Calcium Dependent Variance of Chloride Current Fluctuations in *Xenopus laevis* Oocytes

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Abstract. Calcium induced chloride currents were studied in Xenopus oocytes using the two-electrode voltage clamp technique. Fluctuations of chloride currents measured under voltage clamp were elicited by acetylcholine. Fluctuations were superimposed on a larger chloride current. These chloride currents are due to an increase of intracellular calcium. However, an injection of calcium into the cytoplasm evoked a current which was smooth. The presence of fluctuations in Xenopus oocytes, when only $InsP_3$ is involved in the calcium release, suggests that fluctuations are primarily due to calcium release from $InsP_3$-sensitive calcium stores. Power spectra density of fluctuations have been analyzed. Variances of parts of records are well correlated with the corresponding average chloride currents, and thus with the calcium concentration in the cytoplasm beneath the membrane. These results characterize the mechanism of oscillatory calcium release from internal sources into the cytoplasm.

Key words: *Xenopus laevis* oocytes — Chloride currents — Acetylcholine — Calcium injection — Power spectra density — Variance

Introduction

One of the native (endogenous) ionic channels of the *Xenopus laevis* oocyte surface membrane is a calcium-dependent chloride channel (Barish 1983). At least two population of chloride channels have been suggested to exist there (Boton et al. 1990): fast and slow chloride channels. Both these types are activated by intracellular calcium.

Oocytes challenged with acetylcholine, which acts through muscarinic receptor, show a large chloride current with two phases (Kusano et al. 1977). The second phase consists of a slow chloride current with superimposed fluctuations (Dascal et al. 1985). When injected, calcium activates only the slow chloride conductance (Miledi and Parker 1984). Injections of as little as 0.5 pmol/l of calcium

produce measurable responses (Oron and Dascal 1992). This can be repeated several times without either desensitization or potentiation. A number of consecutive threshold injections results in delayed small depolarizing current with pronounced fluctuations (Gillo et al. 1987).

Chloride currents are also evoked by intracellular injections of inositol 1,4,5-trisphosphate ($InsP_3$) into oocytes of *Xenopus laevis* (Oron et al. 1985). $InsP_3$ is believed to act through a specific receptor which functions as an intracellular, ligand-gated Ca channel. This glycoprotein of 260 kDa appeared to be structurally similar to (but functionally distinct from) the ryanodine receptor in skeletal muscle (Berridge 1993). Under physiological conditions, $InsP_3$ is a messenger which connects the membrane challenged by exogenous ligand to internal calcium stores, which are specialized parts of the endoplasmic reticulum (ER). The amount of endoplasmic reticulum present in the cytosol of a given cell type does not correlate with the sensitivity of this cell to a challenge with $InsP_3$. In most cell types, it appears that only a fraction of the non-mitochondrial Ca stores are responsive to the action of $InsP_3$. There is some sub-specialization of Ca-metabolizing endoplasmic reticulum (Sitia and Meldolesi 1992).

We have aimed to contribute to the explanation of the control of chloride channels by calcium ions in *Xenopus laevis* oocytes and to characterize properties of the oscillatory calcium release from internal stores. Chloride current fluctuation were analyzed in correlation with the availability of calcium ions in the cytoplasm.

Materials and Methods

Immature oocytes removed from *Xenopus laevis* were maintained in modified Barth's solution as described previously (Kristian et al. 1991). For electrical recording, single oocytes were held in a Perspex chamber constantly perfused with physiological saline (in mmol/l): 116 NaCl, 2 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5 Tris-HCl (pH 7.2). The experiments were performed at room temperature (20—22 °C). Acetylcholine was used at concentrations in a range from 3×10^{-7} to 10^{-5} mol/l.

Electrophysiological recordings were made with a conventional two-microelectrode voltage clamp amplifier at a constant membrane potential. The actual schemes were presented elsewhere (Kristian and Poledna 1990). The cell was impaled with two standard 3 mol/l KCl electrodes. The microelectrode for membrane potential measurement had a resistance in the range of 2–5 MΩ, the tip of the other had resistance in the range of 0.5–1 MΩ. In experiments with injected calcium, the voltage microelectrode was simultaneously used as an injecting electrode and filled with 50 mmol/l $CaCl_2$ and 500 mmol/l KCl. For these experiments defoliculated oocytes were preferred. The injected concentration was estimated from the diameter of a solution drop injected into paraffine oil under the same pressure. Oocyte volume was assumed to be 0.9 mm^3.

Membrane current records were filtered at 5 Hz by a low-pass filter (−3 dB, 4-pole Bessel type), then digitized at a 25 Hz sampling rate. The records of 2048 samples were divided into four sections containing 512 samples each. From each section the DC level

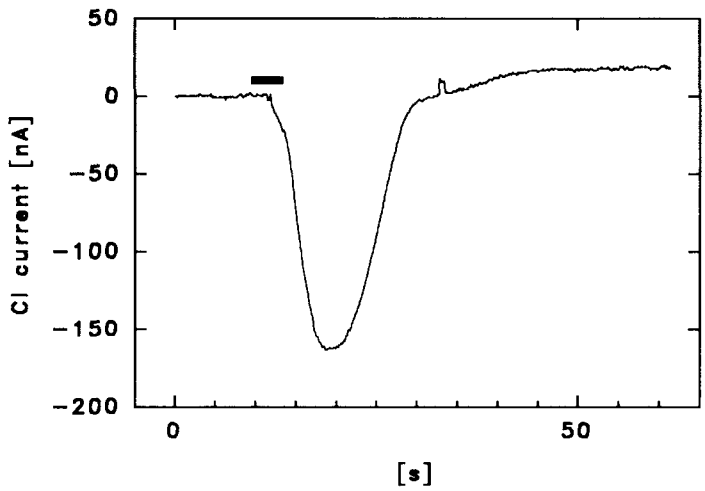

Figure 1. Chloride membrane current recorded under voltage clamp at −60 mV was evoked by injection of calcium ions (2.0×10^{-11} mol) into the cytoplasm. Duration of the injection (marked by the bar) was 3 s.

was subtracted, the spectral densities were calculated and subsequently averaged to obtain the mean power spectrum.

The power spectral density function of Poisson wave with unit events equal to the difference between two exponential functions (Kristian et al. 1991) gave the best fit of the resulting power spectral density calculated from the recorded data. The function of a unit event (unit current) is

$$\imath(t) = h(e^{-t/T1} - e^{-t/T2}) \tag{1}$$

and the power spectral density function is given by

$$S(f) = \frac{2 \cdot vh^2 \cdot (T1 - T2)^2}{1 + w^2(T1^2 + T2^2) + w^4 T1^2 T2^2} \tag{2}$$

where $w = 2\pi f$ and v is the average frequency of the event occurrence.

The values of $T1$, $T2$, and $S(0)$ were estimated by a direct fit of the resulting power spectra. The spectra were used to calculate the variance σ^2 given by

$$\sigma^2 = \int S(f)\mathrm{d}f \tag{3}$$

Results

An increase of intracellular calcium concentration upon a calcium injection evokes the Cl^- current in voltage clamped oocytes at −60 mV. Amplitudes of currents are dependent on the amount of injected calcium as has already been described by Miledi and Parker (1984). For short pressure pulses, the chloride current peaks behind the end of pulse. There is a large variability of responses among oocytes. This response is also dependent on the depth of penetration of the injecting pipet, which affects the initial change rate of the current. This current is typically smooth (Fig. 1). Only after either repeated injections or an injection of a large amount of calcium, current fluctuations appear (Fig. 2). Probably, this is a result of overloading of calcium stores and a spontaneous release takes place. This may indicate presence of calcium stores with the ryanodine receptor/calcium channel.

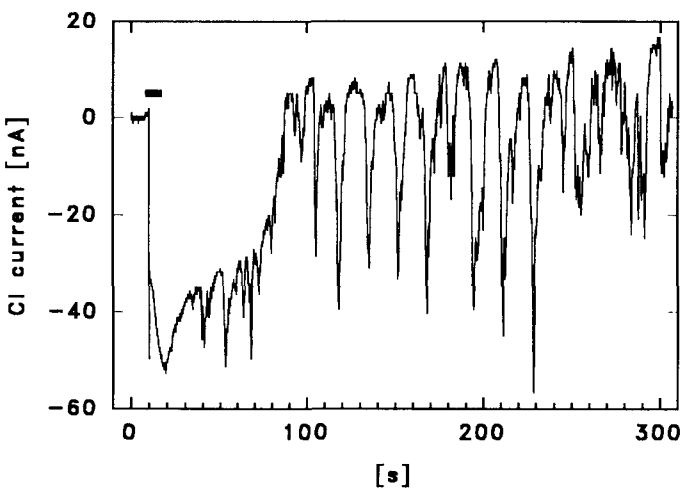

Figure 2. Chloride membrane current recorded under voltage clamp at −60 mV was measured after overloading the oocyte with repeated injections of calcium ions. At these conditions, a small amount of injected calcium (marked by the bar) was able to initiate transient spikes.

Applications of acetylcholine (ACh) to an oocyte clamped at −60 mV evokes a chloride current usually with two phases (Kusano et al. 1977). The first one is transient and the second one is slow with superimposed fluctuations (Fig. 3). A ligand-elicited increase of calcium concentration mediated by $InsP_3$ increases the chloride current which shows superimposed fluctuations because of fluctuations of calcium release (Osipchuk et al. 1990). This is possible in the presence of $InsP_3$.

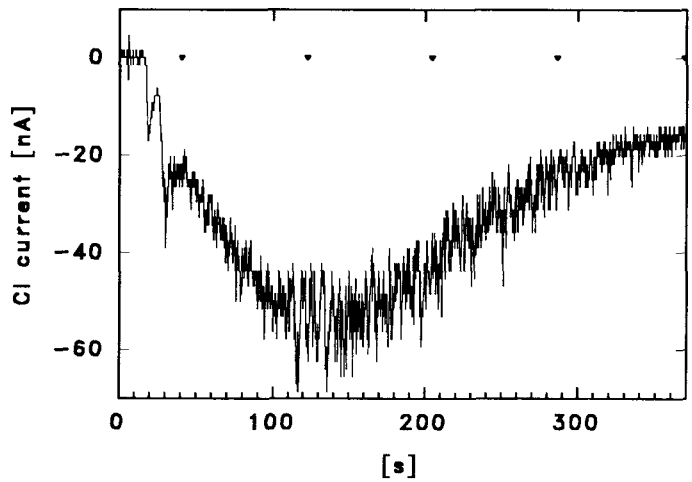

Figure 3. Chloride membrane current recorded under voltage clamp at −60 mV in an oocyte challenged by ACh at concentration 10^{-5} mol/l Triangles mark parts of the record which were used for the computation of the power spectra density

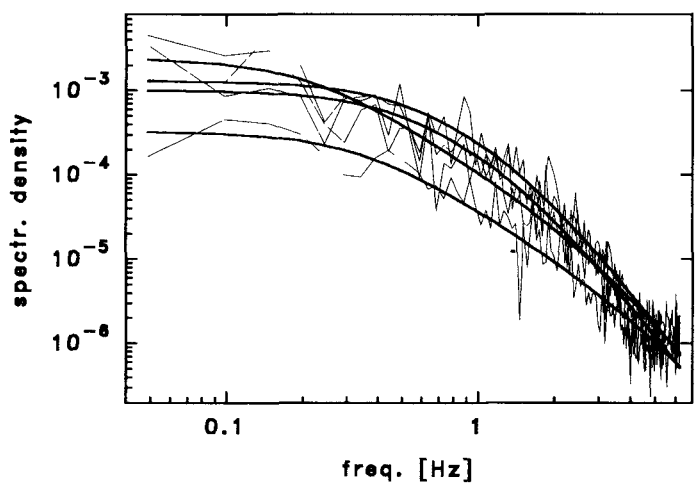

Figure 4. The power spectra density in [pA^2s] units from record in Fig 3 The spectra were averaged from four intervals Each interval contained 512 samples taken at frequency 25 Hz Each spectrum corresponds to one interval marked in Fig 3 Smooth curves represent approximations of spectra by equation (2)

Fluctuations can be characterized by both the power spectra density function and the variance of the chloride current record Power spectra density functions from

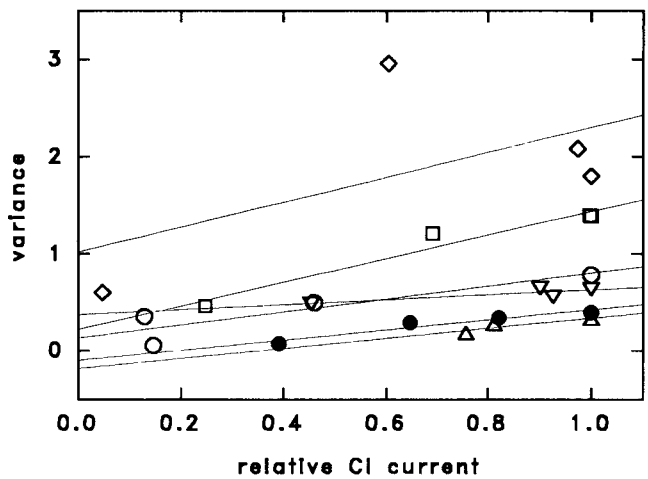

Figure 5. Normalized variances of fluctuating current records plotted against an average current at corresponding intervals. Data from one record are marked with the same symbol and a linear regression line is added. The filled symbols correspond to the record in Fig. 3.

different parts of the record are clearly distinct (Fig. 4). These differences can be expressed by the variance of the chloride current record. This variance was determined according to relation (3) by numerically integrating the power spectral density.

The variance of these fluctuations is related to the average level of the chloride current which reflects the level of cytoplasmic calcium concentration in the space beneath the surface membrane (Fig. 5). Parker and Ivora (1992) showed a linear relation between the amount of calcium injected and the resulting chloride current. These fluctuations correspond to repeated transient release of calcium from the ER stores. Such a release is possible in the presence of $InsP_3$. Higher concentrations of calcium increase frequency of fluctuations.

Discussion

From the experiments with injecting of calcium ions into the cytoplasm we can conclude that increased calcium concentration activates a chloride current which is smooth. Its amplitude is dependent on the cytoplasmic calcium concentration (Parker and Ivora 1992). A higher calcium concentration provides a higher chloride current. When surface membrane receptors are activated by extracellular ligand binding, $InsP_3$ is produced with a subsequent release of calcium from intracellular stores into the cytoplasm (Berridge 1993). The effect on the activation of chlo-

ride currents would be expected to be the same. However, permeability of the $InsP_3$-activated calcium release channel is dependent on calcium concentration in the cytoplasm (Bezprozvanny et al. 1991). There are two types of calcium compartments in the endoplasmic reticulum. One of them is $InsP_3$-sensitive, where the ligand gated calcium release channel is activated by $InsP_3$. The other one is $InsP_3$-insensitive, where the ligand gated calcium release channel is activated through ryanodine receptor by increased calcium concentration. Therefore, calcium concentration can locally oscillate due to either $InsP_3$-sensitive calcium release (Poledna 1993) or due to calcium-induced calcium release (Poledna 1991). However, $InsP_3$-insensitive stores are scarce in Xenopus oocytes (Berridge 1991). This effect of calcium ion is locally confined because the free diffusion path for calcium is very short (Allbritton et al. 1992). $InsP_3$ is responsible for spreading of activation due to its larger diffusion coefficient. On the other hand, calcium with a very short diffusion path acts locally.

The presence of chloride current fluctuations in Xenopus oocytes when only $InsP_3$ is involved in the calcium release suggests that fluctuations are primarily due to the calcium release from $InsP_3$-sensitive calcium stores. However, the Ca release from $InsP_3$-sensitive and $InsP_3$-insensitive stores can mutually interfere. A high calcium concentration beneath the surface membrane increases basal level of chloride channel activation (Osipchuk et al. 1990). On the other hand, it recruits a larger amount of $InsP_3$-sensitive calcium stores to oscillate and locally increases frequency of oscillations (Poledna 1993). Because frequency of oscillations and its increase are local properties, the overall effect is an increase of the signal variance. The variance increases due to an increase of both the amplitudes and the frequencies of oscillations. These results support the idea that the frequency of intracellular calcium oscillations is also determined by available intracellular calcium (Poledna 1991, 1993).

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