J. Membrane Biol. 158, 159–166 (1997)

The Journal of
Membrane
Biology

Large-Conductance Cation Channels in the Envelope of Nuclei from Rat Cerebral Cortex

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Received: 12 November 1996/Revised: 18 February 1997

Abstract. Eucaryotic nuclei are surrounded by a double-membrane system enclosing a central cisterna which is continuous with the endoplasmic reticulum and serves as a calcium store for intracellular signaling. The envelope regulates protein and nucleic acid traffic between the nucleus and the cytoplasm via nuclear pores. These protein tunnels cross through both nuclear membranes and are permeable for large molecules. Surprisingly, patch clamp recordings from isolated nuclei of different cell species have revealed a high resistance of the envelope, enabling tight seals and the resolution of single ion channel activity. Here we present for the first time single-channel recordings from nuclei prepared from neuronal tissue. Nuclei isolated from rat cerebral cortex displayed spontaneous long-lasting large conductances in the nucleus-attached mode as well as in excised patches. The open times are in the range of seconds and channel activity increases with depolarization. The single-channel conductance in symmetrical K^+ is 166 pS. The channels are selective for cations with $P_K/P_{Na} = 2$. They are neither permeable to, nor gated by Ca^{2+}. Thus, neuronal tissue nuclei contain a large conductance ion channel selective for monovalent cations which may contribute to ionic homeostasis in the complex compartments surrounding these organelles.

Key words: Nuclear ion channels — Cation channel — Cell nucleus — Patch clamp — Ion selectivity – Nuclear envelope

Introduction

The nuclear envelope separates the nucleoplasm from the cytoplasmic domain. It consists of an outer and an inner membrane with an associated filamentous lamina (Gerace & Burke, 1988; Goldberg & Allen, 1995). The perinuclear space between the two membranes defines a third compartment continuous with the endoplasmic reticulum. Molecules are transported in both directions between the cytoplasm and the nucleoplasm via the nuclear pore complex, a 125 MDa protein assembly with a central channel spanning both membranes and the perinuclear space (Miller, Park & Hanover, 1992).

Recent studies with patch clamp pipettes have shown that nuclear membranes possess a variety of ion channels. These include inositol-triphosphate (IP3)-receptors (Mak & Foskett, 1994; Stehno-Bittel, Lückhoff & Clapham, 1995*a*), Cl^--channels (Tabares et al., 1991; Rousseau et al., 1996) and K^+-selective ion channels (Mazzanti et al., 1990; Maruyama, Shimada & Taniguchi, 1995). The high-resistance tight-seal patch clamp recordings and the existance of ion-selective channels imply that the nuclear envelope resembles a barrier against free diffusion of small ions between the cytoplasm and the nucleus (Bustamante, 1994). This is underlined by the potential gradient between the nucleus and the cytoplasm as well as by the possibility to induce osmotic gradients over the nuclear envelope (Mazzanti et al., 1990; Mazzanti, DeFelice & Smith, 1991).

The nuclear pore allows for the free transition of molecules of up to 70 kD (Miller et al., 1992) and thus had previously been believed to be far beyond the size of any ion-selective channel. Therefore, the tight sealing of glass micropipettes to nuclear membranes is difficult to understand, since nuclear pore complexes are highly abundant and should cause multiple large membrane perforations underneath each pipette (Mazzanti et al., 1990,

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1991; Innocenti & Mazzanti, 1993). Recent evidence suggests that ion translocation through nuclear pore complexes can be observed as large current fluctuations with a conductance of more than 400 pS (Bustamante 1994; Bustamante et al., 1995*a*) or even 1000 pS (Matzke et al., 1992). However, smaller conductance values up to 200 pS from other reports have also been attributed to the nuclear pore complex (Mazzanti et al., 1991). At present, the relation between nuclear pore structure and the observed ionic currents remains unclear. It is feasible that the smaller conductance values reflect the gating of eight putative channels surrounding the central pore which is at least temporarily plugged by a protein (DeFelice & Mazzanti, 1995; Perez-Terzic et al., 1996).

The functional significance of most presently characterized nuclear envelope ion channels is unknown. Only currents gated through nuclear pores and IP3-receptors can be correlated to cellular functions like molecular traffic and intracellular calcium signalling. So far, nuclear ion channels have been described in amphibian (Mak & Foskett, 1994) and mammalian oocytes (Mazzanti et al., 1991), mammalian cardiac cells (Bustamante et al., 1995*a*,*b*), liver cells (Tabares, Mazzanti & Clapham, 1991; Mazzanti et al., 1991; Innocenti & Mazzanti, 1993), rat pancreatic azinar cells (Maruyama et al., 1995), murine pronuclei (Mazzanti et al., 1990, 1991), avian erythrocytes (Matzke et al., 1990) and plant cells (Matzke et al., 1992). Nuclei from nervous tissue, however, have not been directly examined with electrophysiological techniques. Here we show that ion channels are present in the envelope of nuclei isolated from rat cerebral cortex. Analysis of their biophysical properties reveals that they conduct monovalent cations with large conductance and low selectivity and are different from nuclear channels described in other tissues.

Materials and Methods

Isolation of Nuclei

Nuclei from rat cortex were prepared using a modified protocol as described by Otto et al. (1992). Adult Wistar rats of either sex were deeply anesthetized with ether, decapitated and the brain removed. The tissue was cooled for 5 to 10 min in ice-cold, carbogen- (95% O_2, 5% CO_2)-gassed artificial cerebrospinal fluid (in mM): NaCl 124, NaH_2PO_4 1.25, KCl 3, $MgSO_4$ 1.8, $CaCl_2$ 1.6, glucose 10, $NaHCO_3$ 26, pH 7.35 and then mechanically dissected. We isolated cortical tissue free from blood vessels, dura and white matter. The pieces were transferred into sucrose buffer (in mM): sucrose 320, Hepes-Na 20, $MgCl_2$ 1, dithiothreitol 1, aprotinin 10 mg/l, phenylmethylsulfonylfluoride 0.5, pH 7.4 and were subsequently minced in a glass-teflon potter. The homogenate was filtered through 120-mesh nylon gauze and centrifuged at 500 g for 5 min. The pellet was resuspended in hyperosmolaric sucrose buffer (containing 500 mM sucrose, Hepes, $MgCl_2$ and pH as above) and centrifuged again at 500 × *g* for 5 min. The pellet was then resuspended in sucrose buffer with 800 mM sucrose (composition as above) which was carefully underlaid with 2 ml 1.32 M sucrose buffer and centrifuged at 1800 × *g* for 40 min. The resulting pellet was gently resuspended in a small volume of 320 mM sucrose buffer, filtered through 30 mesh gauze and stored on ice. All steps of the preparation were carried out at 4°C.

Patch Clamp Electrophysiology

Small volumes of the suspension containing the nuclei were transferred to a perspex chamber filled with standard potassium-solution (in mM) (KCl 140, $MgCl_2$, 2$CaCl_2$ 1.63, EGTA 2, HEPES 10, pH 7.2, free Ca^{2+} 1 μM). After about 10 min single nuclei free of cytoplasmic structures were easily visible at the chamber bottom using a ×40 objective and Normarski-optics. A small number of contaminating cell bodies could be visually excluded by a cytoplasmic penumbra around the nucleus resulting in a double contour. Intact nuclei had a single smooth surrounding and at least one nucleolus (Beckmann et al., 1992). We recorded from large diameter (ca. 15–20 μm) nuclei which are most likely to stem from neurons (Thompson, 1987). Patch-clamp recordings (Hamill et al., 1981) were performed at room temperature (20–23°C) using an EPC-7 amplifier (List, Darmstadt, Germany) and borosilicate glass pipettes (o.d. 2.0 mm, i.d. 1 mm, resistance 3–5 MOhm). Most recordings were from isolated patches (*see below*) under symmetric ionic conditions, i.e., the pipette solution was identical to the bath solution (KCl standard solution as detailed above). Ca^{2+}-permeability was tested with assymetric solutions: a high-calcium pipette solution (in mM): KCl 50, $CaCl_2$ 51.92, Choline-Cl 15, (290 mOsmol) and a low-calcium bath solution (KCl 50, $CaCl_2$ 1.63, Choline-Cl 90, (310 mOsmol) pH 7.2. Both solutions contained (in mM): $MgCl_2$ 2, EGTA 2, and HEPES 10. The calcium-dependence of channel gating was tested with bath solutions containing different free calcium concentrations: 0.1 μM free Ca^{2+} (0.176 mM total $CaCl_2$), 1 μM free Ca^{2+} (standard solution, 1.63 mM total $CaCl_2$), 10 μM free Ca^{2+} (1.9 mM total $CaCl_2$) and 100 μM free Ca^{2+} (2.02 mM total $CaCl_2$). The free calcium concentrations were calculated using the computer program BUFFER (Schubert, 1996) which was a kind gift from Dr. R. Schubert, University of Rostock, Department of Physiology, 18055 Rostock, Germany. All these solutions contained $MgCl_2$, KCl and HEPES as in the standard KCl-solution (*see above*).

After seal formation, spontaneous currents could be recorded in the ''nucleus attached'' configuration. To gain control of the ion concentrations at the inner side of the membrane(s) we then excised membrane patches by careful backword movement of the pipette. All data in the figures and the Results section are from excised patches if not stated differently. The currents were low-pass filtered at 1 kHz (4-pole Bessel filter) and digitized at 3 to 5 kHz using the TIDA data acquisition program (HEKA, Lambrecht, Germany). Membrane potential values are defined with respect to the cytoplasmic side of the membrane for both nuclear-attached and excised patch recordings (i.e., membrane potential = pipette potential, *cf.* Stehno-Bittel et al., 1995*a*). Thus, at positive potentials and symmetrical solutions cations will flow out of the pipette through the membrane into the nuclear interiour or into the cisterna or (in excised patches) directly into the grounded bath. Such currents will be described as outward currents (upward deflection of current trace).

Data Analysis

Data were analyzed offline after the experiments. Single-channel currents could be identified by stepwise current changes. Current amplitudes were measured by cursor adjustment as the difference between two distinct open levels or between the first open level and the baseline value. In some cases all-point-histograms were used to verify the

multi-Gaussian distribution expected for multiple current levels of equal amplitude (*see* Fig. 1).

Channel activity was estimated as the product of the (unknown) number of channels in the patch (N) and the open probability P_o. The current was integrated over 10 to 30 sec from traces where the leak current level (all channels closed) could be clearly identified. We then calculated

$$N \cdot P_o = \text{Int}(I)\text{dt/i} \cdot \text{t}$$

where i is the single channel current amplitude and t the time of integration. Mean channel conductance and reversal potentials were calculated from linear regression fits to current-voltage relations between −40 and +60 mV. Data are given as mean ± SE. The significance of differences in channel activity or conductance at different voltages was calculated with Student's t-test for paired data. Error probabilities of $P < 0.05$ are regarded as significant.

Results

The preparation from rat cerebral cortex contained a variety of differently sized and shaped nuclei. We established patch clamp recordings from the largest fraction (about 15–20 μm in diameter) with one clearly visible nucleolus in the nucleoplasm. After approaching the membrane with the patch pipette and applying mild suction we obtained tight seals with resistances above 2 GOhm in more than 80% of the nuclei examined (n = 160). Spontaneous long-lasting, high-conductance channel openings were observed in about half of the patches when the command potential was different from 0 mV (symmetrical standard KCl-solution). Upon careful withdrawal of the pipette most nuclei remained attached to the chamber bottom and we were able to isolate a tightly sealed membrane patch. In this configuration the channel activity continued and was apparently unchanged with respect to the nucleus-attached configuration (Fig. 1). We restricted our analysis to the prominent large events as visible in Fig. 1*A* irrespective of the superimposed shorter, low-conductance openings. To yield optimal control of the ionic environment, all following data have been recorded from isolated patches (for the topology of membrane(s) in this configuration *cf.* Discussion).

Channel Conductance

All-point histograms from isolated patches revealed clear equidistant peaks indicating the discrete and reproducible nature of the observed current levels (Fig. 1*B*). Conductance was measured by changing the pipette potential stepwise in both directions from 0 mV to values between −60 and +60 mV (Fig. 2*A*). We also performed voltage jumps from conditioning negative or positive prepotentials which did not change the gating or conductance behavior of the channels. Currents were recorded for 10 to 60 sec at each potential. Within this time, most

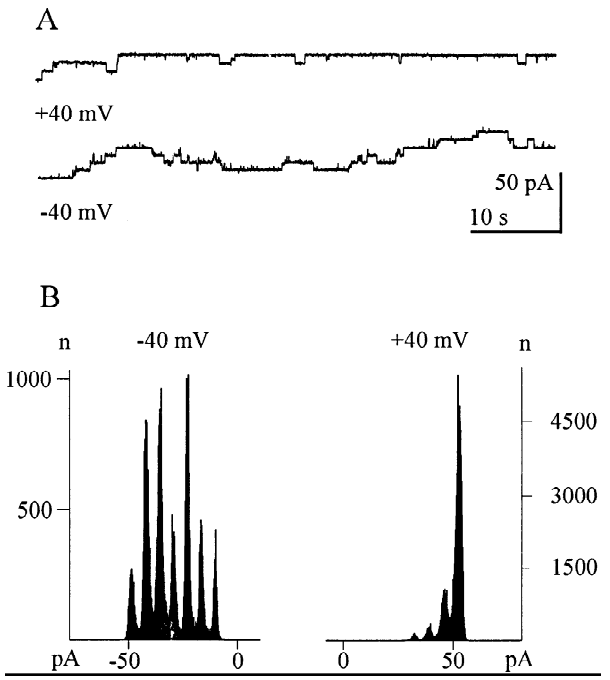

Fig. 1. Spontaneous activity of nuclear ion channels. (*A*) Current recordings from an isolated patch at −40 and +40 mV. Various overlapping long-lasting channel openings are clearly distinguishable. More different levels are apparent at −40 than at +40 mV. (*B*) All-point histograms of the recordings in A show seven discrete peaks at −40 mV and four peaks at +40 mV. Ordinates show the number of points within each corresponding current range (0.25 pA). Note that at +40 mV the lowest current value is at an elevated level compared to −40 mV, indicating that at least three channels are permanently open.

active patches allowed for the distinction of separate (one to eight) current levels (Fig. 2*A*). Extreme potential values, especially at +60 mV, tended to destroy the membrane seal and therefore some patches could only be recorded from −40 to +40 mV. Figure 2*B* summarizes the data from 19 patches measured in symmetrical KCl. Between −40 and +60 mV current amplitudes were linearly dependent on the voltage and a linear regression analysis revealed a single-channel conductance of 166 pS at room temperature and a reversal potential of −0.7 mV. A mild outward rectification was found at −60 mV pipette potential, where the current amplitude was slightly lower than the corresponding value at +60 mV. The rectification coefficient $I_{+60\text{mV}}/I_{-60\text{mV}}$ was 1.23 ± 0.13 (n = 9 patches; $P < 0.01$).

In three patches, we measured the current-voltage relation at three to eight different temperatures ranging from 19.8 to 39°C. Linear regression analysis of the conductance increase with temperature yielded a Q_{10} (relative conductance change per 10°C) of 1.24 ± 0.03.

Voltage Dependence of Channel Activity

It was apparent from the current-voltage measurements that channel activity increased with positive pipette po-

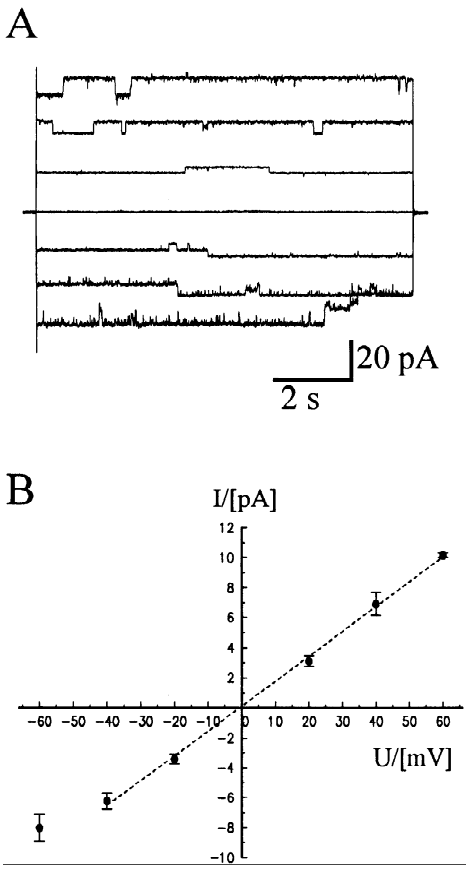

Fig. 2. Current-voltage relation of nuclear ion channels in excised patches. (*A*) Current responses to stepwise changes in pipette voltage (0, ±20, ±40, ±60 mV) from an isolated nuclear membrane patch (symmetrical K^+). No activity is visible at 0 mV, inward currents appear at negative (lower traces) and outward currents at positive (upper traces) pipette potentials. (*B*) Current-voltage diagram from 19 patches from different nuclei ($n = 3$ (−60); $n = 19$ (−40); $n = 10$ (−20); $n = 12$ (+20); $n = 14$ (+40); $n = 3$ (+60)). The relation is largely linear between −40 and +60 mV and the interpolated linear regression line yields a slope conductance of 166 pS. The reversal potential of the regression line is −0.7 mV.

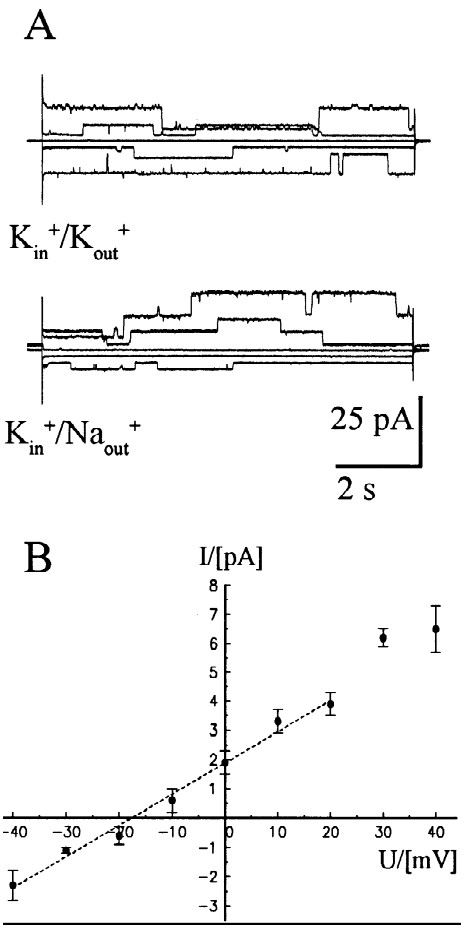

Fig. 3. Relative permeability of K^+ *vs.* Na^+. (*A*) Original recordings from an isolated patch in symmetrical KCl (upper traces) and after replacement of K^+ by Na^+ in the bath (lower traces). Note the clearly asymmetric event sizes after the ion substitution. (*B*) Current-voltage relation for three recordings with KCl in the pipette and NaCl in the bath. The linear regression between −40 and +20 mV shows a reversal potential of −17.6 mV, indicating higher conductance for K^+ than for Na^+ (*see* Results).

tentials. Figure 1 shows a recording from the same patch at +/−40 mV. The all-points histograms (Fig. 1*B*) reveal the existence of multiple current levels at −40 mV, whereas at +40 mV most of the channels are permanently open. In six patches we compared $N \cdot P_o$ at −40 and +40 mV. In all these recordings, the baseline (all channels closed) could be clearly defined because the minimal conductance was smaller than the individual current contribution *i*. The resulting ratio $N \cdot P_{o,+40}/N \cdot P_{o,-40}$ was 1.34 ± 0.23 ($P < 0.05$). Thus, channel activity is enhanced at positive potentials on the cytoplasmic side.

Ion Selectivity

Replacing half of the KCl in the bath solution by choline-chloride resulted in strong outward rectification of the currents. The reversal potential shifted to −21.2 ± 1.4 mV ($n = 3$) which is close to the value expected from the Nernst equation if potassium was the only permeable ion in this solution. The Cl^- concentration (147 mM in pipette and bath) was not changed, indicating that this anion does not contribute to the current. We concluded that the channels are selective for cations and impermeable to choline.

To find out whether the channels selectively conduct potassium ions we established current-voltage relations in symmetrical KCl-solution and subsequently replaced the K^+-ions in the bath by Na^+. This treatment clearly reduced the inward current amplitude, whereas the outward currents remained largely unchanged (Fig. 3*A*). The current-voltage data from three patches were fitted by linear regression between −40 and +20 mV yielding

an extrapolated reversal potential of −17.6 mV (Fig. 3*B*). Feeding this value into the Goldmann-Hodgkin-Katz equation for two monovalent ions (Hille, 1984) results in a relative permeability of 1.99 for K^+/Na^+. Thus, the nuclear channels are two times more permeabel to K^+ than to Na^+.

The nuclear envelope functions as a calcium-store and it is therefore feasible that ion channels located in nuclear membranes are permeable to Ca^{2+}. Such conductances have indeed been observed (Mak & Foskett, 1994; Stehno-Bittel et al. 1995*a*). Therefore we also performed ion-replacement experiments for Ca^{2+} *vs.* K^+. High concentrations of free calcium turned out to increase the noise and frequently destabilized the seals. However, we got sufficient clear channel openings from 5 patches recorded in asymmetric solutions which allow for the detection of calcium-permeability. In these recordings, the bath contained (in mM): 50 KCl and the pipette 50 KCl and 50 $CaCl_2$ (Fig. 4*A*). The osmolarity of the both solutions was adjusted with the impermeable cation choline (*see* Materials and Methods). Under these conditions a significant outward current should have been observed at 0 mV if Ca^{2+} was an efficient charge carrier in the nuclear channels (Mak & Foskett, 1994). However, a linear regression to the current-voltage relation from 5 patches revealed a reversal potential of +4.5 mV (Fig. 4*B*). The conductance decreased from 166 to 56 pS compared to the 140 mM standard KCl-solution which is consistent with a K^+-selective ion channel-. Thus ion channels in the envelope of nuclei isolated from neuronal tissue are impermeable to Ca^{2+}.

Consistent with the low selectivity of the channels, the specific potassium channel blocker TEA was almost ineffective. Adding 5 mM TEA to the bath solution did not change outward currents and reduced inward current amplitudes at −60 mV by only 13%.

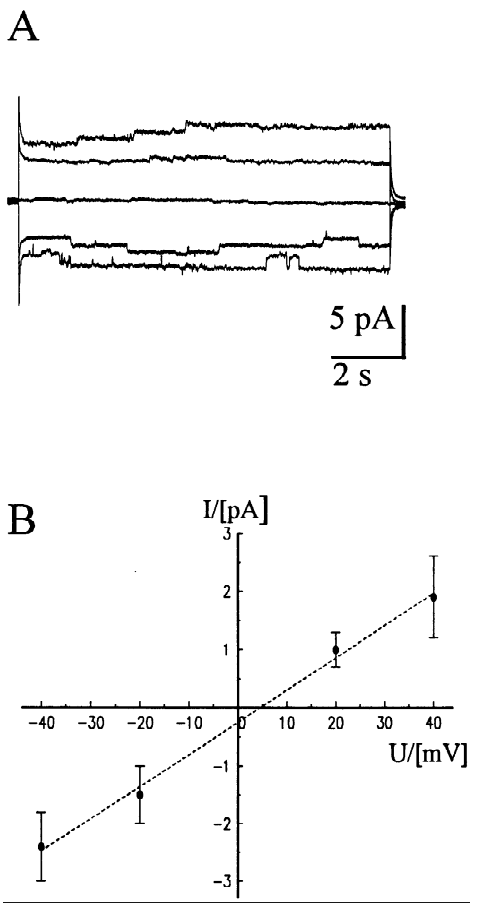

Fig. 4. Channels are impermeable to Ca^{2+}. (*A*) Original recordings from an excised patch at holding potentials between −40 and +40 mV. The pipette contained 50 mM KCl and 50 mM $CaCl_2$ whereas only 50 mM KCl served as charge-carrier in the bath (*see* Materials and Methods). Openings at potentials different from 0 mV appear largely symmetrical in amplitude. (*B*) Current-voltage relation for three distinct patches recorded with the KCl/$CaCl_2$-solution in the pipette. A linear regression reveals a reversal potential of +4.5 mV and the slope corresponds to 56 pS.

Activity is Calcium Independent

Ca^{2+} is not only a potential charge carrier in cation channels but also activates K^+ channels in numerous cells. To find out whether the gating mechanism or open probability of the channels is calcium-dependent, we changed the concentration of free Ca^{2+} in the bath between 0.1 μM and 100 μM (Fig. 5*A;* solutions *see* Materials and Methods). In four patches the activity at 1 μM Ca^{2+} could be compared with $N \cdot P_o$ at one or more other concentrations (0.1, 10, 100 μM. Normalizing $N \cdot P_o$ to the value obtained at 1 μM for each patch revealed no systematic influence of $[Ca^{2+}]$ on the channel activity (Fig. 5*B*).

Discussion

The present data show that nuclei from rat neuronal tissue contain large-conductance cation channels with characteristic properties. The channels conduct the monovalent cations K^+ and Na^+ but are not permeable to Ca^{2+}. Channel activity is weakly voltage-dependent and is not influenced by Ca^{2+}.

Ion Channels in Nuclear Envelopes Isolated from Rat Cerebral Cortex Cells

Single-channel recording is usually employed to analyze signaling mechanisms located on outer cell membranes. However, during recent years patch clamp recordings have been performed on the membranes of various cell organelles (Keller & Hedrich, 1992). Particularly, the complex system of the nuclear envelope has raised interest due to its central role in intracellular signaling

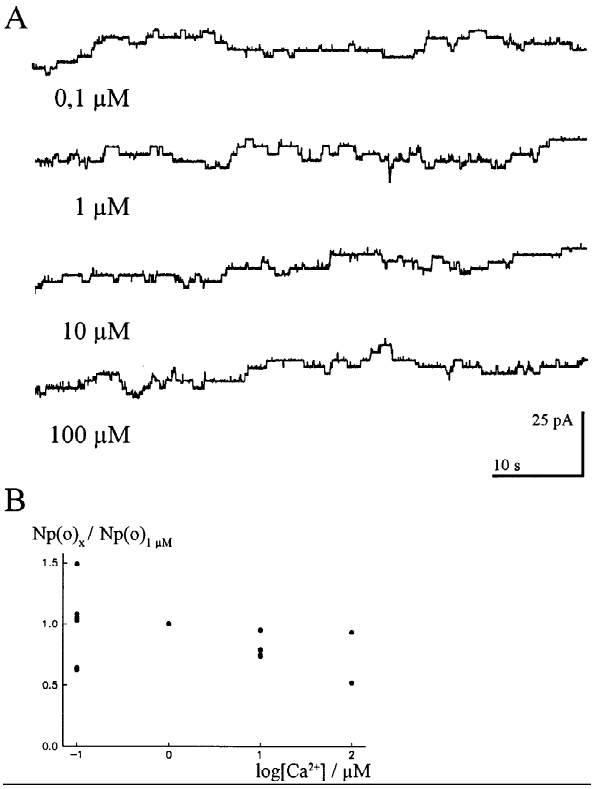

Fig. 5. Calcium does not influence the channel gating. (*A*) Original recordings from an isolated patch, recorded with different concentrations of free Ca^{2+} in the bath solution. Pipette solution contained 1 μM free Ca^{2+}. No obvious changes in the channel activity are apparent between 0.1 μM and 100 μM free Ca^{2+}. (*B*) Scatter plot showing the activity $N \cdot P_o$ at various free calcium-concentrations. The values have been normalized to $N \cdot P_o$ at 1 μM from the respective patch. No consistent calcium-dependent activation or inactivation of channel openings is apparent.

(e.g., Bustamante, 1994). Here we describe for the first time ion channels in the membrane of nuclei isolated from neuronal tissue. The preparation from rat cerebral cortex allows for some differentiation between neuronal and glial nuclei (Thompson, 1987). Microglia and oligodendrocytes contain small nuclei, whereas the large-diameter nuclei analyzed here are most likely neuronal and astrocytic. According to Thompson (1987), neuronal nuclei are large (*ca.* 15 μm) and contain one to two nucleoli whereas astrocytic nuclei contain two or more nucleoli. We selected for nuclei with one nucleolus and thus have most likely recorded from neuronal organelles, although a contamination with astrocytic nuclei cannot be excluded.

Supposed Channel Topology

A presently unresolved problem is the topology of the channel in the nuclear double-membrane system. In principle, three possible localizations are feasible: the channels might be integrated in the outer or the inner membrane or cross through both membranes linking the cytoplasm and the nuclear interior. In the first two cases, the channels would connect the nucleoplasm or the cytoplasm to the perinuclear space which is an extension of the compartment formed by the endoplasmic reticulum. The outer nuclear membrane is a direct extension of the endoplasmic reticulum and it is well feasable that both regions contain similar ion channels. Sarcoplasmic reticulum contains K^+-selective cation channels with large conductance values in the range observed here (Criado & Keller, 1987; Rousseau et al., 1992; Picher, Decrouy & Rousseau, 1996). In reconstitution experiments from sheep cardiac cells, Rousseau and colleagues found a high-conductance (180 pS) K^+ channel in membrane fractions derived from outer but not from inner nuclear membrane (Rousseau et al., 1996). The activity of the neuronal channels in isolated patches here was largely similar to nucleus-attached recordings. If the channels were located in the outer nuclear membrane we would therefore have to assume either that the inner nuclear membrane is extremely leaky under our conditions or that the perinuclear space contains roughly the same K^+ concentration as the standard recording solution (140 mM). Based on the potential of murine pronuclei it has been suggested that the K^+ concentration may be 20% higher in the nucleus than in the cytoplasm (Mazzanti et al., 1990) which would be roughly in the range of our standard solution. However, we do not know the K^+ concentration in the cisterna and thus cannot judge the driving force for potassium ions at the outer nuclear membrane.

If the channels span both membranes, they would have the same topology as nuclear pores, which would raise the question of the relation between these macromolecular gates of protein and nucleic acid traffic and the channels described here. Moreover, the maintenance of functional channels in isolated patches would require that the entire double membrane system can be withdrawn from the nucleus without gross alterations. Various authors have tried to relate their electrophysiological data from nuclear envelopes to nuclear pores (Matzke et al., 1992; Mazzanti et al., 1990, 1991; Bustamante et al., 1995*a*,*b*). The observed maximal conductance values range from about 200 pS to 1000 pS, and often smaller substates have been reported. The unusual high values of about 500 pS (Bustamante, 1995*a*) to 1000 pS (Matzke et al., 1992) are close to the theoretical limit expected from calculations based on the nuclear pore geometry (Mazzanti et al., 1990; Hinshaw, Carragher & Milligan, 1992; Goldberg & Allan, 1995; Bustamante et al., 1995*a*). The smaller values (200 pS) have been related to nuclear pore complexes because the increasing density of nuclear pores in murine cells during development is paralleled by an increasing number of conductance states in each patch. (Mazzanti et al., 1991). They

might reflect subconductance states of the central pore. Alternatively, it has been suggested that the 200 pS conductance may be due to ion transport through the eight channel-like structures surrounding each nuclear pore (DeFelice & Mazzanti, 1995). Thus the neuronal nuclear conductance described here might equally well mediate currents through nuclear pore complexes as through channels in the outer nuclear membrane.

Nuclear pores in rodent tissue appear at varying densities which increase with developmental age and metabolic activity. In adult tissue up to 70 pores can be expected under each patch pipette (Mazzanti et al., 1991). Regardless of the nature and topology of the channels we would therefore have to assume that most nuclear pores remain closed under our conditions allowing to resolve single ion channel activity. Imaging of nuclear pore complexes from *Xenopus laevis* oocytes indicates that NPCs can be closed by a central plug structure upon depletion of the cisternal calcium (Perez-Terzic et al., 1996). This gating mechanism might account for the relatively tight appearance of nuclear membranes in many preparations.

Nature of the Channels

The rapid transitions to reproducible current levels, the ion selectivity and the Q_{10} of 1.24 observed in the nuclear membrane recordings are typical features of ion channel proteins (Hille, 1984). Do the biophysical properties of the channels match with other known data from nuclear or cell membranes? Intracellular cation channels with conductances in the range observed here have been described by various authors. Mazzanti et al. (1990, 1991) found a cation-selective channel with 200 pS conductance in murine pronuclei. Rousseau et al. (1996) reconstituted a 181 pS cation-selective channel from the outer nuclear membrane fraction of sheep cardiac cells. Ca^{2+}-activated K^+-channels with 200 pS conductance have been found in nuclear membranes from rat pancreatic acinar cells (Maruyama et al., 1995). Thus, channels with some similarities to the neuronal tissue nuclear ion channels exist. However, the conductance described here is not identical with any of the observed channels. In contrast to Mazzanti et al. (1990, 1991) and Rousseau et al. (1996) we did not observe any clear substates, as indicated by the all-point-histograms (*cf.* Fig. 1). The long-lasting openings which are almost uninterrupted by short intermediate closures (''flickering'') are different from the kinetics observed by the other authors. Finally, the nuclear channels described here are definitively not Ca^{2+}-activated. Thus, neuronal tissue nuclear cation channels share properties with some other intracellular ion channels but seem to form at least a novel subtype.

Possible Function

The channels described here are permeable to monovalent cations, have a large conductance and long open-times and thus can transfer multiple positive charges without influencing calcium signaling. The functional significance of such channels is presently unclear and can only be adequately addressed once the channel topology is known. If the channels bridge cytoplasm and nucleoplasm, they would allow for a rather selective traffic of K^+ between these compartments. This might serve to maintain the potential gradient between the nucleus and the cytosol (Mazzanti et al., 1990). Such a mechanism requires that other ions are not freely diffusible between both compartments which is still controversial, especially for Ca^{2+} (Santella, 1996). Cytoplasmic calcium transients spread directly into the nucleoplasm during neuronal activity (O'Malley, 1994), but differences in the dynamics of Ca^{2+} transients have also been reported (Al-Mohanna, Caddy & Bolsolver, 1994). Depletion of calcium from the endoplasmic reticulum (or, likewise, the nuclear cisterna) results in a block of the nuclear pores (Gerace & Foisner 1994; Greber & Gerace, 1995; Stehno-Bittel, Perez-Terzic & Clapham, 1995*b;* Perez-Terzic et al., 1996) which might affect the flow of ions between the compartments. If the channels really span through both membranes, they might be specifically important in such situations of restricted ion diffusion or closed nuclear pores. A high K^+ conductance could then serve to reduce possible osmotic differences and maintain the potential gradient between nucleus and cytoplasm (Mazzanti et al., 1990).

Alternatively, the channels are located in one (probably the outer) nuclear membrane. They are impermeable to Ca^{2+} and thus are certainly not related to the calcium release through IP3- or ryanodine receptors (Ross et al., 1989; Walton et al., 1991; Mak & Foskett, 1994; Beckmann et al., 1995; Stehno-Bittel et al., 1995*a*). However, a steady flow of K^+ through spontaneously opening cation channels might result in a potential gradient between cytoplasm and cisterna and thus maintain a high driving force for the extrusion of Ca^{2+}. This function has also been suggested for Ca^{2+}-activated K^+ channels in the outer nuclear membrane which will be permanently opened by the high cisternal Ca^{2+} content (Maruyama et al., 1995).

We therefore conclude that nuclear membranes of rat cerebral cortex cells contain a new cation channel with high conductance and low selectivity for monovalent ions. However, more information about topology and gating mechanism is needed to understand its functional role.

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