Metabolism of the biologically active inositol phosphates \( \text{Ins}(1,4,5)P_3 \) and \( \text{Ins}(1,3,4,5)P_4 \) by ovarian follicles of *Xenopus laevis*

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The metabolism of biologically active inositol phosphates in developed ovarian follicles from *Xenopus laevis* was investigated. Techniques used were microinjection of tracer into the intact oocyte coupled by gap junctions to follicle cells, as well as addition of tracer to homogenates of ovarian follicles and to homogenates of oocytes stripped of outer follicle-cell layers. Metabolism was similar to that previously described for other types of cell and tissue, with several unusual features. Homogenates of ovarian follicles were shown to contain an apparent 3'-phosphomonoesterase capable of converting [\(^{3}H\)]Ins(1,3,4,5)\( P_2 \) predominantly into a substance with h.p.l.c. elution characteristics of \( \text{Ins}(1,4,5)P_3 \). In intact ovarian follicles, little \( \text{Ins}(1,4,5)P_3 \) was formed but the esterase was activated by the phorbol ester activator of protein kinase C, PMA (phorbol 12-myristate 13-acetate; 60 nM), as well as by acetylcholine (200 \( \mu \)M). In follicle homogenates, this enzyme also appeared to be active in converting [\(^{3}H\)]Ins(1,3,4,5)\( P_2 \) into a substance eluting as \( \text{Ins}(1,4,5)P_3 \). The apparent 3'-phosphomonoesterase activity was not inhibited by intracellular (or higher) levels of Mg\(^{2+}\). Although PMA activated this enzyme in intact oocytes relative to 5'-phosphomonoesterase activation, it did not enhance overall metabolism, in contrast with reports on other tissues. Compared with the processing of inositol phosphates injected into the intact follicle, homogenization in simulated intracellular medium appeared to alter the activity and/or accessibility of several enzymes. The metabolism of inositol phosphates appears to occur predominantly in the follicle cells surrounding the oocyte, as collagenase treatment followed by defolliculation greatly diminished the rates of metabolism of several inositol phosphates. The presence in *Xenopus* ovarian follicles of a 3'-phosphomonoesterase activated by protein kinase C in addition to the well-known 3'-kinase suggests that, by forming a reversible interconversion between \( \text{Ins}(1,4,5)P_3 \) and \( \text{Ins}(1,3,4,5)P_4 \), this tissue may have the potential to prolong stimulatory signals on binding of appropriate agonists to receptors.

INTRODUCTION

Binding of agonists to receptors which results in the activation of phospholipase C causes the formation of \( \text{Ins}(1,4,5)P_3 \) and of the protein kinase C activator diacylglycerol. \( \text{Ins}(1,4,5)P_3 \) induces a rise in intracellular Ca\(^{2+}\) which in many tissues is essential for transduction of the agonist signal into cellular action (reviewed by Berridge & Irvine, 1984). \( \text{Ins}(1,3,4,5)P_4 \), a metabolite of \( \text{Ins}(1,4,5)P_3 \), is also believed to be biologically active, causing changes in the level of intracellular Ca\(^{2+}\) (Irvine & Moor, 1986; Hill et al., 1988). The accepted general schemes for the metabolism of \( \text{Ins}(1,4,5)P_3 \) and \( \text{Ins}(1,3,4,5)P_4 \) have been deduced from experiments carried out by many investigators working with diverse tissues from several species. Here we compare some unusual metabolic aspects of these biologically active inositol phosphates in the ovarian follicle of *Xenopus laevis*, both with intact follicle morphology and after homogenization.

The ovarian follicle and oocyte of *Xenopus laevis* have proved useful for investigation of the responses both of endogenous receptors (e.g. Lacy et al., 1989) and of exogenous receptors which have been processed, translated and rendered active from injected mRNA extracted previously from mammalian tissues (e.g. McIntosh & Catt, 1987). In immature *Xenopus* ovarian follicles, inositol phosphates have been shown to be produced on activation of both an endogenous receptor [for acetylcholine (Oron et al., 1985; McIntosh & Catt, 1987)], and exogenously expressed receptors [for angiotensin II and thyrotropin-releasing hormone (TRH) (McIntosh & Catt, 1987; Oron et al., 1987), and serotonin (Nomura et al., 1987)], whereas no inositol phosphates were formed in response to these hormones by control follicles not injected with mRNA. The morphology of the ovarian follicle may be expected to influence the metabolism of inositol phosphates which are produced by receptor activation. The follicle includes the very large oocyte cell itself (> 1 mm diameter) and a layer of normalized follicular cells surrounding it, which are in close electrical contact through gap junctions (Browne et al., 1978). The siting of endogenous receptors is predominantly in the follicular layer of cells (Dascal, 1987; Smith et al., 1987), whereas exogenous receptors are expected to be expressed in the oocyte itself. Intracellular messengers such as inositol phosphates, cyclic AMP and Ca\(^{2+}\) are likely to be transferred through gap junctions between the two cell types (as between hepatocytes; Sáez et al., 1989), and also to influence such transfer through the junctions (Browne et al., 1978; Spray et al., 1985). In addition to follicular cells, the ovarian follicle, when manually detached from the ovary, includes also the theca (containing collagen thread with capillaries and fibroblasts) and an outer layer of ovarian epithelial cells (Dumont & Brummet, 1978).

Metabolism of inositol phosphates which are known to be biologically active was investigated here in homogenates of ovarian follicles (containing several cell types), after micro-injection of labelled precursors into intact oocytes still in contact with the surrounding follicular cells through gap junctions, and
in homogenates of oocytes from which all surrounding cells had been stripped by collagenase treatment.

**EXPERIMENTAL**

**Animals**

Mature female *Xenopus laevis* were obtained from NASCO (Fort Atkinson, WI, U.S.A.) or from the Universities of Christchurch or Auckland, New Zealand. They were fed minced beef liver twice a week and maintained at about 20 °C in dechlorinated tap water containing 50 mg of EDTA/l, with a 14 h/10 h light/dark cycle. They were anaesthetized with 3-aminobenzoic acid ethyl ester methanephosphonate salt (0.2% w/v) or on ice during surgical removal of ovarian tissue.

**Materials and methods**

3H-labelled Ins (61.2 Ci/mmol), Ins(1,4)P2 (1.5 Ci/mmol), Ins(1,4,5)P3 (4 Ci/mmol) and Ins(1,3,4,5)P4 (0.1 and 17 Ci/mmol) were obtained from Dupont/NEN Products. [3H]Ins(1,3,4)P3 was formed from [3H]Ins(1,3,4,5)P4 using red-blood cell-membrane 5'-phosphomonoesterase (as described by Irvine et al., 1986b). [3H]Ins phosphates were purified by h.p.l.c. and eluted from Dowex-8 with ammonium formate, and salt was removed by lyophilization in polypropylene tubes (to avoid losses of the more extensively phosphorylated phosphoinositols) before uptake in water. [3H]Ins was purified by h.p.l.c. and lyophilization immediately before use.

Samples of approx. 20 ovarian follicles at stages 5 and 6 (Dumont, 1972) were dissected from ovarian tissue in OR-2 medium (Wallace et al., 1974). All test conditions and incubations were carried out at room temperature (~ 20 °C). In stripped samples, follicular cells were removed manually from oocytes after incubation in Ca**2+**/Mg**2+-free OR-2 medium with collagenase (Sigma Type 1A, no. C-9891; 4 mg/ml) for 4 h. The oocytes were then incubated for 18–24 h before testing. Samples of 20 follicles were homogenized by sonication for 0.5 s with a microprobe in 55 μl of a solution containing 250 mM-sucrose, 50 mM-Tris/HCl, pH 7.3, 1 mM-EGTA, 1 μg of leupeptin/ml, 10 mg of bovine serum albumin/ml and 50 μM-phenylmethylsulphonyl fluoride. The volume was made up to 200 μl to give final concentrations of 50 mM-potassium glutamate, 28 mM-NaCl, 1 mM-EGTA, 4 mM-MgSO4, 5 mM-ATP and 7 mM-Hepes, pH 7.2 (Hawkins et al., 1986), unless otherwise stated. About 15 nCi of [3H]Ins phosphate was added to homogenates. Ins(1,3,4)P3 (0.5 nCi, about 0.3 fmol), Ins(1,4,5)P3 (0.5 nCi, 0.13 fmol) or Ins(1,3,4,5)P4 (0.5 nCi, 0.3 fmol or 5 fmol) were microinjected in 50 nl into whole ovarian follicles. These solutions were taken up into a glass needle (aperture 20 μm) which was filled with paraffin oil and attached to a Hamilton Microlab-P programmable pipette set to deliver 50 nl. Phorbol 12-myristate 13-acetate (PMA; Sigma; 60 nm) was added to the medium 2–5 min before injection of tracer into the follicles. Reactions were terminated and Ins phosphates were extracted as described by McIntosh & Catt (1987), but with phytic acid hydrolysate (35 μg of P2/sample) added to the extraction medium to minimize loss of Ins(1,4,5)P3 (Wreggett et al., 1987).

Ins phosphates were analysed by h.p.l.c. (Morgan et al., 1987) using a Perkin-Elmer Series 410 BIO pump and controller and 250 mm x 4.6 mm SAX columns (Alltech or Hichrom) with linear or concave gradients from 0–1.6 mM-ammonium phosphate, pH 3.35 (79.3 g of diammonium hydrogen phosphate/litre plus about 60 ml of orthophosphoric acid) over 70–90 min, designed to optimize resolution of the peaks of interest. Eluent (1 ml/min) was mixed continuously with ACS II scintillant (Amersham) and counted for radioactivity using a Nuclear Enterprises Isosof 1 flow liquid scintillation detector, or collected in 0.5 ml fractions for prolonged counting in a Beckman liquid scintillation spectrometer. Counts from the Isosof 1 were summed over 6 s intervals using a serializing interface and Macintosh microcomputer that displayed results in real time and stored the data for later analysis. Peaks were identified by comparison of their elution volumes with standards of [3H]Ins, [3H]Ins(1,4)P2, [3H]Ins(1,3,4)P3, [3H]Ins(1,4,5)P3, and [3H]Ins(1,3,4,5)P4 run under identical conditions about every fourth sample. Elution conditions were suitable for resolving isomers of InsP2 in selected samples only. Confirmation of the resolution of the InsP2 peaks and their relative identification were obtained by running them consecutively with samples in which [3H]Ins phosphates had been extracted from stimulated ovarian follicles already shown to form multiple bisphosphates. Results from compared samples were normalized for total counts and usually smoothed (Cleveland & McGill, 1984) before presentation. All experiments were repeated 2–4 times with qualitatively the same results.

**RESULTS AND DISCUSSION**

**Metabolism of Ins(1,4,5)P3**

Some features of the metabolism of Ins(1,4,5)P3 by ovarian follicles observed in the present study were similar to previously published observations on the metabolism of this transduction intermediate in other tissues from other species. Peaks eluting with characteristics of Ins(1,3,4,5)P4 (Batty et al., 1985) and InsP2 (Downes et al., 1982) were formed rapidly (in less than 3 min), and Ins(1,3,4,5)P3 from Ins(1,3,4,5)P4 (Batty et al., 1985; Shears et al., 1987)). InsP2 and inositol (Storey et al., 1984) were produced more slowly, both in homogenates and after injection of Ins(1,4,5)P3 into follicles. In the homogenizing medium, no InsP2 was detected in the absence of added ATP (n = 3) (as shown by Irvine et al., 1986a, in brain homogenates and by Dean & Moyer, 1988, using GH3 cells).

Ins(1,4,5)P3 injected into follicles was metabolized faster than by homogenates [99.0 ± 0.8% (n = 4) compared with 74.5 ± 0.4% (n = 3) in 25 min]. The total intracellular concentration of injected Ins(1,4,5)P3 after mixing by diffusion (0.3 μM) was probably less than physiological levels, calculated to be about 1 μM after stimulation in other cells (Irvine et al., 1986a).

In other tissues the 5'-phosphomonoesterase acting on Ins(1,4,5)P3 has been shown to be stimulated by PMA (Connolly et al., 1986; Molina y Vedia & Lapetina, 1986). Neither PMA (60 nm) nor acetylcholine (200 μM) enhanced the rate of metabolism of Ins(1,4,5)P3 injected into cells [85 ± 1% conversion (n = 4) in PMA-treated and 86 ± 2% (n = 2) in controls in 15 min; 61% conversion (n = 1) in acetylcholine-treated and 58% (n = 1) in controls in 3 min].

**Metabolism of Ins(1,3,4,5)P4**

Ins(1,3,4,5)P4 added to follicle homogenates was not only metabolized to Ins(1,3,4)P4 but also produced a peak of comparable size eluting at the position of Ins(1,4,5)P3 (four experiments; Fig. 1). Whereas all the metabolites eluting at the positions of InsP2, Ins(1,3,4)P3 and Ins(1,4,5)P3 increased with time, the proportion of the later InsP2 peak diminished during 40 min (Fig. 1). There was an apparent 3'-phosphomonoesterase activity present which, acting with the Ins(1,4,5)P3 3'-kinase activity observed above, might be expected to produce reversible interconversion of Ins(1,4,5)P3 and Ins(1,3,4,5)P4.

In the absence of addition of ATP to the homogenate, the rate of metabolism of Ins(1,3,4,5)P4 was higher (80% conversion compared with 50% conversion in the presence of added ATP over 30 min). This might be expected, both because of enhanced
**Inositol phosphate metabolism in *Xenopus* ovarian follicles**

Fig. 1. Time course of the metabolism of Ins(1,3,4,5)P$_4$ in homogenates of ovarian follicles

Samples were extracted at 3–40 min ( ), 3 min ( ), 15 min ( ), 30 min ( ), 40 min ( ) after addition of [3H]Ins(1,3,4,5)P$_4$ (0.1 Ci/mmol) to homogenates, and analysed for InsP$_2$, Ins(1,4,5)P$_3$, Ins(1,4,5)P$_4$ and Ins(1,3,4,5)P$_4$ to reveal the time course of metabolism. The inset shows elution patterns at three reaction times; elution times of standards are shown by arrows.

![Graph showing the time course of metabolism of Ins(1,3,4,5)P$_4$.]

Fig. 2. Effects of PMA and acetylcholine on formation of apparent Ins(1,4,5)P$_3$ from Ins(1,3,4,5)P$_4$ injected into whole follicles

Samples were extracted 30 min after addition of tracer to controls ( ) or PMA (60 nM)-treated ( ) follicles, and subjected to h.p.l.c. Results are shown as means ± S.E.M. of nine samples from four experiments [calculated after normalization to total counts in each sample and approximate alignment of elution times of Ins(1,3,4)P$_3$ standards]. The relative positions of standards are shown by the bars. The inset compares the effect of adding acetylcholine (200 μM) ( ) with results from a control experiment ( ).

![Graph showing the effects of PMA and acetylcholine on Ins(1,4,5)P$_3$ formation.]

5'-phosphomonoesterase activity affecting metabolism of Ins(1,3,4,5)P$_4$ and Ins(1,4,5)P$_3$ as shown in rat liver (Shears et al., 1987), and also because any Ins(1,4,5)P$_3$ formed would be unable to be converted back to Ins(1,3,4,5)P$_4$, thereby increasing its rate of removal.

The production of a large proportion of Ins(1,4,5)P$_3$ from Ins(1,3,4,5)P$_4$ in whole-cell homogenates has not been reported previously. Small amounts of Ins(1,4,5)P$_3$ (less than 3%) were formed in GH$_3$ cell homogenates at 4 mM-Mg$^{2+}$ (Dean & Moyer, 1988) and in homogenates of rat basophilic leukemia cells (RBL-2H3; 3–6%; Cunha-Melo et al., 1988). Larger proportions of Ins(1,4,5)P$_3$ have been produced by cell fractions, i.e. by membranes prepared from the RBL-2H3 cells (30–55% at 4 mM-Mg$^{2+}$; Cunha-Melo et al., 1988) and by partially purified phosphatase preparations from porcine brain cytosol at 1 mM-Mg$^{2+}$ (Høer et al., 1988). An inhibitory effect of Mg$^{2+}$ on apparent 3'-phosphomonoesterase was indicated by Doughney et al. (1988) who reported that production of Ins(1,4,5)P$_3$ increased from 0.8% to 40% when Mg$^{2+}$ levels were decreased from 2 mM to nanomolar levels in human erythrocyte membrane preparations.

In homogenates of *Xenopus* ovarian follicles in a Mg$^{2+}$-free solution, total metabolism of Ins(1,3,4,5)P$_4$ was decreased to 9% (n = 2) from 40% (n = 2) in 7 min at 4 mM-Mg$^{2+}$. The peaks of InsP$_2$ were small (in all, 6% of total counts compared with 34%), with a predominance of Ins(1,4,5)P$_3$. When 13 mM-Mg$^{2+}$ was included in the homogenization solution (n = 1), metabolism of Ins(1,3,4,5)P$_4$ and the ratio of apparent Ins(1,4,5)P$_3$ to Ins(1,3,4,5)P$_4$ produced (1:1) did not differ significantly from that at 4 mM-Mg$^{2+}$ (P = 0.72, Wilcoxon test). Although both 5'- and 3'-phosphomonoesterase activities were decreased in Mg$^{2+}$-free solution, the apparent 3'-phosphomonoesterase activity was not inhibited by elevated concentrations of Mg$^{2+}$, unlike the enzyme described in human erythrocyte membranes (Doughney et al., 1988).

Injection of Ins(1,3,4,5)P$_4$ into follicles resulted in negligible radioactivity eluting at the position of Ins(1,4,5)P$_3$ thus preventing clear detection of apparent 3'-phosphomonoesterase activity. However, as seen in Fig. 2, enzyme activity appeared to be enhanced in the presence of PMA (60 nM) and on stimulation with 200 μM-acetylcholine, both of which would be expected to activate protein kinase C. This suggests a possible activation of the enzyme during hormone binding to those receptors which cause stimulation of protein kinase C. If activation of this 3'-phosphomonoesterase occurs on hormone binding to receptors, the enzyme would be expected to prolong the presence of Ins(1,4,5)P$_3$ and Ins(1,3,4,5)P$_4$ by their interconversion in conjunction with the 3'-kinase, thereby extending influences on intracellular Ca$^{2+}$ levels during stimulation of follicles by agonists. The other possibility, of PMA and hormone action increasing levels of Ins(1,4,5)P$_3$ by decreasing 5'-phosphomonoesterase activity, was not supported by results reported in the previous section, and below.

The large difference in apparent 3'-phosphomonoesterase activity between follicle homogenate and unstimulated intact follicles (in which the oocyte and follicular cells linked via gap junctions are likely to be accessible to the inositol phosphates) may have several explanations. Homogenization may expose or activate appropriate enzymes by destroying cellular compartmental structures (including possible siting on the extracellular face of the plasma membrane) and thereby increase the metabolism of added inositol phosphates or metabolites relative to when tracer is injected into the intact follicle [Cullen et al. (1989) suggested that electroporation of L1210 cells activates or exposes 3'-phosphomonoesterase activity]. The homogenization medium may contain activators not present in the oocyte or follicular cell intracellular medium, or may lack an inhibitor usually present. It is less likely that the enzymes occur at very different levels in the inner ovarian epithelial cells, capillaries and scattered fibroblasts of the theca which are homogenized in the whole ovarian follicle, in addition to the oocyte and associated follicular cells which are accessible to injected tracer.

Metabolism of Ins(1,3,4,5)P$_4$ of low specific activity (0.1 Ci/mmol) injected into follicles was considerably slower than in homogenates [13.1 ± 0.5% (n = 10) compared with 33.0 ± 1.5% (n = 6) over 30 min], but the intracellular concentration of Ins(1,3,4,5)P$_4$ introduced in this way (10 μM) was probably much greater than physiological levels. At a higher specific activity of 17 Ci/mmol, 16% of the injected label was metabolized in 7 min, compared with 40% in the homogenate.
(n = 1), while 76 ± 3% (n = 3) of the injected label was metabolized at 45 min compared with 75% in the homogenate. Oocytes caused to mature by treatment with 1 µM-progesterone metabolized injected Ins(1,3,4,5)P₄ more rapidly (70%) during 20 min than did immature follicles (36 ± 3%; n = 3).

The 5'-phosphomonoesterase acting on Ins(1,4,5)P₃ in erythrocyte membranes also hydrolyses Ins(1,3,4,5)P₄ (Connolly et al., 1987). In other tissues this enzyme has been shown to be stimulated by PMA (Connolly et al., 1986; Molina y Vedia & Lapetina, 1986). Whereas stimulation of protein kinase C enhanced the activity of the 3'-phosphomonoesterase in intact follicles, neither PMA (60 nM) nor acetycholine (200 µM) increased the activity of the 5'-phosphomonoesterase (measured as overall rate of metabolism) either in follicle homogenates [with the low-specific activity-isomer: 31 ± 2% conversion with PMA (n = 3), 34% conversion with acetycholine (n = 1) and 35 ± 2% (n = 3) by controls in 30 min] or when injected into cells [with the low-specific activity isomer: 13.5 ± 0.7% conversion (n = 6) with PMA, 12% conversion (n = 1) with acetycholine and 12.2 ± 0.6% (n = 5) by controls in 30 min; with the high-specific-activity isomer: 40 ± 1% conversion (n = 3) with PMA and 36 ± 3% conversion (n = 3) by controls in 20 min]. These results are in agreement with the lack of effect of PMA on the metabolism of Ins(1,4,5)P₃ injected into ovarian follicles described above.

Homogenates of one out of the four batches of ovarian follicles used metabolized Ins(1,3,4,5)P₄ to a third peak in the region expected for an InsP₄ eluting more slowly than Ins(1,4,5)P₃ (three samples). This peak was undetectable after injection of Ins(1,3,4,5)P₄ into follicles from this batch in the absence or presence (60 nM) of PMA; the latter condition produced Ins(1,3,4,5)P₄ as well as Ins(1,3,4)P₃ (7 min, n = 1; 20 min, n = 6) similar to results in Fig. 2. Different batches of follicles vary in many properties, including endogenous responsiveness to hormones and the unstimulated levels of baseline inositol phosphates formed on loading or injecting them with freshly purified [3H]Ins (e.g. McIntosh & Catt, 1987). Such variation in amounts and kinds of enzymes or other cell components is presumably related to the season and the maturity of follicles, which can be only partially controlled in animals maintained in the laboratory.

**Metabolism of Ins(1,3,4)P₃**

Ins(1,3,4)P₃ was metabolized slowly in follicle homogenates, giving a peak eluting at the position of Ins(1,4)P₃ after 15, 30 and 90 min of incubation (two experiments; Fig. 3), but not after 5 min. No evidence of the other expected InsP₄ isomers, i.e. Ins(1,3)P₃ and Ins(3,4)P₂ (e.g. Dean & Moyer, 1988), was found up to 90 min of reaction, although the h.p.l.c. conditions used clearly resolved InsP₄ peaks formed on stimulation of [3H]Ins-labelled follicles with acetycholine (200 µM) in samples chromatographed consequently. This further indicated the presence of a 3'-phosphomonoesterase activity.

No metabolites of InsP₄ nor InsP₄ were evident after 20% metabolism of Ins(1,3,4)P₃ for 90 min in homogenates of follicular oocytes, nor after injection of Ins(1,3,4)P₃ into intact follicles when 60% and 100% metabolism to InsP₄ and Ins occurred over 10 and 90 min respectively. This contrasts with more highly phosphorylated metabolites of Ins(1,3,4)P₃ or Ins(1,3,4,5)P₄ observed in homogenates or permeabilized preparations of other cell types (e.g. Balla et al., 1987; Shears et al., 1987; Stephens et al., 1988).

**Effect of removal of the cells surrounding the oocyte**

When surrounding cell layers were removed from the oocytes, the metabolism of Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ by homogenates

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**Table 1. Effect of removing outer follicle cell layers by collagenase treatment on metabolism of inositol phosphates by homogenates of ovarian follicles**

Results are expressed as the proportion of added tracer metabolized to other inositol phosphates. Oocytes were prepared by incubating ovarian follicles in Ca²⁺/Mg²⁺-free OR-2 medium with collagenase (4 mg/ml) for 4 h before manual stripping of outer layers. After resting for 18-24 h, 20 oocytes or follicles were homogenized per sample, tracer was added, incubation was carried out at room temperature and metabolites were extracted and analysed by h.p.l.c. as described in the Experimental section. Results are means ± s.e.m., with numbers of samples tested given in parentheses.

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was greatly decreased, as shown in Table 1. No metabolism of Ins(1,3,4)P₂ was seen in homogenates of oocytes treated with collagenase to remove surrounding cells, although 20% metabolism was observed in homogenates of ovarian follicles over the same period (90 min; Table 1). The oocyte itself, therefore, appeared to be a minor site of metabolism of the inositol phosphates Ins(1,4,5)P₃, Ins(1,3,4,5)P₄ and Ins(1,3,4)P₃ in ovarian follicles. [This may not apply to the matured oocytes, where follicle-cell-to-oocyte attachments have apparently been dissolved (Dumont & Brummet, 1978), but the rate of metabolism of Ins(1,3,4,5)P₃ is greater than in intact follicles as reported above.] It is also possible, but less likely, that the collagenase treatment may have damaged the metabolic enzymes. Oocytes stripped of outer layers of cells as described in the Experimental section retained their resting membrane potentials (R. P. McIntosh & J. E. A. McIntosh, unpublished work) when measured electrophysiologically (Lacy et al., 1989), showing that many cell-surface functions were maintained after such treatment.

General discussion

Stimulation of protein kinase C and application of hormones stimulating phospholipase C have been shown to enhance electrophysiological responses to injected InsP₃ and InsP₄ in Xenopus oocytes (Ito et al., 1988; Lupu-Meiri et al., 1988; Mahlmann et al., 1989). Activation of the 3'-phospho-monoesterase activity reported here may contribute to the explanation of these results. Formation of Ins(1,4,5)P₃ from Ins(1,3,4,5)P₄ injected into unstimulated Xenopus oocytes may cause, or contribute, to the development of the resulting chloride current described by Parker & Miledi (1987) in a manner similar to the release of Ca²⁺ by tetrakisphosphate in electroporated L1210 cells as discussed by Cullen et al. (1989).

Metabolism of inositol phosphates in oocytes of Xenopus laevis is also of interest because transient waves of increased free cytosolic Ca²⁺ occur for several minutes after fertilization of the eggs (Busa & Nuccitelli, 1985), and some fertilization events are produced by microinjection of Ins(1,4,5)P₃ (Picard et al., 1985). It is possible that the 3'-phosphomonoesterase activated by protein kinase C as described here may prolong stimulatory signals from Ins(1,4,5)P₃ Ins(1,3,4,5)P₄ and any active higher inositol phosphates formed during stimulation by fertilization or appropriate agonists.

It is noted that small peaks eluting with chromatographic characteristics of InsP₃ were also detected consistently on metabolism of Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ by Xenopus follicular oocytes after 25 min, in addition to those metabolites described above (McIntosh & McIntosh, 1990).

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REFERENCES


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