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Sodium-selective Channels in Membranes of Rat Macrophages

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Abstract. With the use of the patch-clamp technique, highly selective nonvoltage-gated sodium channels were found in the membrane of rat peritoneal macrophages. The inward single channel currents were measured in cell-attached and outside-out mode experiments at different holding membrane potentials within the range of −60 to +40 mV. The channels had a unitary conductance of 10.2 ± 0.2 pS with 145 mM Na^+ in the external solution at 23–24°C. The results of ion-substitution experiments confirmed that this novel type of cation channel in macrophages is characterized by high selectivity for Na^+ over K^+ (as for Cs^+, NH_4^+, Ca^{2+}, Ba^{2+}) ions, whose conduction through these sodium-permeable channels was not measurable. Lithium is the only other ion that is transported by this pathway; the unitary conductance was equal to 3.9 ± 0.2 pS in the Li^+-containing external solution. Single channel currents and conductance were found to be linearly dependent on the external sodium concentration. Sodium channels in macrophage membrane patches were not blocked by tetrodotoxin (0.01–1 μM). Single sodium currents were reversibly inhibited by the external application of amiloride (0.1–2 mM) and its derivative ethylisopropil-amiloride (0.01–0.1 mM). The mechanism of channel block by amiloride and its analogue seems to be different.

Key words: Patch clamp — Macrophage — Sodium-selective channel — Amiloride

Introduction

Macrophages, found in virtually every tissue, play a major role in mediating the body's immune and inflammatory responses. The patch-clamp technique has enabled characterization of a variety of ion channels in cells of the immune system. Several types of potassium, chloride and nonselective cation channels were identified in macrophages (*for review, see* Gallin, 1991). Recently, receptor-dependent channels, possibly providing essential calcium influx from the extracellular medium, have been found (Naumov et al., 1992). Although intensive studies especially those with the use of the patch-clamp technique were carried out, no information concerning electrically unexcitable sodium-specific channels has yet been reported either in macrophages or in other leukocyte cells.

In our study, we have found highly selective nonvoltage-gated sodium channels of 10 pS conductance in the membrane of rat macrophages. Selective properties, ion concentration-conductance dependence, some pharmacological characteristics of channels, including the effects of potential blocking agents, were examined in excised outside-out patch measurements. This novel channel type described in macrophages appeared to have some features including selectivity and conductance value, similar to those of the well-known sodium channels in the apical membranes of epithelia. At the same time, there is an obvious difference in the amiloride affinity and ion-concentration dependence of sodium channels in macrophages, compared with those in epithelial cells.

Materials and Methods

Cells

Peritoneal resident or elicited macrophages were obtained from Wistar rats of either sex (5–10-wk old) from the cavity of animals, as described in Conrad (1981). Elicited macrophages were obtained from rats by intraperitoneal injection of 3 ml of 3% Tripton (Ferac, FRG)

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medium 3–4 days before. Cell suspension from the peritoneal cavity was placed into the plastic dishes with 4 · 4 mm pieces of coverslips. After the 40–60 min incubation at 37°C, dishes were gently agitated, and the supernatant medium was aspirated. Adherent cells were washed three times with fresh portions of medium with antibiotics. This procedure ensured the washing and removal of all nonadherent cells which may be present in the intraperitoneal fluid. The cells on coverslips were then cultured in medium 199 containing 10% fetal bovine serum and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin) at 37°C. Cultured macrophages were used in patch-clamp experiments 1–15 days after plating.

In the patch-clamp experiments, we used an inverted microscope with Nomarsky optics at magnification of 256× to examine the macrophage population and to position the patch pipette. This optical arrangement enabled us to observe distinctly the shape and borders of cells. For giga-seal formation, typical cells were used without any preferable selection. In parallel with patch experiments, it was determined with the use of α-naphthyl acetate esterase staining (Monahan, Dvorak & Dvorak, 1981) that no less than 98% of cells in monolayers were macrophages.

Patch-Clamp Method

Ionic currents were studied in cell-attached or outside-out configurations (Hamill et al., 1981). Patch pipettes having a resistance of 10–15 MΩ were fabricated with Pyrex capillary tubing, coated with Sylgard 184 and polished immediately before use. Pieces of coverslips with adhered cells were transferred into a recording chamber (0.1 ml). At the beginning of the experiment, the chamber was filled with a bath solution containing (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/TrisOH, 10 glucose (pH 7.3), in which the giga-seal was formed. For cell-attached mode measurements, sodium solution was substituted with a potassium bath solution. When working in outside-out configuration, the pipette with the excised membrane fragment was transferred to a compartment of smaller volume (about 20 μl). Experiments were carried out at room temperature 23–24°C.

The ground electrode was an Ag-AgCl pellet separated from the bathing solution by a short Ringer-agar bridge, the potential inside the pipette was clamped at the desired level. Membrane voltage is the potential of the intracellular membrane side minus the potential of the extracellular one.

The patch-clamp amplifier designed and constructed in our laboratory had a conventional resistive feedback in the headstage of 20 GΩ. Currents were low-pass filtered with a Bessel 4-pole filter using cut-off frequency ranging from 200 to 1,000 Hz. Signals were first registered on an FM recorder N067 and subsequently transferred to the computer for analysis. The signals were digitized at 1 msec/pt with 12-bit accuracy and analyzed off-line. Software was written on Pascal language to perform standard single channel analysis including baseline drift removal, digital filtering, current amplitude histograms, open/closed time distribution, and fitting to histograms. Measurements of open and closed times were done with the use of half-amplitude threshold-crossing event detection (Colquhoun & Sigworth, 1983).

The probability for the channel being open (P_o) was calculated from the formula: $P_o = I/(N \cdot i)$, where I is the mean current; i is the unitary current amplitude; N is the number of functioning channels in the patch. I was calculated by averaging the amplitude histograms obtained for 5–20 sec intervals. N was determined from the maximum number of simultaneous openings over the whole period of observation.

Averaged data are given as the mean ± SE (number of experiments).

Solutions

The bath solution for cell-attached measurements contained (in mM) 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/TrisOH (pH 7.3). Normal sodium external solution for outside-out recording contained (in mM) 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/TrisOH (pH 7.3); the same solution was in the pipette in case of cell-attached measurements. In the ion-substitution experiments, all sodium ions in the external solution were substituted with an equimolar (145 mM) quantity of monovalent cation (K^+, Cs^+, NH_4^+, Li^+) or with 100 mM of bivalent cation (Ca^{2+}, Ba^{2+}). In the external solutions with lower sodium concentration (108.7, 72.5, 36.2 mM), part of Na (25, 50 or 75% of normal concentration 145 mM, respectively) was replaced with Cs^+ ions. The pipette solution for the outside-out experiments contained (in mM) 63 K_2SO_4, 10 KCl, 20 HEPES/KOH (pH 7.3), 1 $MgCl_2$, 2 EGTA/KOH and appropriate quantity of $CaCl_2$ to establish final free calcium concentration ($[Ca]_i$) 0.01–0.1 μM, 1 ATP and 0.1–0.2 mM 5′-*O*-(3-thiotriphosphate) (GTPγS), when necessary. HEPES and EGTA were from Serva. GTPγS, tetrodotoxin (TTX) and amiloride were from Sigma, ethylisopropilamiloride (EIPA) was from Merck. EIPA was prepared as stock solution in dimethylsulfoxide and added to the tested external solutions to yield the desired final concentration.

Results

Cell-Attached Experiments

We have investigated single channel activity of macrophage membrane using patch-clamp measurements on intact cells. A considerable part of the cell-attached patches was silent. In some cases, single channel recordings in different patches revealed the activity of one or several channel types. Nonselective cation channels, potassium and chloride currents should be mentioned similar to those reported earlier for leukocyte membrane (Gallin, 1991). Of special interest is a new type of single channel activity seldom observed and easily distinguishable from the above-mentioned currents. Figure 1 shows one of the experiments in cell-attached configuration with the normal sodium solution in the pipette. Channel activity of such a type was observed in 6 (about 7%) out of 86 cell-attached patches. Single channel recordings (Fig. 1*A*) reveal inward current events at different holding potentials from −60 to +30 mV. The first amplitude level corresponds to one channel opening; the second level of double amplitude indicates that two channels open simultaneously. The unitary current-voltage relation (Fig. 1*B*) shows a single channel conductance value of 9.7 pS determined from the slope of its linear part in negative potential range. When extrapolated, it intercepts the x axis at about +44 mV. Actual value of the reversal potential may be assumed to be a higher positive value than that obtained by extrapolation of the linear section. In any case, it is likely to be no less than +40 mV. This fact, together with the obvious existence of large enough inward currents at potentials near zero, give evidence

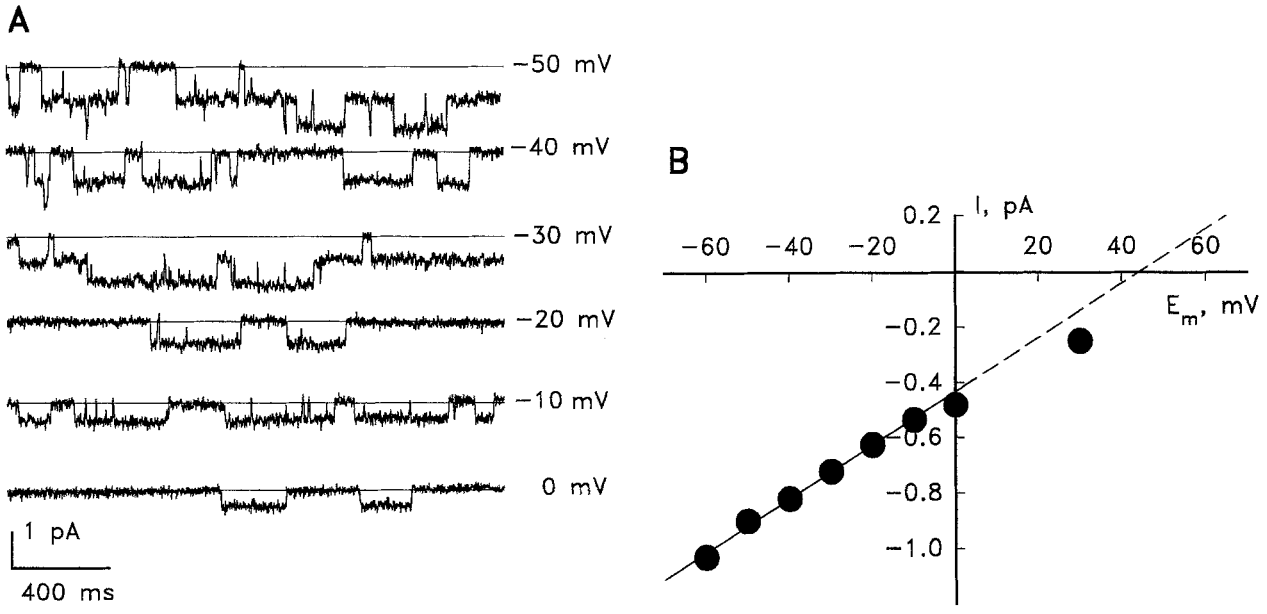

Fig. 1. Single channel activity in a cell-attached patch with 145 mM Na^+ in the pipette. (*A*) Representative current records; filter was 200 Hz. Holding membrane potentials are indicated near traces. (*B*) Current-voltage relation. Unitary conductance: 9.7 pS.

against a possible anion preferential permeability of channels. As Na^+ is the main cation in the pipette solution, the observed channel activity in cell-attached mode recordings is most likely to represent the influx of sodium ions from the pipette into the cell through fluctuating channels. We have tried to study the characteristics of these sodium-conducting channels in macrophage cell membranes with the use of excised outside-out patch measurements.

Single Sodium Channel Activity in Outside-Out Excised Patches

Figure 2 shows the results of the typical outside-out experiment. Pipette solution (*see* Materials and Methods) contained 0.1 mM GTPγS, a nonhydrolyzable analogue of guanosine triphosphate (GTP); $[Ca]_i$ was 0.1 μM buffered with EGTA. Single channel activity in normal (145 mM Na^+) external solution and the corresponding current-voltage relation are presented in Fig. 2*A* and *C*. Inward current events observed at different holding potentials (Fig. 2*A*) are likely to be due to the sodium-permeable channel activity. Current-voltage curve (Fig. 2*C*) is seen to be linear at the negative potential range. As a rule, some deviation from the linear character occurred at positive membrane voltages. The slope of the linear part of the current-voltage curve corresponds to the unitary conductance of 9.5 pS (for normal external solution, Fig. 2*A, C*). When extrapolated, it intercepts the x axis at +54 mV. It should be noted that these potential values obtained by extrapolation characterized the position of the current-voltage curve and may reflect only the left boundary of the presumably higher positive reversal potential values. Moreover, we did not observe outward currents through these channels even at large positive membrane voltage. The current-voltage relation determined in an outside-out experiment for sodium (145 mM Na^+) external solution (Fig. 2*B*) practically coincides with that for channel openings registered in the cell-attached configuration (Fig. 1*B*, 145 mM Na^+ in pipette). This fact, together with the similarity of the kinetic behavior of channels (*see* current records in Fig. 1*A* and in Figs. 2*A*, 4*A*, 5, and 6 for normal conditions), enables us to assume that sodium-permeable channel openings observed in outside-out macrophage membrane patches belong to the same type as measured in the cell-attached mode records.

It made no difference to the current amplitude whether or not the normal external solution contained 2 mM Ca^{2+} (*not shown*). Therefore, in further experiments all measurements were performed in control and other analogous external solutions containing 2 mM Ca^{2+}, which is close to the normal physiological extracellular medium. The following ion-substitution experiments fully confirmed that these sodium-conducting channels are absolutely impermeable for external calcium ions (even at 100 mM concentration).

Sodium-permeable channels with similar characteristics were observed in 18 out of 71 outside-out patches at 0.1 μM $[Ca^{2+}]_i$ and 0.1 mM GTPγS in the pipette solution. Typical inward sodium channel activity dis-

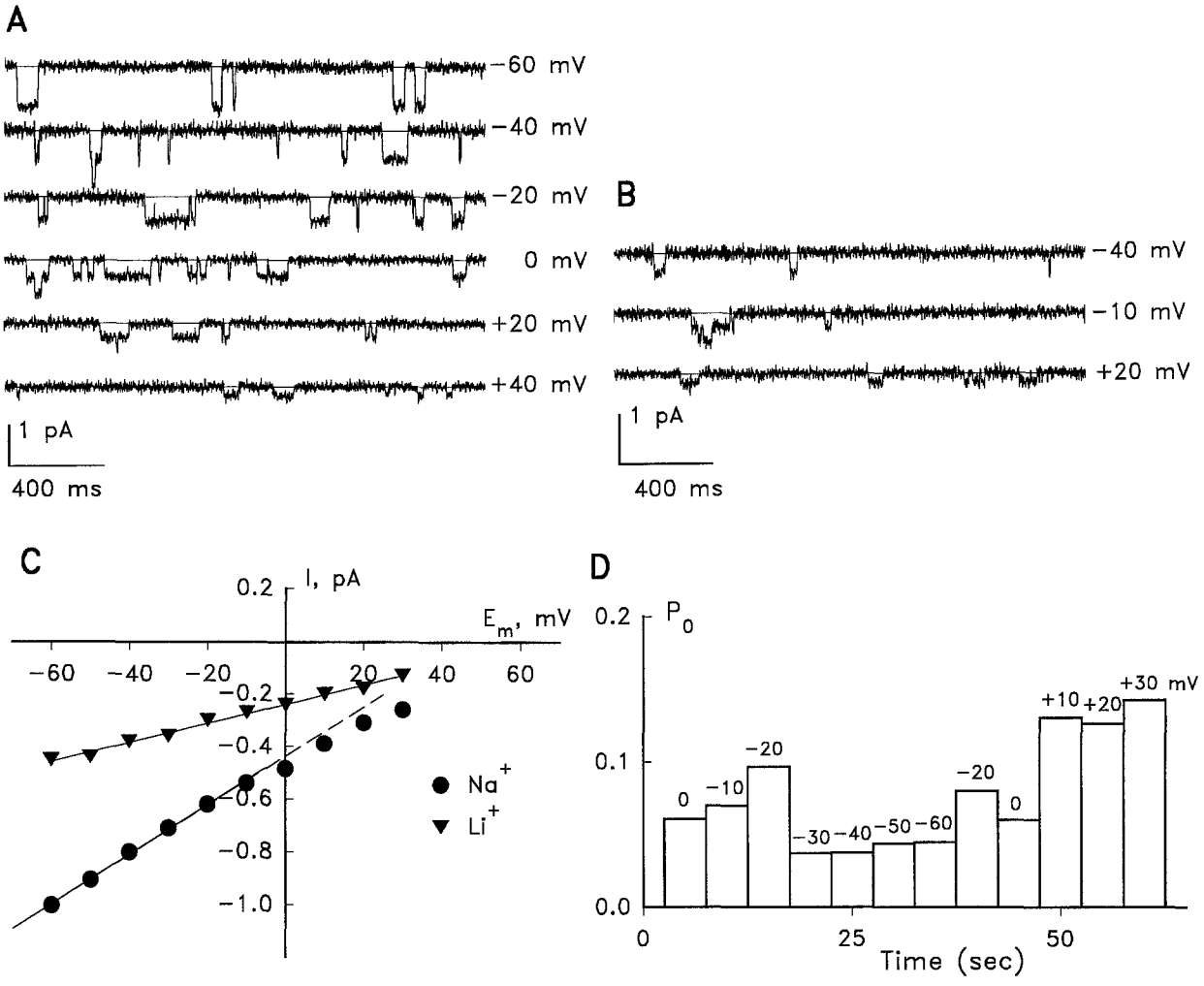

Fig. 2. Single sodium channel activity in the excised outside-out patch. Pipette solution contained 0.1 μM free Ca^{2+} and 0.1 mM GTPγS. (*A*) Current records in the normal external solution (145 mM Na^+). Membrane potentials are indicated near traces; filter was 200 Hz. (*B*) Current records on the same patch in the Li^+ external solution. (*C*) Current-voltage relations for normal sodium and for lithium external solutions. Unitary conductances: 9.5 and 4.2 pS, respectively. (*D*) Open probability P_o in time corresponding to different holding potentials (pointed above columns).

played in 3 out of 13 patches at 0.01 μM $[Ca^{2+}]_i$ and 0.1 mM GTPγS in the pipette. Thus, 84 outside-out patches were recorded in the presence of GTPγS and in 21 of these we could observe sodium channel activity. Other patches (75%) with GTPγS in the pipette did not reveal sodium currents.

Mean value of the single sodium channel conductance was equal to 10.2 ± 0.2 pS (n = 11) in the control external solution at room temperature 23–24°C. Mean open time was about 20–50 msec (*see* Fig. 2*A*). It should be noted that sodium channel activity usually increased slightly with time after patch formation and reached some average level in 2–3 min characterized with small oscillations during subsequent recordings. One of the factors modulating channel activity may be a membrane potential. We used the probability of the channel to be open (P_o) as a measure of the channel activity. It was observed that P_o values were larger at the holding potential levels between 0 and +30 mV, compared with P_o in the potential range of −30 to −60 mV (Fig. 2*D*).

In the absence of a nonhydrolyzable analogue of GTP in the "intracellular" pipette solution, outside-out patches with sodium channel activity were more rare. This was examined in a separate series of parallel experiments with or without GTPγS (0.1 mM) on cells from the same passage. We have observed sodium channels in 4 out of 10 outside-out patches with GTPγS, and in one of 10 patches devoid of GTPγS in the pipette. To increase the probability of displaying sodium-permeable channel openings in outside-out patches, GTPγS was added in the pipette solution.

In part of the experiments, the currents through other channels made it difficult to measure those through sodium channels, especially at large positive potentials. Figure 3 shows the records with two different

channels from the outside-out patch in normal external solution at three potentials. Sodium currents of inward direction and outward potassium currents can be seen and are easily distinguishable under recording conditions; conductance value can be well estimated in this case. To obtain reliable quantitative description of other sodium channel properties, patches containing no other channel types were most suitable. The examination of selectivity (with the use of cation-substitution experiments), ion concentration dependence, block effects (*see below*) and P_o measurements were carried out on patches displaying only sodium currents.

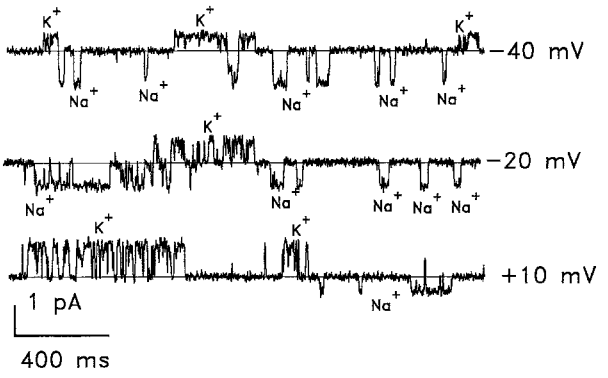

Fig. 3. Current records from the outside-out patch showing the activity of two different channel types. Normal external solution. Unbroken horizontal lines indicate zero current level as in all other recordings. Sodium currents (downward) and potassium currents (upward) can be seen at three different membrane potentials.

Selective Properties

In the outside-out experiments with about 145 mM K^+ in the pipette (internal) solution and 145 Na^+ in the external one the absence of the outward openings and, consequently, of the visible current reversion to large enough positive potentials indicated a rather high Na^+/K^+ channel selectivity. To estimate directly the selective properties of channels, the ion-substitution measurements were performed. The equimolar substitution of all sodium with potassium in the external solution resulted in a full abolishment of the inward currents even at large negative membrane potentials (*not shown*). After the replacement of normal Na^+-containing solution, the typical sodium channel activity with the same amplitude was fully restored. It can be concluded that (i) inward single channel currents in the sodium external solution are not due to anion efflux; (ii) these channels are characterized with high Na^+/K^+ selectivity since potassium conduction through them was not measurable.

The same effect of reversible abolishment of the inward currents was observed in the outside-out experiments when Na ions were substituted with 145 mM NH_4^+ and Cs^+ or with 100 mM Ca^{2+} and Ba^{2+} in the external solution (*not shown*). These results fully confirmed cation specificity of channels and their high sodium selectivity. Thus, sodium-specific channels in macrophage membranes were shown to be practically impermeable for the monovalent (K^+, Cs^+, NH_4^+) and bivalent (Ca^{2+}, Ba^{2+}) cations tested.

Figure 2*B* shows single channel currents measured in the same outside-out patch when all Na^+ ions were substituted with Li^+ in the external solution. In the Li^+ solution, inward current openings have been preserved but their amplitude is seen to decrease approximately 2–3 times, compared with corresponding values in normal sodium solution. The unitary conductance determined from the slope of the current-voltage relation was equal to 4.2 pS (Fig. 2*C* for Li^+ solution). Similar results were obtained in other four outside-out experiments with lithium substitution. Mean conductance value was equal to 3.9 ± 0.2 pS (n = 5) which constitutes 0.4 of the conductance in the normal sodium solution.

Concentration Dependence

The single channel conductance and currents of the epithelial sodium channels were reported to saturate as functions of permeant (sodium or lithium) ion activity (Olans, Sariban-Sohraby & Benos, 1984; Palmer & Frindt, 1988). To study possible saturation effects of sodium channels in macrophage membranes, a series of single current measurements at different external sodium concentrations was performed. The results of the representative experiment are shown in Fig 4. External solution tested contained 145 mM Na^+ (normal) and 108.7, 72.5, and 36.2 mM. To maintain the constant ionic strength of the external solution, part of Na^+ (25, 50 or 75%) was substituted with impermeant Cs^+ ions. It can be seen that an inward current amplitude at every potential diminished proportionally to a decrease of the sodium concentration in the external solution, and is approximately equal to 0.75, 0.5, 0.25 of the normal amplitude value (at 145 mM Na^+), respectively (Fig. 4*A,B*). Both the unitary conductance and the amplitude depended linearly on the external sodium concentration up to 145 mM. No saturation effect of unitary currents and conductance was found for sodium-selective channels in macrophages in the whole range of physiological concentrations.

The Effect of Blocking Agents

We have examined the pharmacological properties of sodium-selective channels in macrophage membranes with the use of the drugs applied traditionally as tools

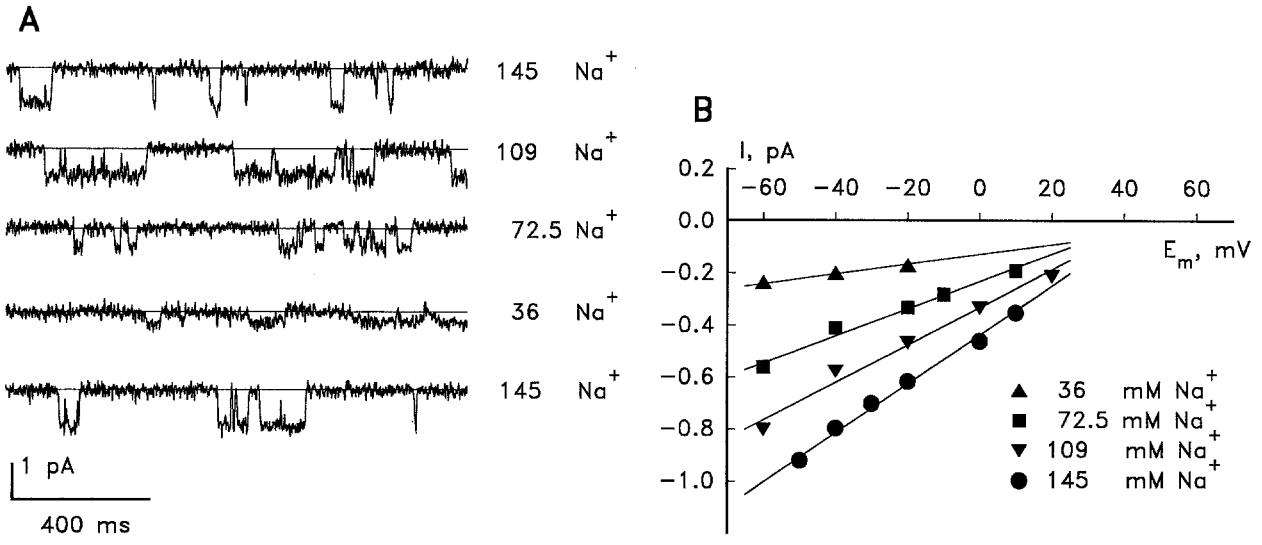

Fig. 4. Concentration dependence of sodium currents and conductance. Outside-out configuration. Pipette solution contained 0.1 mM GTPγS and 0.01 μM free calcium. (*A*) Current records at the normal external sodium concentration 145 mM (before and after change) and at three lower concentrations: 108.7 mM Na^+ (or 75% of the normal 145 mM), 72.5 mM (50% of normal) and 36.2 mM (25% of normal). Membrane potential was −20 mV; filter was 200 Hz. (*B*) Current-voltage relations obtained on the same patch for different external sodium concentrations. Unitary conductance values: 10.1 pS (for normal sodium), 7.8 pS (for 75% of normal), 5.5 pS (for 50% of normal), about 2 pS (for 25% of normal).

for searching different sodium-permeable channel types: tetrodotoxin, diuretic amiloride, and its derivative ethylisopropilamiloride (Kleyman & Cragoe, 1988).

It was found that single sodium channels in outside-out macrophage membrane patches were not blocked by the externally applied tetrodotoxin at 0.01–1 μM concentrations (*not shown*).

Figure 5 shows the effect of the application of amiloride-containing external solution on the sodium-selective channels. Amiloride in a concentration less than 0.1 mM resulted in no variations of the amplitude or the time parameters of single openings. In the presence of 0.1–2 mM amiloride, sodium currents were reversibly inhibited (Fig. 5*A*). A visible decrease of the single channel amplitude was observed to nearly complete suppression of inward currents at amiloride content about 2 mM. This is accompanied by a slight increase of open state noise level. The time parameters of the sodium channel openings were seen to stay unchanged. Figure 5*B* shows the concentration dependence of open channel current inhibition using a Hill plot for the data presented in Fig. 5*A*. Regression line was determined and indicated a Hill number (n) of 1.7 and K_{10} of 0.87 mM.

Figure 6*A* shows that the addition of 50 μM EIPA also caused a reversible inhibition of single channel currents, although the mechanism of action appeared to be different. The probability of channels to be open and apparently the mean open time decreased, whereas the unitary amplitude and conductance were unaffected. For the experiment presented in Fig. 6*A*, P_o was about 0.23 in the normal external solution before EIPA application. In the EIPA-containing solution, P_o value decreased to 0.06 and then increased after washing of drug. The application of 0.1 mM EIPA usually resulted in a complete block of the channel openings (*not shown*), Figure 6*B* shows plot of P_o *vs.* EIPA concentration in the range of 10–50 μM obtained in another experiment.

It can be concluded that sodium-selective channels in macrophages are sensitive to diuretic amiloride and its derivative EIPA, although they are characterized with low affinity to these drugs (compared with typical values known for epithelial channels). It is seen that the effects of these two related chemicals were obviously distinguished on the level of single current measurements.

Discussion

The data presented allow a conclusion that a novel type of cation channels has been found in the plasma membrane of primary macrophages. We used the patch-clamp technique to identify and characterize these channels. A reliable identification of the single channel events as Na^+ inward currents was made on the basis of the ion-substitution experiments. These sodium-conducting channels in macrophage membrane patches were shown to be highly selective; they seemed to be virtually impermeable to K^+, Cs^+, NH_4^+, Ca^{2+}, Ba^{2+}. It is natural to compare highly selective sodium chan-

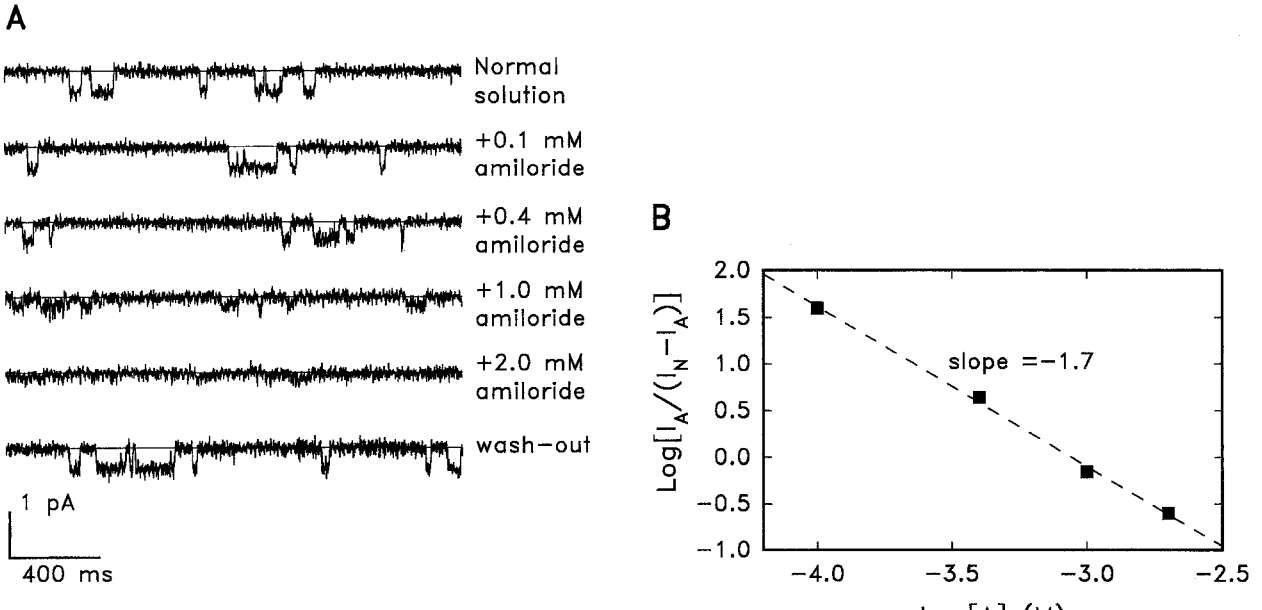

Fig. 5. The effect of amiloride on single sodium channels in the outside-out patch. (*A*) Current records before in presence of and after amiloride-containing external solution. Membrane potential was 0 mV; filter was 200 Hz. The concentrations of amiloride [A] added to the normal solution (145 mM Na^+) are pointed out near traces. (*B*) Hill plot of the data shown in *A*. $Log[I_A/(I_N-I_A)]$ is plotted on the ordinate, and the abscissa axis is log[A]. I_N and I_A are the mean amplitudes of sodium channel currents in normal and amiloride-containing external solution, respectively. The data can be described by a straight line with the slope of 1.7 and $K_{1/2}$ of 0.87 mM.

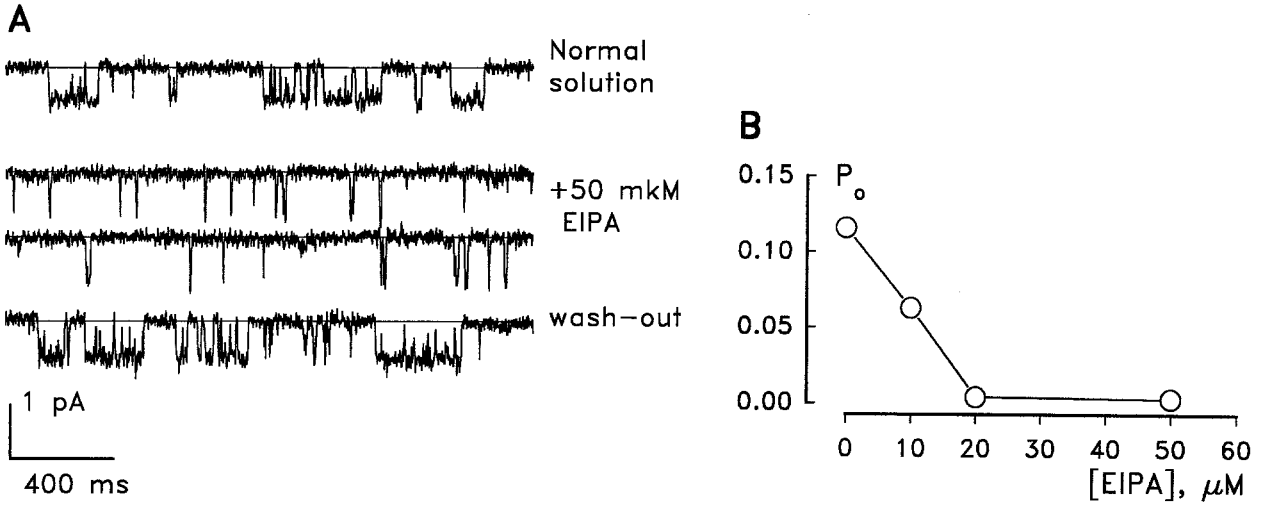

Fig. 6. The effect of ethylisopropilamiloride (EIPA) on single sodium currents in the outside-out patch. (*A*) Current records before (P_o = 0.23), during (P_o = 0.06) and after (P_o = 0.41) the external application of 50 μM EIPA. Membrane potential was −30 mV; filter was 200 Hz. (*B*) Dependence of the open channel probability (P_o) on [EIPA] concentration obtained for another patch. Membrane potential was −20 mV. P_o for normal external solution was equal to 0.12.

nels found in macrophages with the well-known types: (1) voltage-activated sodium channels in nerve and muscle membrane (*for review, see* Hille, 1992) and (2) amiloride-sensitive sodium channels in Na^+ reabsorbing epithelia (*for review, see* Smith & Benos, 1991). The selective properties of sodium channels described here are similar to both types mentioned: Li^+ proved to be a single cation passing through these channels with the conductivity comparable to Na^+. It should be noted, however, that the group of epithelial sodium channels were reported to be characterized with the considerable diversity of their properties including selectivity, conductance value and amiloride sensitivity (*see* Eaton & Hamilton, 1988; Smith & Benos, 1991).

The unitary conductance value of channels obtained in our cell-attached and outside-out experiments (about 10 pS) is somewhat lower than that of voltage-gated Na channels in nerve cells (14–17 pS) measured under comparable conditions (Negulyaev, Vedernikova & Savokhina, 1990; *see also* Hille, 1992).

Unlike the electrically excitable type (1), sodium channels in macrophage membranes failed to be activated by voltage displacement. Single channel activity was observed at different levels of the holding membrane potential. There appeared to be only a slight modulating effect of voltage on the probability of channels to be open.

In contrast to the Na channels (1) of excitable tissues, Na-selective channels in macrophages proved to be insensitive to the tetrodotoxin. This neurotoxin is regarded as a typical inhibitor of electrically gated Na channels in nerve and muscle with the exception of the so-called TTX-insensitive Na channels found in the coelenterate neurons and embryonic cells (*see* Hille, 1992).

Amiloride-sensitive channels (2) with similar conductance 12 pS and high Na/K selectivity have been found in the luminal membrane of rabbit straight proximal tubules (Gögelein & Greger, 1986). Voltage-independent sodium channels (2) characterized by conductance values within the range of 8–15 pS have also been observed in several different epithelial tissues; in a few cases, a variability of selective properties has been reported (Joris et al., 1989; *see also* Eaton & Hamilton, 1988). It may be assumed that sodium channels in macrophages have some characteristics similar to epithelial channels, including unitary conductance, selectivity and kinetic properties, taking into account their diversity in different preparations of specialized reabsorbing epithelia. The most common feature is that sodium channels described here are also voltage independent. They proved to be sensitive to amiloride and one of its hydrophobic analogues—EIPA—but in concentrations three orders higher than respective values for typical high-amiloride-affinity epithelial channels. Amiloride concentrations that are needed to block sodium channels in macrophages ($K_{1/2}$ = 0.87 mM) are also higher than corresponding values for low-amiloride-affinity channels in epithelia (Smith & Benos, 1991).

There is a notable difference between the amiloride and EIPA effects on single channel currents. Amiloride caused an evident decrease of the amplitude of single events and channel conductance in parallel with some increase of the noise level of the open state. This effect agrees with an assumption that the drug-channel interaction represents a very rapid dissociation process; the underlying flickering is too fast to record and the unitary conductance appears lowered. Hill plot representation of the results is consistent with the idea that at least two amiloride molecules are involved in binding to the open channel. In contrast to amiloride, the EIPA application did not result in the reduction of the amplitude, but led to a drastic decrease of the probability of channel opening (in the concentration range 10–20 μM).

The blocking effect of amiloride on sodium channels in different types of reabsorbing epithelia was reported to consist of decreasing the open probability and mean open time, whereas the amplitude of single openings was unaffected (Gögelein & Greger, 1986; Palmer & Frindt, 1986; Joris et al., 1989). This differs from amiloride block observed in our work and is similar to the EIPA effect described here. There is no sufficient data of patch experiments on different cell types to allow comparison of the effect of amiloride and its analogues at the single channel level. A proper understanding of blocking mechanisms of sodium channels by diuretics is lacking now. Zweifach and co-workers (1992) have found the inhibition of Ca-activated K channels by EIPA (0.01–0.1 mM), whereas amiloride had no effect at concentrations as high as 2 mM. Lane and co-workers (1991) supposed that at least two binding sites may be involved in the blocking of the mechanosensitive cation channel of *Xenopus* oocytes by amiloride.

On the basis of our results, we assume that the inhibition of Na current in macrophage membranes by diuretics may involve interaction of drug molecules with two (or more) sites in the channel. The first binding site is near the external membrane surface and the second is in the hydrophobic region. A different inhibitory mechanism for diuretics may be due to preferable interaction of amiloride with the external site and of more hydrophobic EIPA with the internal one. Additional studies appear to be necessary to obtain a description of drug-channel interaction in detail. The finding that macrophages, a highly accessible biological material, may express amiloride-sensitive sodium channels suggests their possible use as model preparation for researching diuretic action.

It remains unclear how sodium channels identified in macrophages may be regulated. By analogy with amiloride-sensitive epithelial channels (Cantiello, Patenaude & Ausiello, 1989; Smith & Benos, 1991), G proteins may be assumed to be involved in the control of sodium channel activity in macrophages. It is in agreement with our observation that the presence of GTPγS in the pipette solution increased the probability of channel activity displayed in the outside-out patch. However, no direct evidence of such a regulation has been obtained for sodium channels in macrophage membranes.

The physiological role of the nonvoltage-gated sodium channels in macrophages may be associated with fluid fluxes and volume regulation, as traditionally assumed. Moreover, the data obtained on different

cell types suggest that intracellular Na^+ plays an important role in the control of the intracellular processes, in particular, by modulating the functioning of other channels in plasma membranes (Liu & Kao, 1990; Balke & Gil Wier, 1992). From their investigations of the extracellular Na effect, Harvey and co-workers (1991) have concluded that intracellular Na modulates the cAMP-dependent regulation of K^+, Cl^- and Ca^{2+} channels in cardiac myocytes. Intracellular Na has been also assumed to affect free cytoplasmic $[Ca^{2+}]_i$ in mononuclear leukocytes (Wehling, Käsmayr & Theisen, 1990). The authors have found that the effect of aldosterone on calcium content was antagonized by 1 μM ethylisopropylamiloride and was not observed in sodium-free external medium. A question arises about the mechanism of sodium entry presumably involved in the process mediating the action of the ionic composition and hormones of the extracellular medium on the cytoplasmic targets. To explain the effect of amiloride and its analogues on Na^+ fluxes, inhibition of the Na-H antiporter is assumed (Wehling et al., 1990). It seems also possible that sodium transport occurs through specific ionic channels in leukocyte cells. Sodium-selective channels in macrophages described in our work may be assumed to provide Na^+ influx through plasma membranes involved in the intracellular regulation processes.

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