordings in Ba-, Ca-, Cs- and Li-containing solutions were followed by wash-out with control Na solution Both channel types were found to be impermeable to bivalent cations (Ca^{2+}, Ba^{2+}) The selectivity of HS and LS channels for monovalent cations appeared to be essentially different (Fig 4) Equimolar substitution of all Na^+ with Cs^+ in the external solution resulted in a fully reversible abolishment of currents through HS channels (Fig 4*A*) In Li-containing external solution, inward currents through HS channels with decreasing amplitude were measured Inward currents of lower amplitude was observed for LS channel in Cs^+ solution LS channels were shown to pass Li^+ as well as Na^+ ions (Fig 4*B*)

Membrane voltage may exert modulatory effect on Na channel activity

To estimate the level of channel activity, the probability of single channel to be open (P_o) was used Fig 5 shows P_o values for HS and LS channels at different membrane potentials The data obtained in cell-attached and outside-out recordings indicate a tendency of the channel activity to increase with the depolarizing membrane voltage Current recordings in Fig 1 may also illustrate this observation

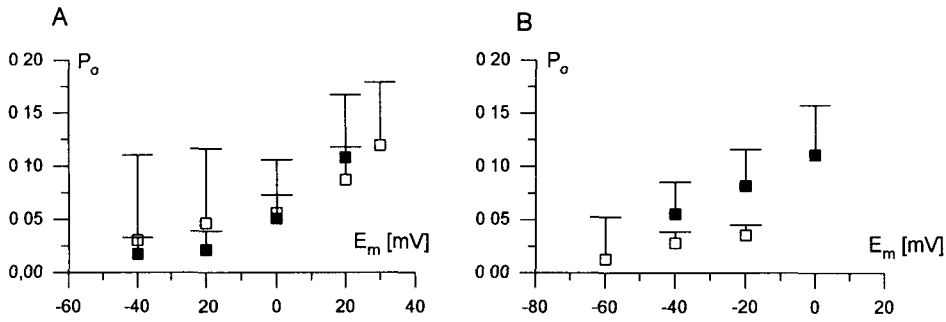

Figure 5. Channel open probability (P_o) at different levels of holding membrane voltage The analysis of (*A*) HS and (*B*) LS Na channel activity is presented, data were collected from 15 patches Filled and open symbols correspond to cell-attached and outside-out measurements, respectively

Background activity of Na channels in K562 cells was too low to obtain reliable open and close time distributions at each potential Nevertheless, the average fraction of time spent in the open state apparently increased with the depolarization both for HS and LS Na-permeable channels

Pharmacological properties of Na-permeable channels

We examined pharmacological properties of Na-permeable channels in K562 cells using tetrodotoxin and the diuretic amiloride, traditional tools to identify known types of Na channels (Hille 1992, Benos et al 1995) Both HS and LS channels were absolutely insensitive to tetrodotoxin (0 1–1 μmol/l) In a concentration range of up to 100 μmol/l, amiloride induced no changes of single currents through HS and LS channels Fig 6 shows the effect of external application of higher amiloride concentrations on HS channels In the presence of 0 1–2 mmol/l amiloride, sodium currents recorded in outside-out patch were reversibly inhibited (Fig 6) A visible decrease of the amplitude of channel openings was observed accompanied by a slight increase of open state noise level LS channels were found to be insensitive to amiloride throughout the concentration range tested

Voltage-gated Na channels in K562 cells

Voltage-gated, TTX-sensitive inward currents carried by Na were reported previously in K562 cell membrane (Schlichter et al 1986, Yamashita et al 1987) In our patch clamp experiments on K562 leukemia cells, HS and LS Na-permeable channels were active at different levels of holding membrane voltage without voltage step However, the level of membrane voltage could have a slight modulatory effect on the channel activity (Fig 5) Conductance and Na/K selectivity of HS channels were rather similar to those of electrically-gated sodium channels

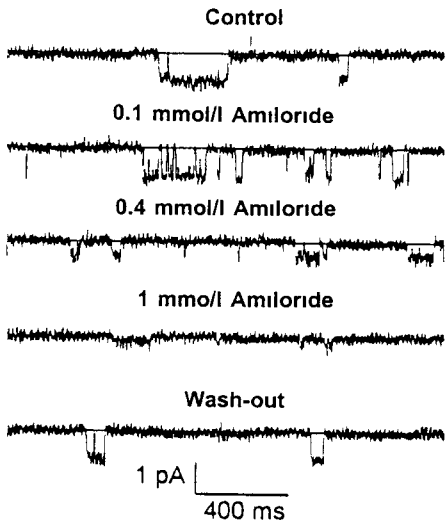

Figure 6. Effect of amiloride on HS Na channels Outside-out current recordings at 0 mV membrane potential Concentrations of amiloride added to the Na-containing external solution are shown at the traces

described elsewhere (Schlichter et al 1986, for review see Hille 1992) If might be assumed that the activity of HS channels represents a non-inactivating minor component of potential-activated inward Na current A special series of whole-cell measurements with voltage step were carried out to explore the pharmacological properties of voltage-gated sodium channels in K562 cells In 51 out of 87 cells, potential-dependent inward currents with properties typical for voltage-gated Na channels were observed Pharmacological tests confirmed that TTX reversibly inhibited these inward integral currents in a dose dependent manner Application of 1 μmol/l TTX resulted in a full block of voltage-gated Na channels We showed also that amiloride (1 2 mmol/l) had no effect on the amplitude or kinetics of potential-activated Na currents These data demonstrate that voltage-gated Na channels displaying rather typical characteristics in K562 leukemia cells are absolutely different from voltage-insensitive Na-selective channels described in the previous sections of the present paper

Discussion

Our results suggest the existence in human myeloid leukemia K562 cells of voltage-insensitive cation channels characterized by preferential sodium permeability They operated at different levels of holding membrane voltage without any external stimulation or voltage step Cell-attached and outside out experiments on K562 cells revealed two types of Na-permeable channels differing by their selectivities for monovalent cations HS (P_{Na}/P_K = 10) and LS (P_{Na}/P_K = 3) channels Under physiological conditions both types of Na permeable channels seem to transport

Na^+ from the extracellular fluid into leukemia cells An important feature of both channel types is their full impermeability to bivalent cations indicating that these channels do not affect directly intracellular calcium level The properties of the newly disclosed Na channels (HS and LS) are absolutely different from those of tetrodotoxin-sensitive voltage-activated channels also identified in leukemia cells (Schlichter et al 1986 and our data) A slight modulatory effect of the membrane potential on HS and LS channel activity observed in our experiments obviously differs from the activation process of voltage gated Na channels

Voltage-insensitive Na-permeable channels in leukemic K562 cells have some similarities with Na channels which are typically present in apical membranes of reabsorbing epithelia (Hamilton and Eaton 1985, Gogelein and Grieger 1986, Joris et al 1989, Benos et al 1995) The main common feature is the absence of voltage dependent gating mechanism Furthermore, unitary conductance of HS and LS channels is near 12 pS, i e similar to the conductance values found for epithelial channels in rabbit straight proximal tubules (Gogelein and Gregei 1986), in cul tured A6 cells (Hamilton and Eaton 1985), and sweat duct cells (Joris et al 1989) The different selectivity of Na channels found in our experiments also resembles the known variability of epithelial channel properties The main difference between epithelial Na channels and voltage insensitive Na channels in leukemic cells is their pharmacological properties LS channels were shown to be insensitive to amiloride amiloride sensitivity of HS channels was extremely low as compared to the epithelial channels (Benos et al 1995) even taking into account their characteristical diversity

Our results indicate a great similarity of voltage-insensitive Na channels in leukemia cells with Na selective channels described in peritoneal macrophages (Negulyaev and Vedernikova 1994), carcinoma cells (Negulyaev et al 1994) and smooth muscle cells (Van Renterghem and Lazdunski 1991) HS channels in K562 cells and Na channels in macrophages displayed the same selectivity related and pharmacological properties, very close conductance values and similar modulating effect of membrane voltage on channel activity Taking together, these data allow us to suppose the existence of a novel family of Na-specific channels in blood cells Pharmacological tests performed on single channel level confirmed that voltage-insensitive Na channels in rat macrophages (Negulyaev and Vedernikova 1994) and human leukemia cells are clearly distinct from both amiloride-sensitive Na channels described in human B lymphoid cells (Bubien and Warnock 1993) and tetrodotoxin-sensitive voltage-gated channels (Schlichter et al 1986 Yamashita et al 1987 and our data) It should be noted that in our experiments on K562 cells, no amiloride-sensitive (in micromolar range) currents were observed similar to those found in lymphoid cells (Bubien and Warnock 1993)

The background activity of Na-permeable channels in K562 cells was rather low As shown on intact cells and on membrane fragments variations of the mem-

brane voltage may cause small changes of P_o value An increased expression Na-permeable channels was observed in leukemia cells in response to long (24 hours) incubation with the mineralcorticoid aldosterone (Negulyaev et al 1996a) Our recent data revealed that actin filament disruption induced activation of LS Na channels in K562 cells (Negulyaev et al 1996b) In general, these effects observed for Na channels in leukemia cells had some similarity with the regulation mechanisms reported previously for epithelial amiloride-sensitive Na channels (Cantiello et al 1991, Benos et al 1995)

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