Cytoplasmic Calcium Fluctuations in Calcium Overloaded *Xenopus laevis* Oocytes

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Abstract. Cytoplasmic calcium fluctuations were studied in calcium overloaded *Xenopus* oocytes. Calcium sensitive chloride currents were recorded using the two-electrode voltage clamp technique. Fluctuations of chloride currents measured under the voltage clamp were elicited by injection of calcium into the cytoplasm. Contrary to infrequent injections of small calcium amounts which evoke smooth transient responses, the fluctuating chloride currents are due to overloading of intracellular calcium stores which then release calcium repeatedly. Chloride current fluctuations in calcium overloaded oocytes can be reversibly suppressed by caffeine. This effect is concentration dependent, and the amplitude decrease of fluctuations is already apparent at 2 mmol/l caffeine. Power spectra density of fluctuations have been analyzed; they exhibited this pronounced effect of caffeine. Other effective inhibitors were tetracaine and heparin. The results of the present work suggest that at least a part of the endoplasmic reticulum in *Xenopus* oocytes is a calcium releasing calcium store which can be activated by calcium at the resting inositol trisphosphate concentration.

Key words: *Xenopus laevis* oocytes — Cytoplasmic calcium fluctuations — Chloride currents — Caffeine — Calcium injection — Power spectra density

Introduction

In a cell, many signalling processes are mediated by repeated transient changes of the cytoplasmic calcium concentration. Therefore, mechanisms of generating temporary elevations of intracellular calcium concentration are subject of permanent attention. The essential components of the cytoplasmic calcium concentration changes are calcium flows through the surface membrane and the membranes of

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specialized parts of the endoplasmic reticulum. These include both passive fluxes through calcium channels and active transport by calcium pumps. To analyze this complicated system, it is useful to study it in a special situation. Such a useful preparations can be a calcium overloaded oocyte exhibiting sustained calcium oscillations.

The calcium-dependent chloride channel is a native (endogenous) ionic channel of the *Xenopus laevis* oocyte surface membrane (Barish 1983). Chloride currents can be used to monitor changes of the sub-plasmalemma calcium concentration (Osipchuk et al. 1990). When injected, calcium activates the slow chloride conductance only (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 result in a delayed small depolarizing current with pronounced fluctuations (Gillo et al. 1987).

Under physiological conditions, inositol 1,4,5 trisphosphate ($InsP_3$) is a messenger which transfers information from the membrane challenged by exogenous ligands to internal calcium stores, which are specialized parts of the endoplasmic reticulum (ER). $InsP_3$ 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). In contrast to the $InsP_3$-sensitive store, the calcium sensitive store is affected by both ryanodine and caffeine (Fewtrell 1993).

Parys et al. (1992) studied the distribution of calcium release channels in *Xenopus laevis* oocytes. Immunofluorescence experiments indicated the presence of the $InsP_3$ receptors in the cortical layer and the perinuclear endoplasmic reticulum of the oocyte. However, immunological and biochemical experiments did not reveal the presence of the ryanodine receptor.

When overloaded with calcium, *Xenopus* oocytes show spontaneous oscillations of cytoplasmic calcium (Poledna et al. 1993). $InsP_3$ concentration should be very low under resting conditions due to rapid degradation. This experimental situation is advantageous for analyzing calcium transport, because calcium oscillations are a sensitive indicator for external intervention into this system. The aim of the present experiments was to study mechanisms of calcium release from internal stores of *Xenopus* oocytes and to determine which of the proposed mechanisms of calcium transients is effective under particular physiological conditions (Poledna 1991, 1993).

Materials and Methods

Immature oocytes removed from *Xenopus laevis* were maintained in modified Barth solution as described previously (Kristian et al. 1991). The oocyte diameters ranged from

1.2 to 1.3 mm. For electrical recordings, 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).

Electrophysiological recordings were made with a conventional two-microelectrode voltage clamp amplifier at constant membrane potential. The voltage microelectrode was simultaneously used as a pressure injecting electrode, and it was filled with 50 mmol/l $CaCl_2$ and 500 mmol/l KCl. The microelectrode for membrane potential measurement had a resistance in the range of 2–5 MΩ, the tip of the other one, filled with 3 mol/l KCl, had resistance in the range of 0.5–1 MΩ. Pressure pulses of 0.2 MPa were applied for 5–30 s. The amount of injected calcium was estimated before oocyte impalement by measuring a drop size at the microelectrode tip immersed in paraffine oil. The volume of the injected solution did not exceed 0.15% of the oocyte volume.

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. Records of 2048 samples were divided into four sections of 512 samples each. From each section the DC level was subtracted, the spectral densities were calculated and subsequently averaged to obtain the mean power spectrum.

Results

Oocytes were voltage clamped at −60 mV and injected with calcium. An increase of intracellular calcium concentration upon a calcium injection evokes the transient Cl^- current. Amplitudes of currents are dependent on the amount of calcium injected as already described by Miledi and Parker (1984). We have shown (Poledna et al. 1993) that a calcium injection exceeding 50 pmol elicits a large transient negative chloride current that declines to the resting level upon the end of injection. This current is followed by frequent negative chloride current fluctuations, lasting for tens of minutes. This is a result of calcium stores overloading, and a repeated spontaneous calcium release takes place. This may indicate the presence of calcium stores with the ryanodine receptor/calcium channel, since there is only resting $InsP_3$ concentration. To test this hypothesis, caffeine was used. Caffeine is known to increase the sensitivity of the ryanodine receptor/calcium channel for calcium (Xu et al. 1989).

Caffeine in the external solution reduced the amplitudes of fluctuations (Fig. 1*a*). This effect was reversible, and its characteristics did not change upon repeated application of caffeine. Experiments were performed with 14 oocytes from 5 donors. Ten mmol/l caffeine suppressed chloride current oscillations in less than 30 s. Power density spectra of the chloride current fluctuations (Fig. 1*b*) showed substantial decrease upon caffeine application. For 10 mmol/l concentration, this decline was more than one order in the frequency range up to 1 Hz.

The effect of caffeine was very specific. Low concentrations of caffeine (such as 2 mmol/l) also had reducing effect (Fig. 2). This caffeine concentration is close to

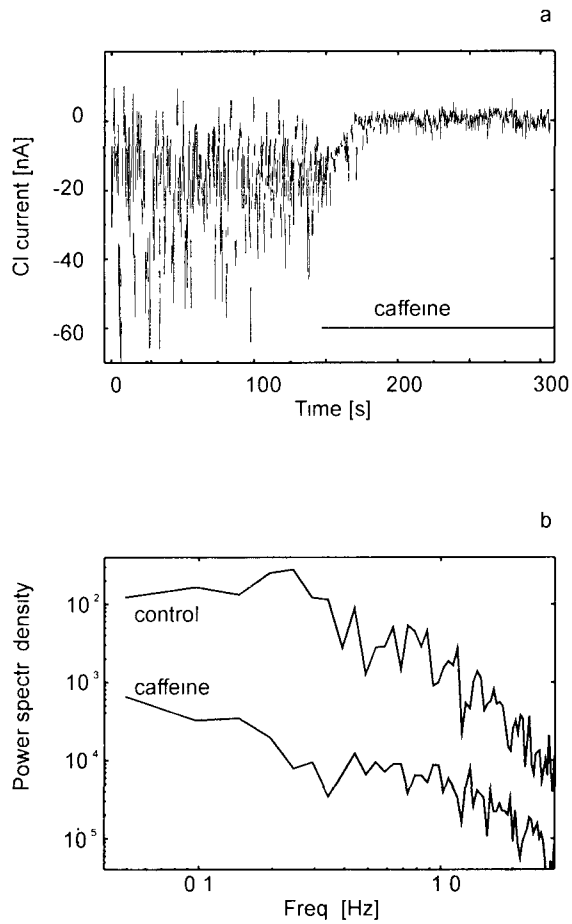

Figure 1. Chloride current fluctuations in calcium overloaded *Xenopus laevis* oocyte. Fluctuations are markedly decreased when 10 mmol/l caffeine is applied extracellularly (*a*). The effect of caffeine is clearly apparent from the power spectra (*b*) of the record shown in (*a*). The spectra (in pA^2s units) correspond to the control record and to that in the presence of caffeine.

its effective level in a skeletal muscle cell. A possible mechanism for the suppression of calcium fluctuations could be the opening of calcium release channels and the resulting disbalance between calcium release and uptake necessary for sustained oscillations. However, caffeine induced calcium release from intracellular stores in *Xenopus* oocytes has not been observed under physiological conditions.

Tetracaine is recognized as very effective in blocking calcium release from sarcoplasmic reticulum (Caputo 1976; Gyorke and Palade 1992) where ryanodin re-

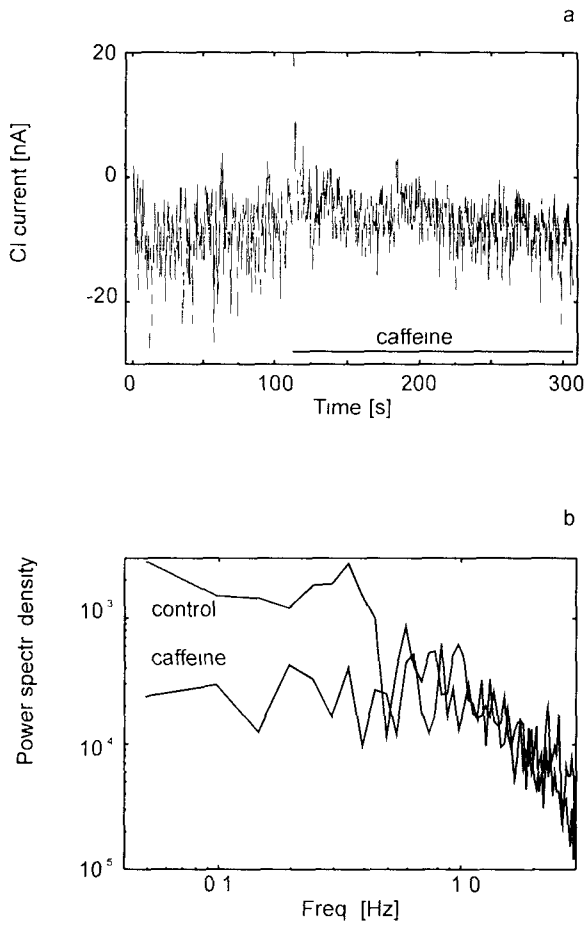

Figure 2. The effect of 2 mmol/l caffeine on the chloride current fluctuations in calcium overloaded *Xenopus laevis* oocyte. Caffeine was applied extracellularly (*a*). Its effect is clearly apparent from the power spectra (*b*) of the record shown in (*a*). The spectra (in pA^2s units) correspond to the control record and to that on the presence of caffeine.

ceptor/calcium release channels are present. Also, tetracaine abolishes chloride current fluctuations in calcium overloaded oocytes. Application of tetracaine into oocyte external solution is shown in Fig. 3.

To asses firmly the type of the calcium release channel participating in oscillations in calcium overloaded oocytes, we also used heparin, which competes with $InsP_3$ in binding to $InsP_3$ receptors. Heparin injected into the cytoplasm decreased oscillations substantially (Fig. 4). This may indicate that in calcium overloaded oocytes, $InsP_3$-receptor/calcium channel is involved in cytoplasmic calcium

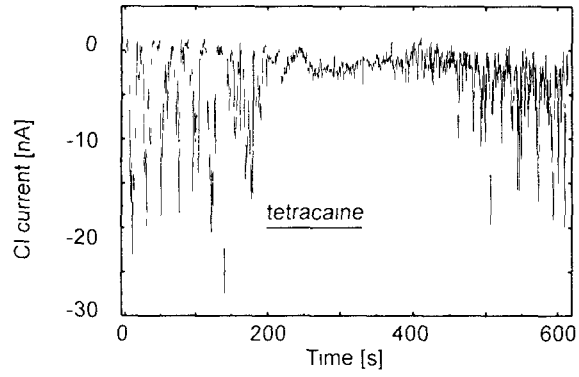

Figure 3. The effect of 0.3 mmol/l tetracaine on the chloride current fluctuations in calcium overloaded *Xenopus laevis* oocyte. Tetracaine application is marked by the line.

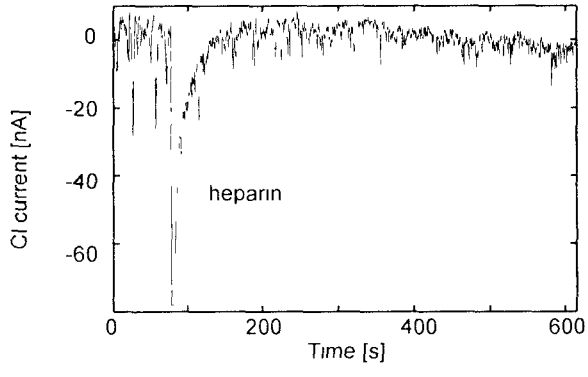

Figure 4. The effect of heparin on the chloride current fluctuations in calcium overloaded *Xenopus laevis* oocyte. Heparin injection was accompanied by the current artifact which rapidly returned to the baseline.

fluctuations and that cytoplasmic calcium can open the channel even at resting intracellular concentrations of $InsP_3$.

Discussion

We studied repeated calcium transients in calcium overloaded *Xenopus* oocytes by measuring chloride currents under voltage clamp conditions. This was justified by the results of Parker and Ivorra (1992) who showed that chloride current amplitudes

were linearly dependent on the amount of both calcium and $InsP_3$ released by photolysis flashes.

When a larger amount of calcium is injected into the cytoplasm of an oocyte, it is being sequestered by endoplasmic reticulum. Increased cytoplasmic calcium concentrations can activate the calcium release channel. This activation can be promoted by increased intraluminal calcium concentration in the endoplasmic reticulum either via increased calcium gradient or via another mechanism. In calcium overloaded oocytes, the mutual relation between calcium release and sequestration maintains repeated calcium transients. The question arises which type of the calcium releasing channels participate in this mechanism. Ryanodine and $InsP_3$ receptors may be considered. Caffeine and heparin have been widely used to study these channels. A number of our results presented herein, including caffeine and tetracaine effects suggest a possible role for ryanodine receptor/calcium releasing channel. This behavior agrees with the hypothesis of $InsP_3$-insensitive calcium stores with calcium-induced calcium release proposed by Berridge (1991b). Berridge (1991a) studied $InsP_3$-induced membrane potential oscillations in *Xenopus* oocytes. Caffeine had no effect on the early $InsP_3$-induced spike but it suppressed the subsequent oscillations.

The ryanodine receptor/calcium release channel has characteristics that fit this type of activity. On the other hand, Parys et al. (1992) have been unable to find any ryanodine receptors in *Xenopus* oocytes by sensitive antibody tests.

Parker and Ivorra (1991) reported that caffeine did not appreciably reduce currents evoked by injection of calcium into *Xenopus* oocytes, whereas measurements using the Ca indicator Rhod-2 showed that it instead inhibited the liberation of calcium by $InsP_3$. Even at high (10 mmol/l) concentrations, caffeine did not itself elicit any clear calcium activated current.

The suppression of calcium oscillation by heparin favors the substantial role of $InsP_3$-receptor/calcium channel in maintaining repeated cytoplasmic calcium fluctuations. However, under the present experimental conditions, $InsP_3$ is at its low resting concentration. Linear dependence of chloride currents on photoreleased calcium (Parker and Ivorra 1992) suggests that this increased intraluminal calcium may be able to decrease the threshold of $InsP_3$-receptor to the resting $InsP_3$ level.

Missiaen et al. (1992) showed that in permeabilized hepatocytes caffeine antagonized the effect of low $InsP_3$ concentrations and abolished calcium spikes, without itself releasing calcium. Their results suggested that luminal calcium might sensitize $InsP_3$-receptor/calcium channel leading to spontaneous calcium release. Similar conclusions can be applied to calcium overloaded *Xenopus* oocytes. However, the possibility that calcium overloaded $InsP_3$-sensitive compartments can be emptied by the resting concentration of $InsP_3$ is challenged by the results of Combettes et al. (1993). Various manipulations in which the load of the calcium pools was varied by a factor of two did not significantly affect the apparent relative efficiency

of $InsP_3$ in releasing calcium Moreover Bezprozvanny and Ehrlich (1994) did not observe any potentiation of $InsP_3$ gated channel activity by intraluminal calcium

Oscillations of cytoplasmic calcium are the result of an interplay between calcium uptake to and calcium release from the endoplasmic reticulum This takes place only within a specific range of release and sequestration characteristics (Poledna 1993) Therefore calcium overloading increases intraluminal calcium concentration inside the endoplasmatic reticulum as well as in the cytoplasm These effects together with the potential saturation of the calcium uptake create favorable conditions for sustained calcium oscillations Any intervention that substantially changes only some participating components can abolish oscillations

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