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Several types of sodium-conducting channel in human carcinoma A-431 cells

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Abstract

Patch clamp method in outside-out configuration was used to search for cation channels which possibly mediate sodium influx through plasma membrane in A-431 carcinoma cells. We found four types of nonvoltage-gated Na-conducting channel. The first of 9–10 pS conductance (145 mM Na^+, 30°C) seems to be Na-selective; three others were characterized with conductance values of 24, 35 and 65 pS and lower selectivity among cations. Na-selective channels (9–10 pS) were not blocked by tetrodotoxin (1 μM). External application of amiloride (0.1–2 mM) resulted in a reversible inhibition of single currents through Na-selective channels.

Key words: Patch clamp; Carcinoma cell; Sodium-conducting channel

1. Introduction

Intracellular Na^+ plays an important role in the control of many cell functions including action potentials in excitable tissues, volume regulation, secondary active transport processes such as Na/H exchange, Na/Ca exchange and Na-coupled uptake of nutrients. In a number of cells Na influx was suggested to be involved in the growth responses to mitogens [1,2]. Cytosolic free sodium concentration was shown to modulate the activity of K, Cl and Ca channels in ventricular myocytes [3,4] and the affinity of receptors to hormones in platelet [5], to activate K^+ channels in cardiac cells [6].

The mechanisms of sodium entry in the unexcitable cells are uncertain. It may be assumed that under physiological conditions Na^+ influx is due at least partially to the activity of Na-permeable channels in plasma membrane. Cation channels of low selectivity have been suggested to mediate vasopressin-induced Na and Ca fluxes in hepatocytes [7]. As reported earlier nonvoltage-gated Na-selective channels were found in specialized reabsorbing epithelia [8]. A novel type of highly selective Na^+ channel recently identified in macrophages [9] may represent a pathway for sodium entry in leukocytes and possibly in other unexcitable cells. Here we report a primary description of four types of Na-conducting channel in carcinoma cells which may be included in the passive Na transport mechanism in the non-renal epithelial tissues.

2. Materials and Methods

Human epidermoid carcinoma cells A-431 (Cell Culture Collection, Institute of Cytology, Russia) were cultured in glass flasks in basal Eagle's medium supplemented with 10% bovine or fetal calf serum and 30 μg/ml gentamycin. For experiments the cells were plated on coverslips (0.4 × 0.4 cm) and grown in the same medium in 6% CO_2 humidified atmosphere.

Ionic currents were measured in outside-out configuration at 30–31°C. Patch clamp experiments were performed essentially as described earlier [9]. Current signals recorded on magnetic tape were low-pass filtered with Bessel 4-pole filter using a cutoff frequency ranging from 200 to 1000 Hz. The signals transferred to the computer were digitized at 1 ms/pt with 12-bit accuracy and analyzed off-line. Averaged data are given as the mean ± S.E.

Abbreviation: GTPγS, guanosine 5′-*O*-(3-thiotriphosphate).

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Pipette cytosol-like solution contained (in mM) 63 K_2SO_4; 10 KCl; 20 Hepes-KOH (pH 7.3); 1 $MgCl_2$; 2 EGTA-KOH and an appropriate quantity of $CaCl_2$ to establish the final free calcium concentration $[Ca]_i$ 0.01–0.1 μM. In most experiments 1 mM ATP and 50–200 μM guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS, a nonhydrolyzable analogue of GTP) were added. Control external solution contained (in mM) 145 NaCl (or 140 NaCl + 5 KCl), 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes-Tris-OH (pH 7.3). EGTA and Hepes were from Serva. Tetrodotoxin (TTX), amiloride and GTPγS were from Sigma.

It should be noted that the choice of appropriate solutions is the most effective way to identify single-channel currents in plasma membrane of carcinoma cells. The anion composition of pipette and bath solutions made it possible to avoid or to minimize possible inward chloride currents through anion channels in outside-out patch. The combination of external Na^+ contained solution and K^+ in the pipette allowed us to estimate the Na/K selectivity of different cation channels.

3. Results and discussion

In the normal sodium external solution 39 of the 67 stable outside-out patches did not display channel activity of inward current direction. In the other 28 patches (42%) we observed inward currents in the potential range of −100 to + 50 mV which appeared to represent Na^+ influx through single channels in the excised membrane fragment. In our experiments with A-431 carcinoma cells we have found four types of nonvoltage-operated Na-permeable channels that differed clearly by their conductance values. Table 1 includes mean conductance values, reversal potentials obtained by extrapolation and the number of patches displaying this type of single channel activity. These four channel types appeared to be impermeable for calcium; their selectivity for monovalent cations seemed to be different.

Table 1
Four types of sodium-permeable channels in A-431 carcinoma cells

Channel type	Unitary conductance (pS)	Extrapolated reversal potential (mV)	Number of patches
I	9.6 ± 0.8	+54 ± 3	7
II	23.7 ± 1.0	+20 ± 3	3
III	35.1 ± 0.6	+18 ± 2	7
IV	64.6 ± 2.0	+12 ± 2	3

Fig. 1A shows representative single channel current records at three holding membrane potential levels in the normal (145 Na) external solution. Inward currents measured were due to Na^+ entry; with sulfate as the major anion in the pipette solution the activity of anion channels would display as outward currents due to the

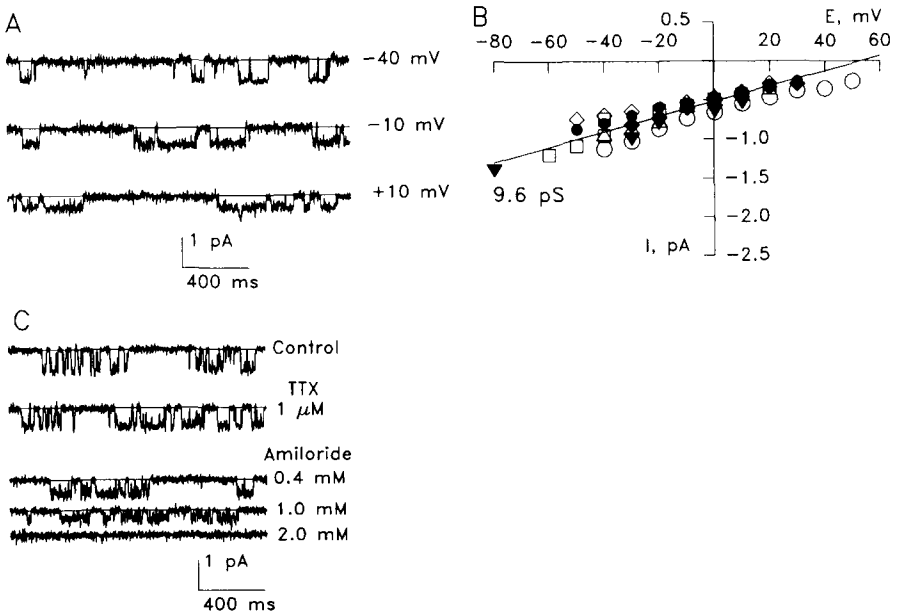

Fig. 1. The activity and properties of sodium-selective channels (type I) in outside-out patch of A-431 carcinoma cells. (A) Single- channel current records in the normal sodium external solution at three holding potentials pointed out near traces. Filtration was 200 Hz. (B) Current–voltage relations for normal solution (30°C) for seven patches indicated by different symbols; data for the experiment shown in A are given by hollow diamonds. Linear regression was calculated using all values in the range from −80 to 0 mV. The slope of the regression line corresponds to the unitary conductance of 9.6 pS and intercepts the voltage axis at + 54 mV. (C) Current records on the same patch in the normal external solution, in the presence of tetrodotoxin (TTX, 1 μM) and then (after washing off of TTX) of amiloride (0.4, 1, 2 mM). Data measured for normal conditions are indicated by filled diamonds in B.

chloride influx with reversion at large negative potential. Replacing the sodium external solution with 100 $CaCl_2$ resulted in the abolishment of typical inward channel activity (not shown). This suggests that sodium currents through cation channels were measured which are impermeable for calcium. Channels with similar properties were found reliably in seven outside-out patches and referred to as type I; in these seven experiments GTPγS was present in the pipette solution. Current–voltage relations measured on different patches marked with different symbols are collected in Fig. 1B (normal sodium external solution, 30°C). Current–voltage curves are seen to be linear at least at the negative potential range. The slope of the regression line corresponds to the unitary conductance of 9.6 pS. The real value of the reversal potential is assumed to be much more positive than that determined by extrapolation (+54 mV, Fig. 1B). We did not observe outward currents through these channels at holding potentials from +50 to 60 mV. The data obtained indicate a rather high Na/K selectivity of 9–10 pS sodium-permeable channels (I).

The results of a typical experiment representing effects of potential blocking agents on sodium-selective channels (I) in outside-out patch are shown in Fig. 1C. In the normal external solution (upper trace in Fig. 1C) sodium channel(s) in this patch displayed a unitary conductance of 10.9 pS (filled diamonds in Fig. 1B). It can be seen that single-channel currents were unaffected by tetrodotoxin (1 μM), which is a well-known inhibitor of voltage-gated sodium channels in nerve and muscles [10]. The application of diuretic amiloride (0.4–2 mM) resulted in a reversible inhibition of sodium currents consisting in a visible decrease of the amplitude of channel openings. This was accompanied by a slight increase of open state noise level and no variation of open and close time parameters. Lower concentrations of amiloride (0.1–10 μM) specifically inhibited Na channels in reabsorbing epithelia [8] but had no effect on 9–10 pS Na channels in A-431 cells.

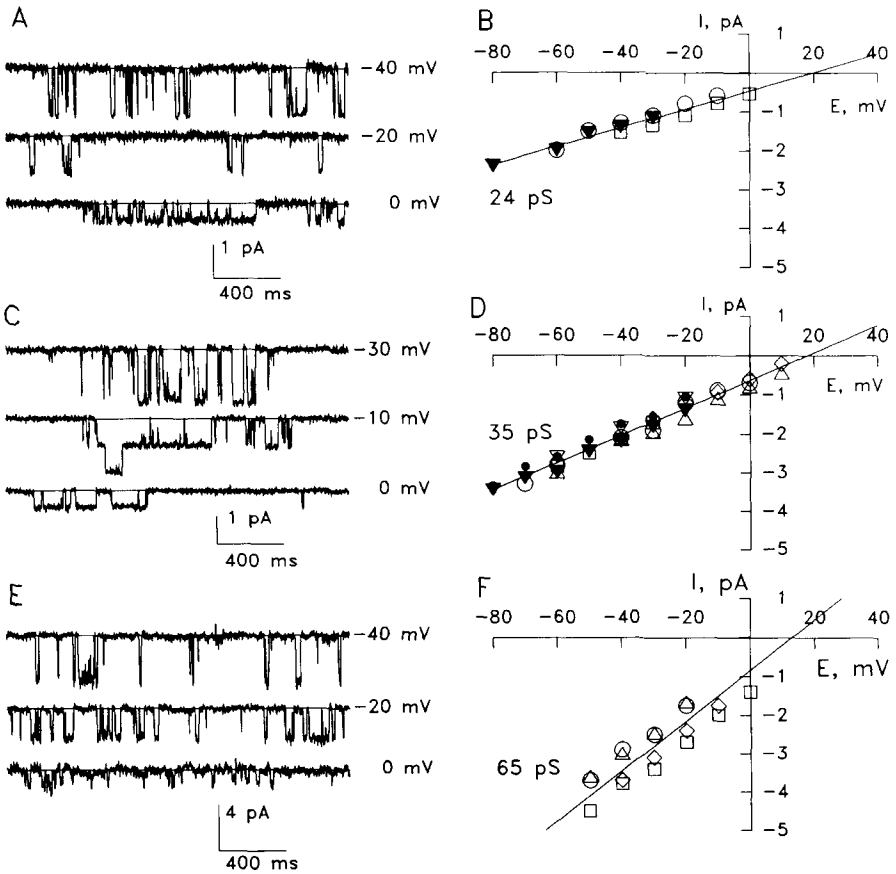

Fig. 2. The activity of three types (II, III and IV) of sodium-conducting cation channel. (A,C,E) Current records in the normal sodium solution for channels of 24 (A), 35 (C) and 65 (E) pS, respectively. Membrane potentials are pointed out near traces. Filtration was 200 Hz. (B,D,F) Corresponding current–voltage relation data for every group of channels. Regression lines were obtained for all potential ranges measured. Following unitary conductance and extrapolated reversal potential values were calculated: B (type II) 23.7 pS, +20 mV; currents shown in A are indicated by hollow squares; D (type III) 35.1 pS, +18 mV; currents shown in C are indicated by hollow diamonds; F (type IV) 64.6 pS, +12 mV; currents shown in E are indicated by hollow squares. Hollow diamonds in F present current–voltage relation on the same patch (E) in the external solution containing 145 Li instead of Na.

Our results suggest that sodium channels of 9–10 pS (type I) in the excised patch of carcinoma cells described here are very similar to those found recently in peritoneal macrophages [9] and in vascular smooth muscle cells [11], including conductance, selective and pharmacological properties. At the same time this novel channel type apparently differs from two well-defined groups of Na-selective channels – the voltage-gated [10] and epithelial amiloride-sensitive [8] types.

Fig. 2A, C and E show current records in normal external solution on three different patches displaying the activity of three other types of sodium-conducting channel, referred to as II, III and IV.

In three patches (GTPγS was added by pipette to all of them) sodium-permeable channels with a unitary conductance of about 24 pS (II) were revealed; corresponding current–voltage relations are collected in Fig. 2B. Representative single-channel currents are shown in Fig. 2A.

Fig. 2C shows representative recordings of the activity of Na-permeable channels (III) with the higher conductance value of 35 pS. The second level of twice the amplitude observed on the middle trace (Fig. 2C) corresponds to the situation when two channels occurring in this patch open simultaneously. Similar channels were found in seven patches (GTPγS was present in six cases) and corresponding current–voltage relations are shown in Fig. 2D using different symbols. Single-channel currents and conductance value decreased slightly when all Na^+ was replaced with Li^+ in the external solution (not shown).

Fig. 2E shows an example of current records for channel type IV with the largest conductance being about 65 pS. Sodium-permeable channels with similar conductance characteristics were observed in three patches (GTPγS was in all of them) and current–voltage relations are collected in Fig. 2F. Inward currents are seen to decrease a little in Li solution. The results shown in Figs. 1 and 2 are summarized in Table 1.

The data obtained allow us to suppose that three types of sodium-conducting channel – of 24 pS (II), 35 pS (III), and 65 pS (IV) – are characterized with similar selective properties. The extrapolated values of reversal potentials were about +10 to 20 mV (Fig. 2B,D,F) unlike the sodium channels of 9–10 pS (about +50 mV and higher, Fig. 1B). All three types (II, III, IV) appeared to fail in discrimination of monovalent cations. They are also permeable for Li^+. Na/K selectivity of these channels is lower than that of 9–10 pS channels (I). However, at zero membrane potential inward currents were no less than −0.5 pA and the reversal potential has always a positive value (Fig. 2). It can be concluded that channels of 24 pS (II), 35 pS (III) and 65 pS (IV) are also characterized by preferential Na^+ passage. When all cations in the external solution were substituted with 100 Ca^{2+} or 100 Ba^{2+} typical channel activity disappeared (not shown). Similarly to channels of 9–10 pS (I) these three types (II, III, IV) may be supposed to be not permeable for bivalent cations. It should be noted that openings of other channels were measured in 100 Ca^{2+} (or Ba^{2+}) external solution in few experiments, possibly representing the activity of calcium channels described before [12,13].

It seemed that the addition of GTPγS in pipette solution raised the probability to observe the activity of all four types of Na-conducting channel in A-431 cells (unpublished observation) that is similar to sodium-selective channels in macrophages described earlier [9].

It may be supposed that sodium channels of different conductance identified in our work participate in the control of the intracellular Na^+ concentration providing Na^+ influx through plasma membrane. Cation channels of rather similar conductances (16 and 31 pS) activated by vasopressin were revealed in hepatocytes [7]. They were likely to account for agonist-stimulated Na^+ and Ca^{2+} entry. Both types of channel in hepatocytes were reported to be permeable for both Ca^{2+} and Na^+ [7] unlike our data. Histamine-activated nonselective cation channels with a conductance of 26 pS (145 Na) that is close to type II were described in endothelial cells [14]. Nevertheless, permeation properties of these channels passing Ca^{2+} [14] appeared to be different.

The regulation mechanisms of sodium-permeable channels in carcinoma cells has not been examined here. It may be assumed that G-proteins are involved in channel activation; however, this hypothesis needs some direct evidence. The exact mechanisms modulating sodium channel activity remain to be elucidated.

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