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Hemispheric asymmetry of rapid chloride responses to inositol trisphosphate and calcium in *Xenopus* oocytes

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Shallow injection of inositol 1,4,5-trisphosphate (IP_3) near the animal pole of the *Xenopus* oocyte resulted in a large depolarizing current that decayed rapidly. A similar injection near the vegetal pole produced a much smaller response characterized by a significantly slower rate of decay. Injection of $CaCl_2$ near the animal pole of the oocyte resulted in a large depolarizing current characterized by rapid rise and decay times. Injection near the vegetal pole of the cell produced responses that exhibited similar amplitudes but much longer rise and decay times. The protein kinase C (PK-C) activator, β-phorbol 12-myristate 13-acetate (PMA), significantly enhanced the rapid responses to IP_3 injections at either hemisphere but did not affect the amplitudes of the responses to $CaCl_2$. The PK-C inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) had no effect on the responses to $CaCl_2$. These results imply an asymmetric distribution of calcium stores and chloride channels between the two hemispheres of the oocyte.

Inositol trisphosphate; Ca^{2+}; Oocyte; Cl^- channel; (*Xenopus*)

1. INTRODUCTION

Since the discovery of the acetylcholine (ACh)-evoked chloride (Cl) responses in *Xenopus* oocytes [1,2], the oocyte system has been extensively used to investigate the mechanism of signal transduction of responses that involve intracellular calcium mobilization. Studies of intermediate steps in signal transduction were substantially facilitated by the relative ease of intracellular injection of putative messenger substances (IP_3, $CaCl_2$). We have previously reported [3] that the pattern of responses to IP_3 injections depends on the depth of injection. Our results also implied that two distinct Cl channel populations may be involved in the two-component Cl response to ACh and IP_3.

We have recently reported that intrinsic responses to ACh in native oocytes and acquired responses to ACh or TRH in cells injected with rat brain or pituitary cell RNA, respectively, display different hemispheric sensitivity upon local application of agonists [4]. These findings suggest an asymmetric distribution of receptors and, possibly, other components of the transduction pathway in the oocyte.

In view of the extensive use of intracellular injections as a tool for the elucidation of molecular mechanisms of signal transduction, it was of interest to probe the possible asymmetry of hemispheric distribution of releasable calcium stores and Cl channels in *Xenopus* oocytes. Our results are compatible with an asymmetric distribution of calcium stores and of two kinetically distinct populations of Cl channels.

2. MATERIALS AND METHODS

2.1. *Preparation*

Adult *X. laevis* females were obtained from South African Snake Farm. The animals were fed beef liver twice a week and maintained on a 12/12 h light/dark cycle at 20°C. Ovary fragments were excised from cold-anesthetized animals and defolliculated by 2–4 h treatment with collagenase (2 mg/ml, Sigma type Ia) in OR-2 medium (see below) from which $CaCl_2$

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was omitted. Oocytes were maintained at 20°C in NDE medium (see below).

2.2. *Electrophysiology*

A single oocyte, in a 0.4 ml perfusion chamber, was impaled with two standard glass electrodes (0.5–3 MΩ resistance) filled with 3 M KCl. Responses were assayed under voltage clamp at −60 to −100 mV using a Dagan 8500 intracellular clamp-amplifier. Current changes were recorded with a flat-bed two-pen recorder.

2.3. *Intracellular injections*

Voltage-clamped oocytes were impaled with a third micropipette with a tip manually broken to 2–4 μm diameter and back-filled with the desired solution. Injection was performed by applying 2–5 lb/inch^2 pressure for 0.1–1 s. The size of the drop (usually 0.1–0.2 mm in diameter) was monitored by injection into an oil droplet under a microscope equipped with a reticle. The injected volume did not exceed 0.5% of the volume of the cell.

2.4. *Solutions and chemicals*

The composition of OR-2 solution was (in mM) NaCl (82), Na-Hepes (5), KCl (2), $CaCl_2$ (1.8), $MgCl_2$ (1); pH 7.5. That of NDE medium was (in mM) NaCl (96), KCl (2), $MgCl_2$ (1), $CaCl_2$ (1.8), Na-Hepes (5), Na-pyruvate (2.5); pH 7.5. Stock solutions of β-phorbol 12-myristate 13-acetate (β-PMA) and of 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) were 0.1 and 10 mM, respectively, in dimethyl sulfoxide (DMSO). The final concentration was obtained by diluting stock solutions into the final volume of the medium with rapid stirring. The final concentration of DMSO was 0.1% and was also included in OR-2 solution. This concentration of DMSO had no effect on holding current or on responses. β-PMA, Na-pyruvate, H-7 and collagenase were purchased from Sigma; IP_3 from Amersham. All other chemicals were of analytical grade.

2.5. *Analysis of results*

All experiments were repeated several times in oocytes from different frogs. The number of oocytes assayed for each condition is denoted by n (n_c, control; n_e, oocytes pre-treated with PK-C-active drugs) and the number of different donors by N. Experiments were performed by assaying 4 or more oocytes within each experiment and mean ± SE values were determined. Statistical significance was determined by paired or unpaired Student's t-test.

3. RESULTS

3.1. *Responses to IP_3*

Injection of IP_3 (0.01–0.1 pmol) near the animal pole of the oocyte resulted in a rapid, transient current (see fig.1A), followed by a delayed slow, prolonged current with superimposed current fluctuations. These results were similar to the previously described responses to IP_3 [3,5]. The mean amplitude of the rapid component was 764 ± 108 nA and it was characterized by a short rise time (time-to-peak 11 ± 1 s, n = 38, N = 7) and rapid decay ($t_{1/2}$ = 26 ± 3 s, n = 24, N = 6).

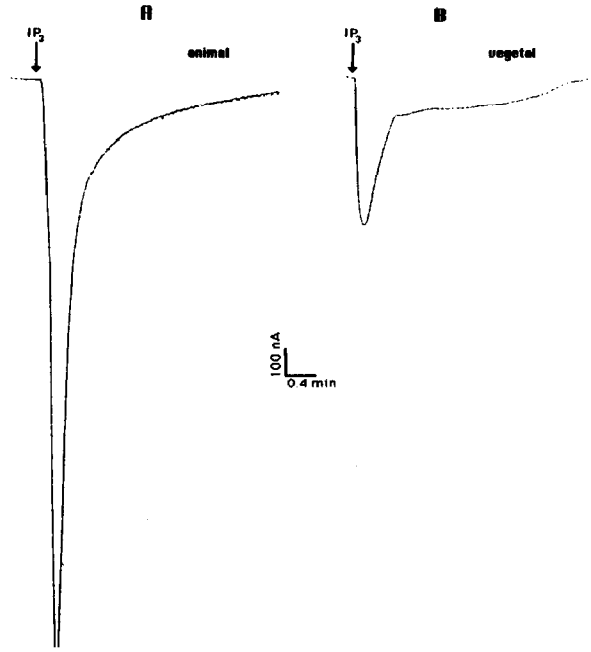

Fig.1. Responses to IP_3 injection. Typical responses to the injection of approx. 100 fmol IP_3 near the animal (A) or vegetal (B) pole in two different oocytes of the same donor voltage-clamped at −70 mV. Depolarizing current is shown as a downward deflection of the trace.

Injection of similar amounts of IP_3 near the vegetal pole of the cell produced responses of significantly smaller amplitudes (139 ± 19 nA, n = 47, N = 7) and of different kinetic pattern (see fig.1B). The time-to-peak was longer (15 ± 2 s, n = 31, N = 7) than that observed in injection at the animal hemisphere but the difference was not statistically significant. The $t_{1/2}$ of the decay of the rapid component of the response was, however, significantly longer (53 ± 11 s, n = 12, N = 5, $p <$ 0.005). These results are summarized in table 1.

3.2. *Responses to $CaCl_2$*

To investigate whether the hemispheric differences in amplitudes and kinetics of the responses to injections of IP_3 reflect asymmetric distribution of Cl channels, we tested responses to $CaCl_2$ injection near the two poles of the oocyte. Injection of large amounts (100–300 pmol) of

Table 1

Responses to IP_3 injections near either pole of the oocyte

	Animal pole (*n*, *N*)	Vegetal pole (*n*, *N*)	*p*
Amplitude (nA)	764 ± 108 (39, 7)	139 ± 19 (47, 7)	<0.0005
Time-to-peak (s)	11 ± 1 (38, 7)	15 ± 2 (31, 7)	<0.05
$t_{1/2}$ of decay (s)	26 ± 3 (24, 6)	53 ± 11 (12, 5)	<0.005

Voltage-clamped (−70 mV) denuded *Xenopus* oocytes were injected near either pole of the cell with 10–100 fmol IP_3 and the resulting current was monitored. The results are presented as means ± SE of the number of assayed cells. *p* values were calculated by unpaired Student's *t*-test for differences in experimental values obtained for the animal and the vegetal poles

$CaCl_2$ near the animal pole of the cell resulted in a rapid, transient current exhibiting short time-to-peak (9 ± 1 s, $n = 33$, $N = 5$) and rapid decay ($t_{1/2}$ = 13 ± 2 s, $n = 17$). Similar injections near the vegetal pole produced responses of the same amplitude but of different kinetic characteristics (see table 1). The time-to-peak of the current was significantly longer (34 ± 3 s, $n = 26$, $N = 5$) and the decay much slower ($t_{1/2}$ = 89 ± 9 s, $n = 10$). Representative traces are shown in fig.2A,B. In several experiments, injection of $CaCl_2$ at the equator of the cell produced a biphasic response that appeared to be a superposition of the responses obtained at the animal and the vegetal hemispheres (fig.2C). Hence, the kinetic characteristics of the responses to $CaCl_2$ injection appeared to be similar to those with IP_3 injection.

3.3. *The effect of activation and inhibition of PK-C on IP_3- and $CaCl_2$-evoked responses*

We have previously observed that the activation of PK-C causes potentiation of the rapid component (D1) and inhibition of the slow, prolonged component (D2) of the response to ACh in *Xenopus* oocytes (Lupu-Meiri et al., submitted). We have also demonstrated that drugs that activate PK-C dramatically potentiate the response to IP_3. We tested the effect of B-PMA on the responses to IP_3 and $CaCl_2$ injections at the two hemispheres of the cell. As expected, pre-incubation of oocytes with β-PMA (0.1 μM for 30 min) significantly

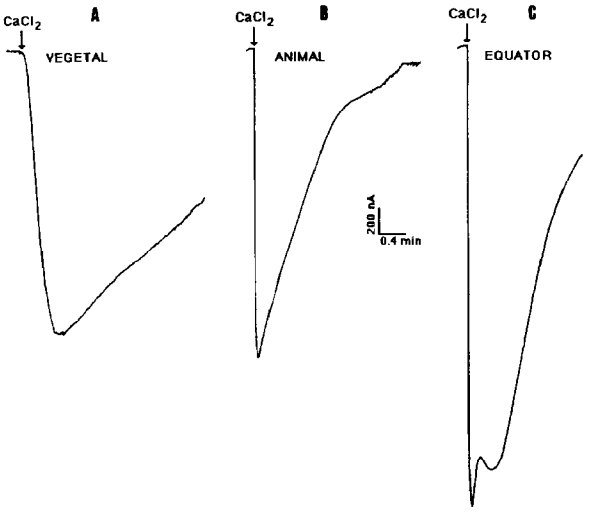

Fig.2. Responses to $CaCl_2$ injection. Three different oocytes of the same donor were voltage-clamped at −60 mV and injected with approx. 200 pmol (each) near the vegetal (A) or animal pole (B), or at the equator (C). Downward deflection of the trace indicates depolarizing current.

potentiated the response to IP_3 injection near the animal pole of the cell (by 279% vs control, $n_c = n_e = 3$), as well as near the vegetal pole (by 419% vs control, $n_c = 5$, $n_e = 10$) without any effect on the kinetics of the response.

The amplitudes of the responses to $CaCl_2$ injections were not affected by β-PMA (116 ± 5% of control, $n_{cl} = 54$, $n_e = 24$, $N = 3$). The injection of large amounts of $CaCl_2$ may be sufficient to activate fully PK-C in the absence of diacylglycerol or its analogs [6]. It was possible, therefore, that the amplitudes of responses to $CaCl_2$ reflected potentiation due to the simultaneous activation of

Table 2

Responses to $CaCl_2$ injections near either pole of the oocyte

	Animal pole (*n*, *N*)	Vegetal pole (*n*, *N*)	*p*
Amplitude (nA)	564 ± 81 (35, 5)	505 ± 77 (29, 5)	>0.05
Time-to-peak (s)	10 ± 1 (33,5)	34 ± 3 (26,5)	<0.0005
$t_{1/2}$ of decay (s)	13 ± 2 (17, 5)	89 ± 9 (10, 5)	<0.0005

Voltage-clamped denuded oocytes were injected with 100–300 pmol $CaCl_2$ near either pole of the cell. For details see fig.2 and table 1

PK-C by Ca^{2+}. To investigate this possibility, we compared responses to $CaCl_2$ in control oocytes and in cells pre-treated for 10–30 min with 10 μM of the PK-C antagonist H-7. These conditions were found to be suitable for inhibiting the endogenous activation of PK-C by cholinergic stimulation (Davidson and Oron, unpublished). There was no effect of H-7 on the amplitude or kinetics of the responses to $CaCl_2$ injections at either hemisphere of the cell (93 ± 6% of control, n_c = 69, n_e = 47, N = 3).

4. DISCUSSION

The *Xenopus* oocyte is a distinctly polarized cell with a clear demarkation between the animal and vegetal hemispheres. There are pronounced morphological differences between the two hemispheres [7,8] and previous reports indicate asymmetric distribution of ionic channels that may result in a continuous generation of current between the animal and vegetal poles [9]. We have recently reported that intrinsic ACh and acquired TRH or ACh receptors (in oocytes injected with GH_3 pituitary tumor cell RNA or rat brain RNA, respectively) display opposite hemispheric asymmetry of responses to local application of the respective agonists [4]. This phenomenon could be a result of asymmetric distribution of the respective membrane receptors or any of the components of the transduction pathway (e.g. phospholipase C, phosphatidylinositol 4,5-bisphosphate, calcium stores or chloride channels). The present work was designed to test the homogeneity of IP_3-releasable calcium stores and calcium-activated chloride channels in *Xenopus* oocytes.

Our results indicate that calcium-evoked Cl currents have different kinetic properties at the two hemispheres of the oocyte. While calcium injections produce sharp, transient responses at the animal hemisphere of the cell, similar injections at the vegetal hemisphere result in responses that display a much longer time-to-peak and decay profiles. Interestingly, injections at the equator often produce responses that appear to be hybrids of the two types of responses obtained at the two hemispheres of the cell (fig.2C). The amplitudes of the responses to $CaCl_2$ injections at the two hemispheres were similar, implying similar density of Cl channels.

Rapid, transient responses to injections of IP_3 at the two hemispheres displayed profiles similar to the characteristic responses to $CaCl_2$ injections. This suggests that the rapid component of the IP_3 response is generated locally by calcium released at or near the site of injection. The much smaller amplitude of the responses to injections at the vegetal hemisphere indicates less IP_3-releasable calcium stores at this location.

Ito et al. [10] have recently observed that activation of oocyte PK-C markedly enhances the responses to IP_3. Here we confirm this finding for IP_3 injections at both hemispheres of the oocyte. We could not detect any effect of either B-PMA or H-7 on the responses to $CaCl_2$ injections at either hemisphere of the cell. This implies that PK-C is not involved in the regulation of calcium-activated Cl channels. The effect of PK-C activators on ACh- or IP_3-evoked responses may represent action at different step(s) of the transduction pathway (e.g. calcium mobilization by IP_3). Alternatively, it is possible that injected $CaCl_2$ activates a different population of channels than Ca^{2+} mobilized by either IP_3 or the hormone. In that case we have to assume that IP_3 liberates stored calcium into a discrete intracellular compartment that is inaccessible to microinjected calcium.

Responses to the stimulation of acquired receptors in oocytes injected with GH_3 cell or brain RNA are characterized by large amplitudes and short time-to-peak and decay $t_{1/2}$ values [11]. They are also produced almost exclusively by local application of the agonists at the animal hemisphere of the cell [4]. Since these characteristics are similar to $CaCl_2$- and IP_3-evoked responses to injections near the animal pole of the cell, it is probable that the acquired responses to ACh or TRH are locally generated by receptors and other components of the transduction pathway situated at the animal hemisphere of the oocyte.

The results described above should be considered when intracellular mechanisms are derived from microinjection of intracellular probes. It is possible that the differences between the two hemispheres of the cell do not represent distinct species of Cl channels, but rather the same channel incorporated into a different membrane milieu. This question should be addressed by monitoring individual channels obtained from patches of membrane from the animal or vegetal hemisphere.

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