

Caffeine Suppresses Chloride Current Fluctuations in Calcium-Overloaded *Xenopus laevis* Oocytes

J. POLEDNA, V. PACKOVÁ

Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic

Received December 13, 1993

Accepted March 22, 1994

Summary

Calcium-induced chloride currents were studied in *Xenopus* oocytes using the two-electrode voltage clamp technique. Fluctuations of chloride currents measured under a voltage clamp were elicited by injection of calcium into the cytoplasm. Contrary to infrequent injections of small amounts of calcium which evoked smooth transient responses, these 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 an amplitude decrease of fluctuations is already apparent at 2 mmol/l caffeine. The analysis of power spectra density of fluctuations have displayed the pronounced effect of caffeine. These results suggest that at least a part of the endoplasmic reticulum in *Xenopus* oocytes is a calcium-releasable calcium store which can be activated at the resting inositol trisphosphate concentration.

Key words

Xenopus laevis oocytes – Chloride currents – Caffeine – Calcium injection – Power spectra density

A 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 only the slow chloride conductance (Miledi and Parker 1984). Injections of as little as 0.5 pmol 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 a delayed small depolarizing current with pronounced fluctuations (Gillo *et al.* 1987).

Under physiological conditions, inositol 1,4,5-trisphosphate (InsP₃) is a messenger, which transfers information from the membrane challenged by exogenous ligands to internal calcium stores that are specialized parts of the endoplasmic reticulum (ER).

InsP₃ is believed to act through a specific receptor which functions as an intracellular, ligand-gated calcium 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 InsP₃-sensitive store, the calcium-sensitive store is affected by 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 InsP₃ receptors in the cortical layer and in the perinuclear endoplasmic reticulum of the oocyte. However, immunological and biochemical experiments did not reveal the presence of the ryanodine-receptor.

Xenopus oocytes, which are overloaded with calcium, accomplish spontaneous oscillations of cytoplasmic calcium (Poledna *et al.* 1993). InsP₃ concentration should be very low under resting

conditions due to its rapid degradation. This implies that besides InsP_3 -sensitive stores, *Xenopus* oocytes also comprise calcium stores emptied by another ligand, which may be calcium itself. The aim of the present experiments was to show that some internal calcium stores of *Xenopus* oocytes possess the calcium-induced calcium release mechanism and to determine which of the proposed mechanisms of calcium transients is effective under particular physiological conditions (Poledna 1991, 1993).

Immature oocytes removed from *Xenopus laevis* were maintained in a modified Barth's solution as described previously (Kristian *et al.* 1991). The oocyte diameter was in the range 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 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 M Ω ; the tip of the other microelectrode, filled with 3 mol/l KCl, had a resistance in the range of 0.5–1 M Ω . Pressure pulses of 0.2 MPa were applied for 5–30 s. The amount of injected calcium was estimated before oocyte impalement by measuring the size of a drop at the microelectrode tip immersed in paraffine oil. 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. The records of 2048 samples were divided into four sections containing 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.

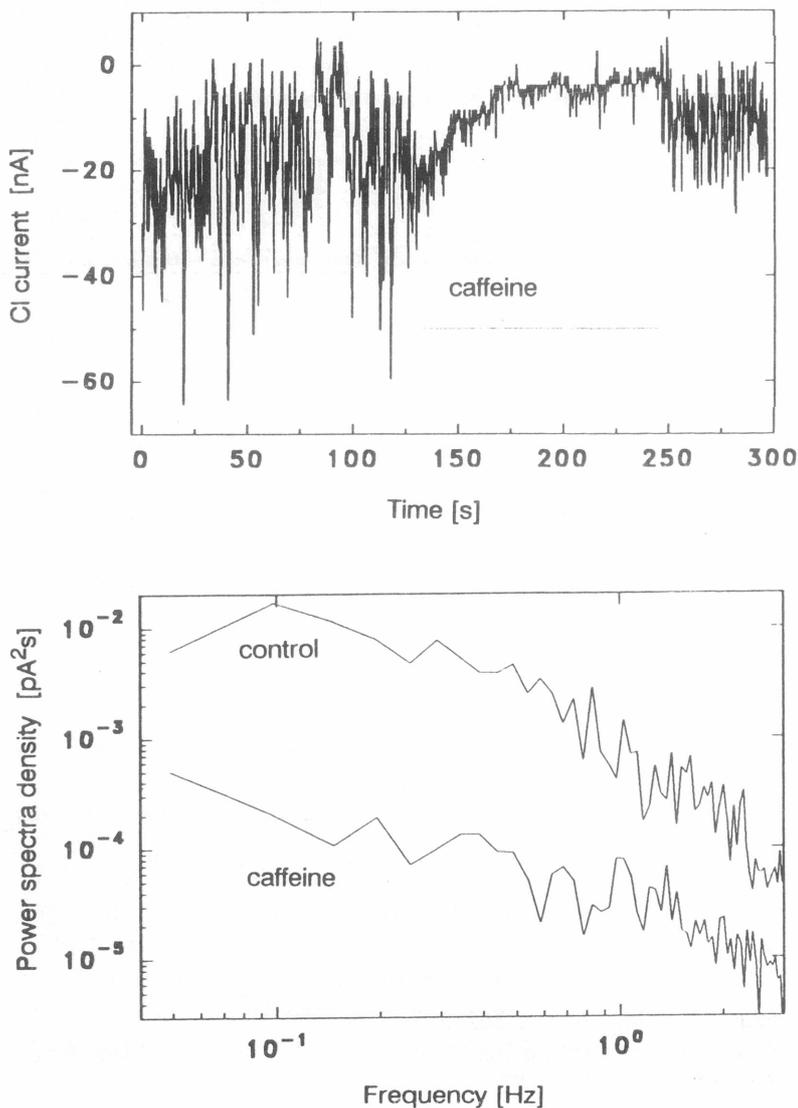


Fig. 1

Chloride current fluctuations in the calcium-overloaded *Xenopus laevis* oocyte. Fluctuations are markedly decreased when 10 mmol/l caffeine is applied extracellularly (upper panel). After the caffeine wash out, fluctuations are restored. The effect of caffeine is clearly apparent from the power spectra (lower panel) of the record shown in (upper panel). The spectra (in pA^2s units) correspond to the control record and the record affected by caffeine, respectively.

Experiments were performed on 13 oocytes from 4 donors. Oocytes were voltage-clamped at -60 mV and injected with calcium. An increase of intracellular calcium concentration after a calcium injection evokes a transient Cl^- current. Amplitudes of currents are dependent on the amount of injected calcium as has already been described by Miledi and Parker (1984). We have shown (Poledna *et al.* 1993) that the injection of calcium, which exceeds 50 pmol, elicits a large transient negative chloride current that declines to the resting level when the injection is discontinued. This current was followed by frequent negative chloride current fluctuations, which lasted for tens of minutes. This is a result of overloading of calcium stores and repeated spontaneous calcium release. This may indicate the presence of calcium stores with the ryanodine receptor/calcium channel, since there is the resting InsP_3 concentration only. To test this hypothesis, caffeine was used. Caffeine increases the sensitivity of the ryanodine receptor/calcium channel for calcium (Xu *et al.* 1989).

Caffeine in the external solution reduced the amplitude of fluctuations (Fig. 1a). This effect was reversible and its characteristics did not change when the application of caffeine was repeated. The concentration of 10 mmol/l caffeine suppressed chloride current oscillations in less than 30 s. Power density spectra of the chloride current fluctuations (Fig. 1b) showed a substantial decrease when caffeine was applied. For 10 mmol/l concentration, this decline was greater than by one order of magnitude in the frequency range up to 1 Hz.

This behaviour 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. Therefore, caffeine discriminates between the InsP_3 -sensitive store, which is responsible for the initial spike, and the InsP_3 -insensitive pool, which is responsible for the repeated calcium release according to the two-pool model. The first is not affected by caffeine, but the second is discharged by caffeine through the ryanodine

receptor/calcium release channel similarly as in the skeletal muscle, so that oscillations are suppressed. The effect of caffeine is very specific. Low concentrations of caffeine such as 2 mmol/l also had a reducing effect. This caffeine concentration is close to its effectiveness in a skeletal muscle cell. The ryanodine receptor/calcium release channel has characteristics that fit this type of activity. On the other hand, a possibility that calcium-overloaded InsP_3 -sensitive compartments can be emptied by the resting concentration of InsP_3 is challenged by the recent results of Combettes *et al.* (1993). Various manipulations in which the load of the calcium pools changed by a factor of two did not significantly affect the apparent relative efficiency of InsP_3 in releasing Ca^{2+} . Therefore, the hypothesis of calcium-induced calcium release is a probable alternative for explaining the present experimental results.

Parker and Ivora (1991) reported that caffeine did not appreciably reduce the currents evoked by injection of calcium into *Xenopus* oocytes, whereas measurements using the calcium indicator Rhod-2 showed that it instead inhibited the liberation of calcium by InsP_3 . Even at high concentrations (10 mmol/l), caffeine did not itself elicit any clear calcium-activated current. However, under present experimental conditions InsP_3 is at its low resting concentration. The linear dependence of chloride currents on photoreleased calcium (Parker and Ivora 1992) excludes the possibility that this calcium is able to decrease the threshold of InsP_3 -receptor to the resting InsP_3 level.

Over a range of caffeine concentrations (1–20 mmol/l) and at different InsP_3 concentrations (3–120 nmol/l) caffeine did not affect the binding of InsP_3 to cerebellar membrane receptors (Toescu *et al.* 1992). These authors have found that caffeine acted by inhibiting the agonist-evoked production of InsP_3 .

It can be concluded that at least a part of the endoplasmic reticulum in *Xenopus* oocytes comprises a calcium-releasable calcium store.

Acknowledgement

This work was partially supported by the Slovak Grant Agency for Science (grant No. 2/68/93).

References

- BARISH M.E.: A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol. Lond.* **342**: 309–325, 1983.
- BERRIDGE M.J.: Caffeine inhibits inositol-trisphosphate-induced membrane potential oscillations in *Xenopus* oocytes. *Proc. R. Soc. Lond. B* **244**: 57–62, 1991a.
- BERRIDGE M.J.: Cytoplasmic calcium oscillations: a two pool model. *Cell Calcium* **12**: 63–72, 1991b.
- BERRIDGE M.J.: Inositol trisphosphate and calcium signalling. *Nature* **361**: 315–325, 1993.
- COMBETTES L., CLARET M., CHAMPEIL P.: Calcium control on InsP_3 -induced discharge of calcium from permeabilised hepatocyte pools. *Cell Calcium* **14**: 279–292, 1993.

- FEWTRELL C.: Ca^{2+} oscillations in non-excitabile cells. *Annu. Rev. Physiol.* 55: 427–454, 1993.
- GILLO B., LASS Y., NADLER E., ORON Y.: The involvement of inositol 1,4,5-trisphosphate and calcium in the two-component response to acetylcholine in *Xenopus* oocytes. *J. Physiol. Lond.* 392: 349–361, 1987.
- KRISTIAN T., KOLAJ M., POLEDNA J.: Analysis of calcium activated chloride current fluctuations in *Xenopus laevis* oocytes. *Gen. Physiol. Biophys.* 10: 265–280, 1991.
- MILEDI R., PARKER I.: Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol. Lond.* 357: 173–183, 1984.
- ORON Y., DASCAL N.: Regulation of intracellular calcium activity in *Xenopus* oocytes. In: *Methods in Enzymology* vol. 207, B. RUDY, L.E. IVERSON (eds), Academic Press, New York 1992, pp. 381–390.
- OSIPCHUK Y.V., WAKUI M., YULE D.I., GALLACHER D.V., PETERSEN O.H.: Cytoplasmic Ca oscillations evoked by receptor stimulation, G-protein activation, internal application of inositol trisphosphate or Ca: simultaneous microfluorimetry and Ca dependent Cl current recording in single pancreatic acinar cells. *EMBO J.* 9: 697–704, 1990.
- PARKER I., IVORA I.: Caffeine inhibits inositol trisphosphate-mediated liberation of intracellular calcium in *Xenopus* oocytes. *J. Physiol. Lond.* 433: 229–240, 1991.
- PARKER I., IVORA I.: Characteristics of membrane currents evoked by photoreleased inositol trisphosphate in *Xenopus* oocytes. *Am. J. Physiol.* 263: C154–C165, 1992.
- PARYS J.B., SERNETT S.W., DELISLE S., SNYDER P., WELSH M.J., CAMPBELL K.P.: Isolation, characterization, and localization of the inositol 1,4,5-trisphosphate receptor protein in *Xenopus laevis* oocytes. *J. Biol. Chem.* 267: 18776–18782, 1992.
- POLEDNA J.: Mechanism of intracellular calcium transients. *Gen. Physiol. Biophys.* 10: 475–484, 1991.
- POLEDNA J.: Model of intracellular calcium oscillations activated by inositol trisphosphate. *Gen. Physiol. Biophys.* 12: 381–389, 1993.
- POLEDNA J., MOJŽÍŠOVÁ A., PACKOVÁ V.: Calcium dependent variance of chloride current fluctuations in *Xenopus laevis* oocytes. *Gen. Physiol. Biophys.* 12: 445–452, 1993.
- TOESCU E.C., ONEILL S.C., PETERSEN O.H., EISNER D.A.: Caffeine inhibits the agonist-evoked cytosolic Ca^{2+} signal in mouse pancreatic acinar cells by blocking inositol trisphosphate production. *J. Biol. Chem.* 267: 23467–23470, 1992.
- XU L., JONES R.V., MEISSNER G.: Activation of the skeletal muscle Ca release channel by the triazine dyes Cibacron Blue F3A-G and Reactive Red 120. *Arch. Biochem. Biophys.* 274: 609–616, 1989.

Reprint Requests

Dr. J. Poledna, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárská 5, 833 34 Bratislava, Slovak Republic.