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Ionic Permeability on Isolated Mouse Liver Nuclei: Influence of ATP and Ca^{2+}

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Abstract. Patch-clamp experiments on isolated nuclei revealed the existence of ionic channels on the nuclear envelope, but their exact localization and function are still unknown. Recent studies have demonstrated that ATP and calcium ions play an important role in nucleocytoplasmic protein traffic. ATP is essential to allow big molecules in and out of the nucleus. However, a cytoplasmic rise of calcium ions above 300 nM decreases both ATP-dependent transport and passive diffusion through the nuclear envelope. The use of isolated nuclei placed in a saline solution provides the possibility for testing only the compounds added in the bath or in the recording pipette. In the present study, we show that ATP is responsible for an increase of nuclear ionic permeability on isolated nuclei. This result not only confirms data previously reported in *in situ* nuclei, but also suggests that ATP is directly involved in the modulation of passive ionic permeability. In these particular experimental conditions, calcium ions decrease the channel current starting from a concentration of 1 μM. The parallelism in the modulation action of ATP and Ca^{++} between nuclear pores and ionic channels present on the nuclear envelope contributes to the support of the idea that an ionic pathway is associated with the pore complex.

Key words: Isolated nuclei — Ionic channels — Patch-clamp — ATP — Ca^{++} — Channel modulation

Introduction

A fascinating problem faced by cell biologists for several years concerns the possibility that the nuclear envelope discriminates between cellular components trafficking in and out of the nucleus. The ability to select between molecules suggests a role of the envelope not only as a source of DNA, but also as an active modulatory site of many cell functions (Dingwall & Laskey, 1992).

The current idea of passive nuclear permeability assigns an upper limit of 40 kD to solutes that should freely permeate the envelope (Dingwall & Laskey, 1992). No restriction occurs, for example, on the nucleocytoplasmic movement of ions, energetic molecules like ATP or GTP and on amino acids. According to this view, increasing intracellular calcium in the cytoplasm could invade the nucleus and trigger many reactions that use divalent ions as a cofactor. It is not clear yet if calcium rises in the cytoplasm and in the nucleus at the same time and by the same amount. Several other groups (Connors, 1993; Al-Mohanna, Caddy & Bolsover, 1994) obtained conflicting results about intracellular Ca^{2+} distribution, and the problem (supposedly due to different preparations, experimental conditions, and techniques) is yet to be clarified.

In the last four years, several research groups demonstrated the presence of ionic channels on the external surface of the nuclear envelope by applying the patch-clamp technique to isolated (Mazzanti et al., 1990; Matzke, Weiger & Matzke, 1990; Bustamante, 1992) and to *in situ* nuclei (Mazzanti, Innocenti & Rigatelli, 1994). The question, still unresolved, concerns the possibility that the nuclear channel could be either part of the nuclear pore, spanning both the membranes of the envelope, or that it is only a communication pathway between the cytoplasm and the nuclear cisternae. Recent studies produced indirect evidence in favor of the first hypothesis (Matzke et al., 1990; Innocenti & Mazzanti, 1993; Bustamante, 1992, 1993, 1994; Mazzanti et al., 1994). The nuclear channel rarely reaches the calculated conductance of the pore (Mazzanti et al., 1990; Matzke et al., 1990). The only certain fact is that, under electrophysiological experimental conditions, nuclear pores are par-

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tially or totally closed (Mazzanti et al., 1990; Matzke et al., 1990; Bustamante, 1992, 1993; Mazzanti et al., 1994). These results are in contrast with the current opinion in which the protein complex appears with a ''crystallized'' opening of 9 nm (Forbes, 1992). The possibility of missing at least one pore in an on-nucleus patch-clamp experiment is negligible, because of their density and the working area isolated by the patch electrode (Innocenti & Mazzanti, 1993; Mazzanti et al., 1994).

There are few hypotheses which can reconcile the two points of view. There is no doubt that, under physiological conditions, the nuclear envelope do not represent a barrier for ions and small solutes. Some authors suggest that under certain conditions it is possible that nucleocytoplasmic pathways modify their size below the 9–10 nm functional diameter creating, as a consequence, two electrically separated environments. In the literature there are examples of changes in nuclear envelope ionic permeability properties. Ito and Loewenstein (1965) measured a resistivity change during hormone stimulation. Many authors measured a nuclear resting potential in intact cells (Loewestein & Kanno, 1963; Mazzanti et al., 1990). The measured voltage difference between nucleus and cytoplasm is around negative 10–15 mV. A Donnan equilibrium might be a good explanation for such a small potential difference. Certainly the presence of a large amount of fixed charges in the nucleus (DNA, RNA and histones) contributes to generate a nucleocytoplasmic charge separation (Oberleithner et al., 1993). Many experiments done on this subject failed to answer whether the nuclear potential is only a Donnan equilibrium or is due to the selective permeability characteristics of the nuclear envelope. Lowenstein and Kanno demonstrated a nuclear potential in different cell types, showing that the rupture of the envelope abolishes the nucleocytoplasmic voltage difference (Lowenstein & Kanno, 1963). In a later study it was shown that increasing the cytoplasmic potassium concentration changes the nuclear resting potential according to the Nernst equation (Mazzanti et al., 1990). Both these results could be interpreted either as a perturbation of a Donnan equilibrium or the presence of a semipermeable membrane. It is reasonable to believe that both the mechanisms are involved and the relevance of one or the other depends on the intracellular environment. It is probably only under particular conditions that the nuclear envelope could represent an ion barrier, giving the possibility of recording ion channels on its surface. There are few molecules that are able to modulate the current flow through the nuclear channels. Zinc, lanthanum and GTP-γ-S depress the ionic movement (Bustamante, 1993) whereas deoxynucleotides, phosphorylation and ATP increase, with different modality, the current flow (Bustamante, 1992, 1993; Mazzanti et al., 1994).

In the present paper we investigated the effect of ATP and tested its mechanism of action on isolated nuclei. We also tested the effect of external (cytoplasmic) calcium on the nuclear ionic conductances to determine if there are interactions or modulations of the current flow.

Materials and Methods

Nuclei Isolation

We isolated mouse liver nuclei by shearing fresh liver tissue in a homogenizer with a Teflon pestle of 0.025 μm clearance, and centrifuged the homogenate on a sucrose pad (in mM: 50 Tris HCl, 25 KCl, 5 $MgCl_2$, 250 sucrose) at 4,000 rev/min in a Labofuge M (Heraeus, Milan Italy) at 0–4°C. This procedure resulted in a pellet of pure nuclei, which we resuspend in the standard bath solution (in mM: 120 KCl, 0.1 $CaCl_2$, 2 $MgCl_2$, 1.1 EGTA, 10 HEPES 5 glucose, pH 7.4) before use, at room temperature. ATP and calcium ions were added to the high K solution in the concentration reported in each figure legend.

Patch-clamp Recording

The patch electrodes were pulled from hard borosilicate glass (Corning 7052) on a Brown-Flaming P-87 puller (Sutter Instrument). The pipettes were coated with Sylgard (Dow Corning) and fire polished to an external tip diameter of 1–2 μm. These electrodes had resistances of 7–10 MΩ. We applied the standard nucleus-attached patch-clamp technique to obtain nucleus-attached patches between 20–50 GΩ in resistance and single-channel recordings. In all the experiments the patch pipette contained 120 KCl solution (*above*). Single-channel currents were recorded with an Axopatch-1D (Axon Instrument, Novato, CA) patch-clamp amplifier and were digitized and stored on a VCR (Panasonic).

Data Analysis

Data were analyzed offline on a Mitsuba computer after filtering at 1,000 Hz using custom made programs developed by W. Gooldby, Department of Anatomy and Cell Biology, Emory University, Atlanta GA.

The stimulus protocol consisted in voltage steps of different duration (3200 and 320 msec) depending on the experiment type: long for the channel kinetics (Fig. 2) and short for ATP and Ca^{2+} effect (Figs. 3, 4). To obtain amplitude histograms over a consistent amount of time, we pasted together many current recordings at the same potential (Fig. 1*a*). This procedure was chosen to emphasize the high number of current sublevels present also within the same experiment.

Results

The performance of ''nucleus-attached'' experiments on isolated liver nuclei did not present particular technical difficulties. The surface of the envelope is clean and the solution in which nuclei are immersed is similar in ionic composition to the cytosol. In these conditions, the nucleus does not show a resting potential. After the seal

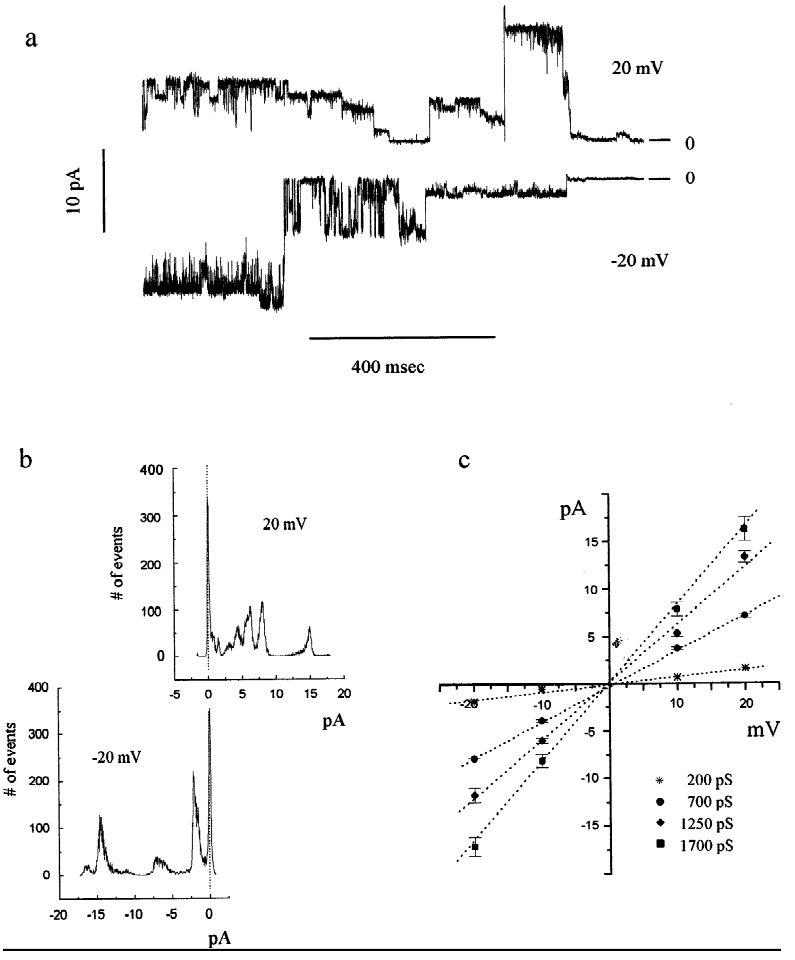

Fig. 1. Multiple conductance states of the nuclear ionic permeability. (*a*) Concatenated on-nucleus current recordings of single-channel at ±20 mV holding voltage. The raw data emphasize different current levels, quantified in the amplitude histograms in (*b*). Using current traces as in (*a*) at different test potentials, analyzing them as in (*b*) it is possible to obtain a current/voltage relationship (*c*) in which we can identify several conductances of nuclear ionic permeability.

formation with 0 mV in the recording pipette, no channel activity is detectable. Presumably, the solution used in the bath permeates inside the nucleus reducing the difference in concentration, if present, across the nuclear envelope. In these experimental conditions, assuming equimolarity of the ionic solution in and out the nucleus, the only driving force could rise from the change in the pipette voltage.

Figure 1*a* shows an example of several single-channel current traces at ±20 mV voltage steps applied alternatively in the patch pipette (*see* Materials and Methods). Amplitude analysis of the current traces produces the two histograms shown in Fig. 1*b*. Both inward and outward currents present many peaks corresponding to several current levels. The voltage protocol was applied at different potentials and the histogram peak amplitudes were displayed on a current/voltage (*i/V*) plot depicted in Fig. 1*c*. The conductances, measured from the line slopes traced through the experimental points, give values that range from 200 to 1700 pS. Similar results were obtained in previous investigations (Mazzanti et al., 1990; Matzke et al., 1990; Bustamante, 1992, 1993). Analyzing 32 other experiments, we calculated an even wider range of conductance, between 100 to more than 3000 pS. In any case, we could not obtain a clear indication about a possible current value that would represent a unitary single-channel conductance. Openings at different levels were a random process within the same experiments and appeared to be disconnected between experiments.

It is still not clear if the current levels recorded at different potentials are separate channels or the same channel with many substates. A tentative study to elucidate these two possibilities was carried out with single-channel analysis and current kinetic observations. In Fig. 2 we present data obtained from three different trials using the same preparation and experimental conditions. A 40-mV voltage step was applied to the pipette to elicit outward current (*a*). From the current traces it is possible to observe that the amplitude, the kinetics and the open/close channel transitions manifest themselves in many ways. The faster time-scale magnification of current recording portions (*b*), shown in the boxes below, reveals some of the characteristics of the nuclear ionic permeability: in the case of outward current, the channel(s) opens and closes either in several steps or in one

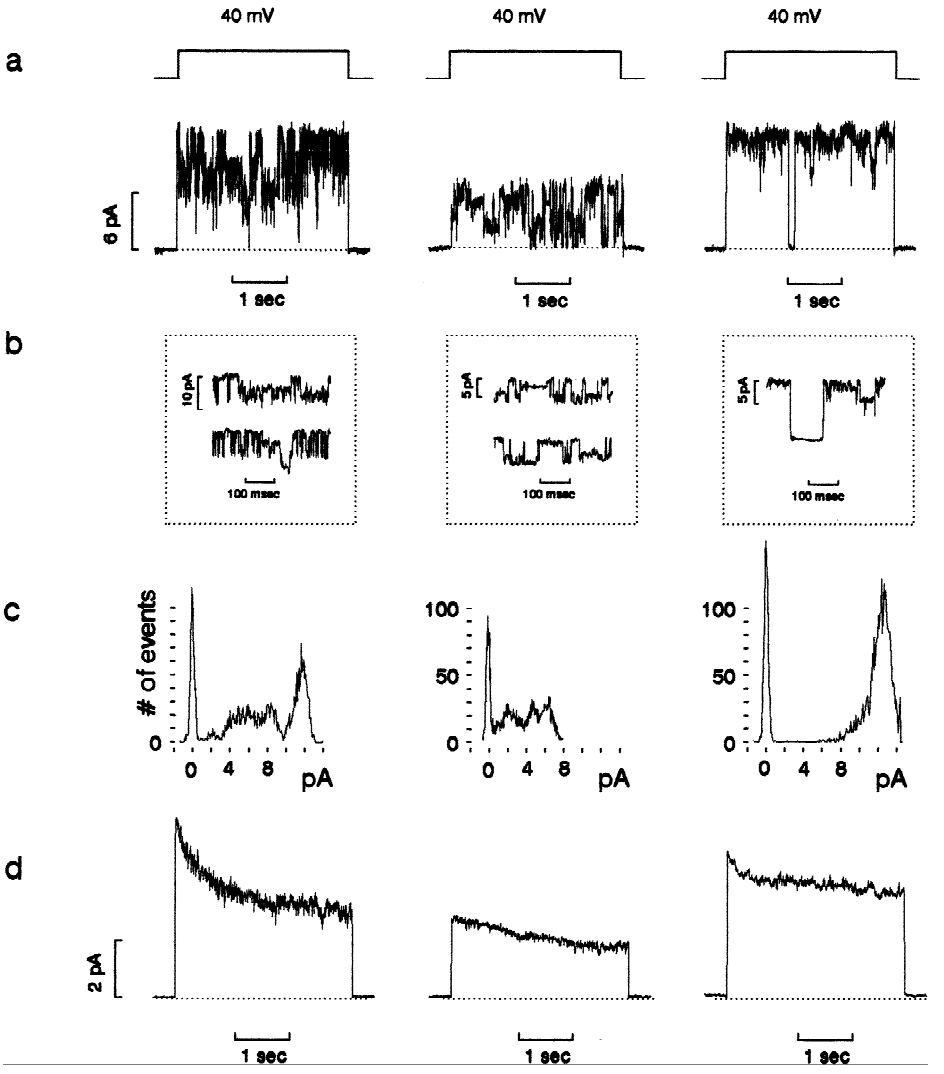

Fig. 2. Single-channel kinetics of nuclear current sublevels. Fig. 2 (*a*) depicts examples, from three different experiments, of current recordings at the same test potential (above), showing different open/close kinetics of the nuclear conductances. The inserts below (*b*), represent a magnification, on a faster time scale, of the most significant transitions extracted from the original current traces. In (*c*) amplitude histograms are plotted showing that, despite ionic channel kinetics, the higher histogram peak corresponds to the maximum current level reached during the recordings. (*d*) Single-channel current averages of 12 recordings in each experiment. All averages show the time-dependent component suggesting a similarity in the basic current pathways responsible for the ionic flow.

only (third box from the left); the different amplitude of current jumps, combined with the possibility for the channel to suddenly change from the close to the open state at the maximum current level, suggests either one large channel with many substates or many cooperative channels. The two hypotheses would be indistinguishable from the single-channel kinetic point of view. Both guesses would explain, in an appropriate way, the fact that the highest peak in the histograms (*c*) often corresponds to the maximum amplitude of the current during each experiment ($n = 27$). This is true for positive single-channel currents (from the nucleus to the patch pipette). Concerning the inward current, opening and closing of the channel are sporadic and total closures of the ionic pathways are rare, suggesting a different kinetics for the nuclear channels depending on the current direction.

The asymmetry in the current kinetics is present also in the single-channel current average. Whereas the inward current average shows a sustained value through all the voltage stimuli (*data not shown*), the outward ionic flow presents a time dependent inactivation (*d*) already described in previous studies (Bustamante, 1992; Mazzanti et al., 1994). The characteristic of asymmetrical single-channel current kinetics, associated with the inactivation of the outward current average, suggests the idea

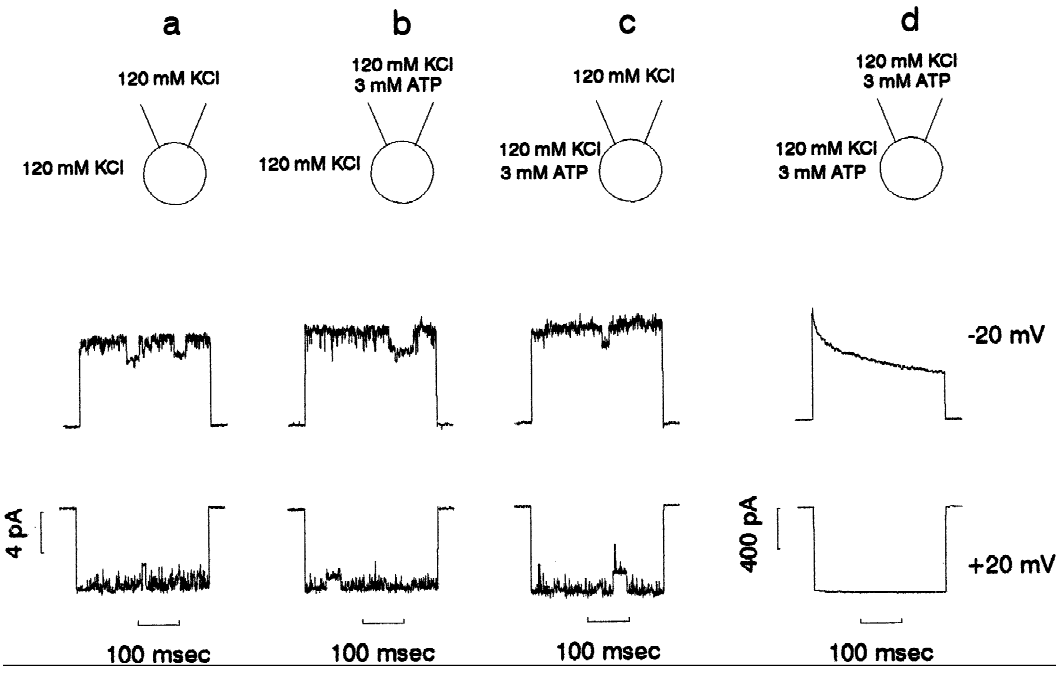

Fig. 3. ATP modulation of ionic nuclear permeability. The four panels present the different experimental conditions in which the nuclear permeability was recorded. In (*a*) the electrode contains only saline solution and the same media surrounds the nucleus. 3 mM of ATP was added alternatively to the electrode (*b*), to the superfusing solution (*c*) or in both the compartments (*d*). The current recordings maintain single-channel characteristics similar to (*a*) in (*b*) and (*c*), but become a macrocurrent when ATP is present at the same time in the patch pipette and in the external solution.

that, using solutions containing only ionic solutes, the detected channel activities could be attributed to several conductances of the same type.

Consideration of the current parameters and the similarity with experiments performed in nuclei of other species (Mazzanti et al., 1990; Matzke et al., 1990; Bustamante, 1994) in connection with the hypothesis that nuclear ionic channels may be part of the nuclear pore complex (Matzke et al., 1990; Innocenti & Mazzanti, 1993), have induced investigation of the role of ATP and calcium ions on the nuclear ionic pathways. At physiological concentrations, ATP increases the current flow through the nuclear envelope of *Xenopus* oocytes (Mazzanti et al., 1994). The role that Ca^{2+} ions have in nucleocytoplasmic transport, on the contrary, is still to be clarified. Among the disparate functions of intracellular calcium ions, there are several reports on the role that this important second messenger plays in the nucleocytoplasmic transport. The aim is to determine if, using isolated liver nuclei, it is possible to study the modulations of the nuclear channels, since this preparation has many technical advantages in comparison with ''*in situ*'' nuclei.

Figure 3 presents the different combination of solutions used to fill the electrode and to bathe the nuclei during the experiments on modulation of the nuclear ionic permeability by ATP. Assuming that under our conditions, small solutes equilibrate in and out of the nuclei in short periods (seconds), we considered the nucleoplasm to be equimolar with the external solution. From the left side of Fig. 3 in the first three experiments ATP was either absent (*a*), present only in the patch electrode (*b*), or just in the external solution (*c*). In all three cases the current traces, both in the inward and in the outward directions, maintained their characteristics even after 15 min of continuous recording, showing the typical openings and closings of a single-channel experiment. In Fig. 3(*d*) ATP was added in the patch pipette and in the external solution. The current increased dramatically in amplitude, showing the characteristic of a macroscopic ion flow and the typical inactivating kinetics of the outward current already reported in other preparations (Bustamante, 1992; Mazzanti et al., 1994).

The modulation by calcium ions is a more complicated investigation. There is no direct evidence concerning an interaction of Ca^{2+} ions with nuclear permeability. In a first series of experiments using a calcium-free solution to fill the nucleus-attached electrode, we gradually increased the external Ca^{2+} concentration starting from 10 μM. Each solution at different concentrations of the divalent ion remained around the nuclei not less than 15 min. To observe a reduction of the current amplitude, continuously recorded, we had to reach 1 mM of Ca^{2+} in the external solution. The effect is partially reversible;

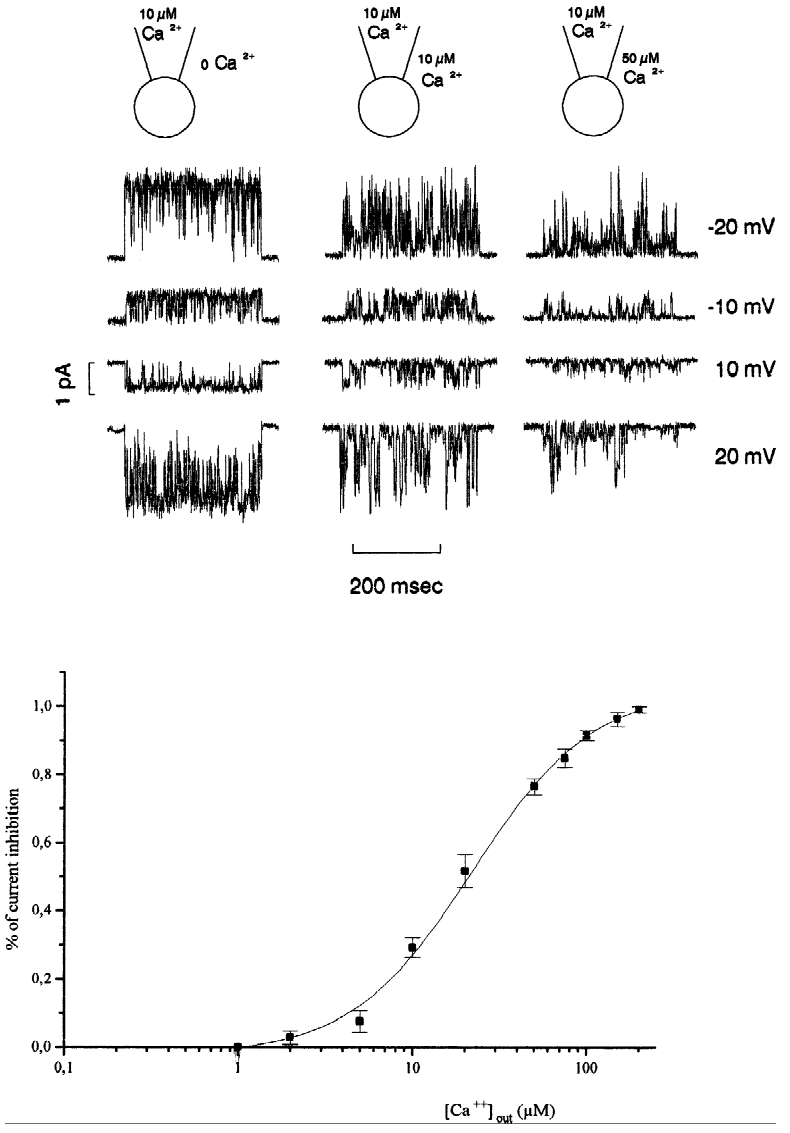

Fig. 4. Ca^{2+} inhibition of nuclear ionic channel. (*a*) On-nucleus recordings of single-channel inhibited by increasing external Ca^{2+} concentration. Experimental conditions are displayed above current traces. Test potentials are reported on the right of the single-channel recordings. (*b*) Dose-response current inhibition curve. The patch pipette always contains 10 μM Ca^{2+} and the external concentration of the divalent cation was increased until no more openings were evident.

over 12 experiments, only 2 showed a recovery of the single-channel current after several minutes of washout. The concentration of the divalent ion necessary to induce a depression of the current is totally unphysiological. We proposed three hypotheses to account for this observation: (i) calcium ions do not modulate the nuclear ionic permeability; (ii) isolated nuclei do not respond to Ca^{2+} concentration; (iii) calcium ions must be present on both sides of the membrane as during the experiment on the ATP modulation.

Figure 4 (*a*) shows the effect of 10 and 50 μM Ca^{2+} when the divalent ion is present in the external solution and in the patch pipette (10 μM). In the left panel we show single-channel recordings at ±10/20 mV when calcium ions are only present in the recording electrode. The current traces in the center depict single-channel openings when the nuclei were perfused with a solution containing 10 μM Ca^{2+} ions. The inhibitory effect of calcium on the channel current appears evident in the reduction of the open time of the channel. The right panel shows the action of 50 μM external Ca^{2+}. In this case the ionic channel opens sporadically and barely reaches the full open amplitude. In most of the experiments the blocking action of calcium results are irreversible. Only 3 experiments out of 11 showed full opening of the channel after intensive washout. Averaging current traces at +20 mV we were able to normalize different experiments and draw a current inhibition dose/response curve shown in Fig. 4 (*b*). The patch pipette in all the experiments contained 10 μM Ca^{2+} while the nuclei were superfused with increasing doses of the divalent ion. The effect on the membrane current starts to be significant after 1 μM external Ca^{2+}, reaching its maximum after 200 μM.

Discussion

The characterization of the nuclear ionic permeabilities is not straightforward, unlike other ionic channels present on the plasma membrane. The exact location of the channel is still unknown; it is undecided if the ionic pathway is part of the nuclear pore complex (Mazzanti et al., 1990; Matzke et al., 1990, 1992; Bustamante, 1992, 1993, 1994; Innocenti & Mazzanti, 1993) or if it is a structure in parallel with the nucleocytoplasmic communication conduit. The particular structure of the nuclear envelope does not help to clarify the nature of the nuclear ionic current. It is arduous to intrepret electrophysiological data obtained from a two-membrane structure in which little is known about the function and the contents of the nuclear cisternae. Recent papers showed that IP3 and Rayanodine receptors, in addition to releasing calcium channels, coexist on the nuclear envelope membranes (Gerasimenko et al., 1995; Stehno-Bittel, Lückhoff & Clapham, 1995). However, the presence of these conductances, using the patch-clamp technique, could be detected only by adding the agonist in the recording pipette. In any case, even if we assume that all the different channels observed are not part of the nuclear pore itself, and take into account the density of nucleocytoplasmic pathways (Innocenti & Mazzanti, 1993), we have to conclude that in particular conditions nuclear pores are not fixed open pores but they have all the capabilities to turn off. From the electrophysiological experiments performed so far and from these new investigations on the role of the nuclear envelope ionic channel, we can conclude that our knowledge here is still very limited, and no direct proof is available to indicate that nuclear ionic channels and nuclear pores are the same entity.

Nuclear Channel Kinetics

There is a constant component in the current flow during nucleus-attached experiments. Many investigations performed on different cell nuclei have shown that averages of single-channel outward currents present a time-dependent inactivation while the inward ionic flows does not (Bustamante, 1992; Mazzanti et al., 1994). Even in the present work, single-channel outward current averages (Fig. 2*d*) show inactivation. Similar time-dependent kinetics is evident in the ''macroscopic'' current stimulated by ATP. The consistent presence of similar kinetic behavior suggests not only that the conductances we are recording are similar to those reported in previous experiments but also that, under our experimental conditions, the population of channels revealed by the patch-clamp technique on the nuclear envelope is homogeneous. Among different ionic pathways probably present on the external nuclear membrane, the permeability structures that show the highest density on the envelope are the nuclear pores. From freeze-fracture images on liver nuclei preparations, the density is, on the average, 14 pores/μm^2 (Innocenti & Mazzanti, 1993). This large number could account, depending on their availability, either for the small or for the large current usually recorded during on-nucleus patch-clamp experiments without any agonist present in the recording pipette solution. The calculated conductances of the numerous current levels range from 100 to more than 3,000 pS. We suggest that permeability structures associated with the nuclear pores are responsible for nucleocytoplasmic passive ionic flow.

Regarding the gating behavior of the nuclear ionic pathways, the experiments of Fig. 2 present results that could be attributed to different mechanisms. The current levels that appear during on-nucleus patch-clamp recordings could be as low as 1 or be more than 20 pA, without any apparent predominant value. The only common tendency between experiments with a few or many current levels is the time-dependent reduction of the outward current. Within the same experiment, it is common to observe opening and closing of many small channels (Fig. 2*b*) and total closure or reopening of the channel from and to the higher current level (Fig. 2*c*). From these results the immediate impression is that certainly these are not independent single channels. Thus, they could be many cooperative ionic pores or substates of bigger channels. Since we failed to identify preferential current level that could represent the channel main state, we favor the idea of many interdependent pathways. Alternatively, this behavior could represent a different concept of a gating mechanism used for a large pore: a molecular diaphragm with many intermediate positions between the open and the closed conformation (Akey, 1990). From the structural point of view, the diaphragm could be formed by the concentric elements described in the spatial arrangement of the proteins constituting the nuclear pore (Hinshaw, Carragher & Milligan, 1992; Akey & Radermacher, 1993; Panté & Aebi, 1993). Taking into account the density of pore complexes and assuming that in our conditions the pores are only partially open, we can imagine the existence of many different current pathways representing the state of each nuclear pore. This hypothesis could explain the absence of a definite value of unitary single-channel current, discrete transition between current level or abrupt opening and closing of large conductances, and finally the great variability of current amplitude observed in different experiments with the same preparation and identical experimental conditions.

ATP and Ca^{2+} Modulation of Nuclear Ionic Permeabilities

Nuclear functions are modulated by many molecules present in the cytoplasm. At the same time the nucleus

accumulates RNAs that have to be transferred to the cytosol. The nuclear envelope operates as a nucleocytoplasmic barrier, discriminating the macromolecules containing specific consensus signal; ATP hydrolysis is essential for the nucleocytoplasmic transport of molecules over 40 kD m.w. (Forbes, 1992). In a previous paper we showed that, using an *in situ* patch-clamp technique on *Xenopus* oocyte nuclei, the ATP binding is sufficient to increase the passive permeability of nuclear membrane patches isolated by the pipette (Mazzanti et al., 1994). Here we confirm such an action. However, we cannot rule out the possibility that other molecules could be implicated as cofactors in the ATP modulation of the nuclear channels. In a recent paper, Bustamante and colleagues (Bustamante et al., 1995) suggest that ATP is needed to dissociate the transcription factors (TFs), and it is actually the latter that interacts with the nuclear envelope increasing its permeability. Even if a nuclei isolation procedure eliminates 80% of proteins in the first 30 sec of washout with a saline solution (Paine et al., 1983), we cannot exclude the possibility that TFs remain attached to the external side of the membrane or are present in the nucleoplasm.

The previous hypothesis about the need for ATP on both membrane sides (Mazzanti et al., 1994) appears confirmed by the present results. Assuming that small molecules equilibrate within a few minutes in the nucleoplasm after they are added to the external solution, we can imagine that ATP maintains the channel open when its concentration is the same inside and outside the nucleus. These results strengthen the hypothesis that passive nuclear permeabilities use the same structures for protein transport. ATP must be present on both sides of the envelope, and this implies a channel spanning the double membrane of the nucleus. The gap-junction channel model could be a good example: there is a diffusional pore formed by two lined-up membrane protein complexes spanning a double membrane, and both subunits must be in the open state to conduct ionic current. This could justify the need for ATP in and out of the nucleus. However the ''gap-junction hypothesis'' is functionally incompatible with the modulation action of Ca^{2+} ions. According to our experiments, the divalent ion must be present on both sides of the nuclear envelope to reduce the current flow at concentrations compatible with intracellular Ca^{2+} oscillations (Fig. 4). If the diffusion nuclear channels have a structure similar to a connexon, it would be sufficient for the action of Ca^{2+} on one side of the envelope to close the passive pathway. Other authors showed a decrease of nuclear passive diffusion in which Ca^{2+} ions are involved. Depletion of divalent ions from perinuclear storage caused a marked decreased of both passive and ATP-dependent nucleocytoplasmic communication (Greber & Gerace, 1995; Stehno-Bittel et al., 1995). Although in this case, since the explanation furnished concerns the absence of a Ca^{2+} interaction with the intramembrane proteins responsible for the anchorage of the pore, it is possible to hypothesize that Ca^{2+} release from nuclear cisternae can reach a critical concentration around the nuclear ionic pathway to reduce its conductance. An indication of such a mechanism comes from Ca^{2+} diffusion upon stimulation (Al-Mohanna et al., 1994) in which above a 300 nM concentration, the distribution of the divalent ions are higher in the cytosol than in the nucleus. For our data on nuclear permeability, (Fig. 4*b*) the critical amount of Ca^{2+} needed to interfere with passive nucleocytoplasmic diffusion is higher than 1 μM. Both the experiments agreed that, if a limitation exists on nuclear passive diffusion due to an increase of Ca^{2+} ions, it can occur only in an uncontrolled release of the cytoplasmic concentration of the divalent ion. We interpret these results as suggesting that nuclear envelope is able to isolate and to protect the genetic material under abnormal cytoplasmic conditions. During pathological cytoplasmic ion imbalance and/or in response to continuous Ca^{2+} release stimulations, decrease of nucleocytoplasmic passive diffusion could prevent DNA damages.

In conclusion, our experiments demonstrate the modulatory action of ATP and Ca^{2+} on passive nuclear envelope permeabilities. In the previous paragraph, we showed that the idea of a classical ionic channel to explain nuclear permeability is kinetically questionable. Even the modulation of the passive pathway points to a molecular mechanism that is different from the classical idea of ionic channels. If opening and closing of the channel is compatible with the functioning of a molecular diaphragm, the need for a channel modulator on both sides of the envelope makes this hypothesis stronger. It is possible that ATP and calcium ions affect the passive flux by interacting with a symmetrical structure. From morphological studies on the nuclear envelope, it is possible to identify such a structure, represented by the outer and the inner ring of the pore complex (Hinshaw et al., 1992; Akey & Radermacher, 1993; Panté & Aebi, 1993). Assuming as legitimate such a modality of permeation, the next question concerns the mechanism by which it should be possible to coordinate the structural change of this hypothetical diaphragm. A multiprotein complex needs a motor to reduce or to increase a large opening. It is not sufficient for a molecular conformational change to close a 9 nm functional diameter pore (Akey, 1990). We can speculate about the role of heavy chain miosin complexes found in association with the nuclear pore (Berrios, Fisher & Matz, 1991) even if, at the moment, there is no evidence of a contractile mechanism within the nuclear envelope or in association with the nuclear pores.

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