Osmo-dependent Cl-currents activated by cyclic AMP in follicle-enclosed *Xenopus* oocytes

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SUMMARY

The role of adenosine 3',5'-cyclic monophosphate (cAMP) in generating the osmo-dependent slow inward membrane currents ($I_{\text{IN}}$) elicited by activation of follicle stimulating hormone (FSH) or acetylcholine (ACh) receptors was studied in voltage-clamped, follicle-enclosed oocytes of *Xenopus laevis* (follicles). Forskolin (FSK) also generated $I_{\text{IN}}$ currents, and in low concentrations it potentiated the $I_{\text{IN}}$ currents elicited by FSH but not those elicited by ACh. Moreover, intra-oocyte injections of cAMP elicited similar slow inward currents (cAMP-$I_{\text{IN}}$) that: (i) were carried mainly by chloride ions; (ii) were abolished by defolliculating the oocytes; and (iii) were dependent on the osmolarity of the external medium. Compared with the Ca$^{2+}$-dependent chloride channels that are located in the oocyte membrane; the cAMP-activated $I_{\text{IN}}$ channels were less permeable to I$^-$ and Br$^-$, and their current–voltage relation did not rectify strongly at negative potentials. Generation of cAMP-$I_{\text{IN}}$ desensitized the FSH-$I_{\text{IN}}$ currents, but did not have effects on both the $I_{\text{IN}}$ and the fast chloride current ($I_{\text{FIN}}$) specifically elicited by ACh. Furthermore, follicular phospholipase C activation through stimulation of angiotensin II (AII) receptors failed to generate the current responses elicited by ACh. We conclude that cAMP acts as a potent second messenger in generating the osmo-dependent Cl$^-$ currents elicited by FSH but not those elicited by ACh. The mechanisms underlying the ACh responses remain unknown. The osmo-dependent chloride channels activated by cAMP may play a role in the control of volume of the follicular cells-oocyte complex.

1. INTRODUCTION

The relations among cell groups of the ovarian follicle are important for oocyte development and maturation (Eppig 1979). In the follicular complex, the oocyte and its surrounding follicular cells display the closest association, since they maintain communication through electrical coupling (Browne et al. 1979) and chemical signalling involving both metabolic coupling and paracrine mechanisms. The importance of this association for oocyte physiology prompted us to conduct a more detailed study of follicular cell–oocyte interactions.

*Xenopus* follicles have proved to be a very useful system in the study of receptors, second messenger pathways and membrane channels, either endogenous or expressed in the oocyte through the injection of mRNA or cRNA (Kusano et al. 1977, 1982; Miledi et al. 1989a). Therefore, knowledge of the native characteristics of *Xenopus* oocytes is fundamental for their proper use as an expression system. Recently we showed that, in follicles, both follicle stimulating hormone (FSH) and acetylcholine (ACh) elicit smooth, slow Cl$^-$ currents ($I_{\text{IN}}$) that appear to have similar characteristics (Arellano & Miledi 1993). Our results strongly suggest that those currents originated in the follicular cell compartment and that they were clearly different from the oscillatory Ca$^{2+}$-dependent Cl$^-$ currents generated in the oocyte membrane itself, through activation of the phosphoinositide system consequent to stimulation of several native receptors (see, for example, Kusano et al. 1977, 1982; Miledi et al. 1989b; Tigyi et al. 1990). The FSH-$I_{\text{IN}}$ or ACh-$I_{\text{IN}}$ chloride currents were not Ca$^{2+}$ dependent, but were strongly dependent on the osmolarity of the external solution, and the channels involved were less permeable to I$^-$ and Br$^-$ than the Ca$^{2+}$-dependent chloride channels. However, the second messenger system(s) involved in activation of the $I_{\text{IN}}$ currents remained unknown.

The main aim of the present experiments was to examine the role of the cAMP and IP$_3$/diacylglycerol pathways in the follicular responses elicited by FSH and ACh. Our results suggest that cAMP activates $I_{\text{IN}}$ currents that may participate in the regulation of cell volume during development and growth of the *Xenopus* oocyte–follicular cells complex, and raise the possibility that some effects produced by ACh in follicles may involve a novel cellular signalling mechanism. Part of this study has been presented in abstract form (Arellano & Miledi 1994).

2. MATERIALS AND METHODS

Follicle-enclosed oocytes (stages V and VI (Dumont 1972)) were isolated from ovaries of adult *Xenopus laevis* (purchased from Xenopus I, Ann Arbor, Michigan, U.S.A., and Nasco, Fort Atkinson, Wisconsin, U.S.A.). The follicles
were dissected free of their inner ovarian epithelium, leaving the thecal basement membrane, and were allowed to equilibrate for at least 5 h at 18 °C in Barth's medium containing (in millimoles per litre): 88 NaCl, 1 KCl, 2.4 NaHCO₃, 0.33 Ca(NO₃)₂, 0.41 CaCl₂, 0.82 MgSO₄, 5 HEPES, adjusted to pH 7.4 and supplemented with gentamicin 70 µg ml⁻¹. In general, the follicles were used during the first or second day after dissection. In some cases the follicles were incubated in a modified Barth's medium (Arellano & Miledi 1993) which preserved the follicle responses for longer periods (3-6 d). For some experiments oocytes were defolliculated by collagenase treatment (1-2 mg ml⁻¹) for 1-2 h in normal frog Ringer solution (NR; see below), or manually by rolling oocytes free of their epithelial and thecal envelopes over a poly-L-lysine coated glass slide (i.e. rolled oocytes (Miledi & Woodward 1989)).

Follicle membrane current responses were routinely monitored in a hypotonic frog Ringer (HR) solution (containing (in millimoles per litre): 88 NaCl, 2 KCl, 1.8 CaCl₂, 5 HEPES, pH 7.0), by using a conventional two-microelectrode voltage-clamp technique (Miledi 1982). Usually, BaCl₂ (1-2 mM) was added to the HR to block the additional K⁺ currents that are activated by the agonists or cAMP. NR solution was similar to HR but with 27 mM more NaCl. The Na⁺ 50%-NR had 50% of the NaCl substituted by Tris-HCl in HR, and in high K⁺-HR 8 mM NaCl was replaced by KCl. K⁺ currents in low-Cl⁻ HR (Cl⁻ 34 mM) solution were blocked by using tetraethylammonium (TEA) chloride, and Cl⁻ was substituted by SO₄⁻. This solution contained (in millimoles per litre): 28 TEA-chloride, 30 Na₂SO₄, 2 KCl, 1.8 CaCl₂, 34 sucrose, 5 HEPES, pH 7.0. Follicular responses elicited in this solution were compared with responses obtained in a TEA-HR (Cl⁻ 94 mM) solution containing (in millimoles per litre): 28 TEA-chloride, 60 NaCl, 2 KCl, 1.8 CaCl₂, 5 HEPES, pH 7.0. The Na⁺-HR and NaBr-HR solutions were made by substituting the corresponding salt by NaCl in HR.

Intrafollicle injection solutions, containing guanosine 3',5'-cyclic monophosphate (cGMP) or cAMP (1-10 mM, plus 20-50 µM EGTA, 5 mM HEPES, and adjusted to pH 7.0 with KOH) were made by pneumatic pressure ejection from micropipettes (Miledi & Parker 1984).

ACh, Angiotensin II (AII), EGTA, cAMP, cGMP, 3-isobutyl-1-methyloxanthine (IBMX), EFS, collagenase type I, and gentamicin were purchased from Sigma Chemical Co. (St Louis, Missouri), TEA-chloride was from Eastman Kodak Co. (Rochester, New York), Forskolin (FSK) and porcine FSH were obtained from Calbiochem (La Jolla, California).

3. RESULTS

(a) Inward currents in follicles exposed to FSH, ACh or Forskolin

Follicles were voltage clamped at -60 mV or -80 mV and tested in HR medium for responses to FSH (1 µg ml⁻¹) and ACh (0.5 µM). As shown previously (Arellano & Miledi 1993), FSH and ACh elicited multiphasic membrane current responses, carried mainly by K⁺ (outward components) and Cl⁻ (inward components). In the present study, we routinely blocked the outward potassium currents by adding Ba²⁺ or TEA to the HR (see figure 3a). In HR, the follicles developed large outward and inward membrane currents upon exposure to 1 µg ml⁻¹ FSH, a concentration that elicited maximal responses in control conditions. Ba²⁺ eliminated the outward current and left an inward slow Cl⁻ current (Iₛ, figure 1a, b). Ba²⁺ had a similar effect on smaller K⁺ currents elicited by ACh.

It is known that activation of FSH receptors, located in the follicular cell membrane, promotes cAMP synthesis (Greenfield et al. 1990), and that this cyclic nucleotide is involved in mediating the follicular K⁺ currents that are activated by hormones and other agents (see, for example, Woodward & Miledi 1987). These effects are mimicked by application of forskolin (FSK), a direct activator of adenylyl cyclase. Therefore, we examined directly the role of the cAMP in mediating the activation of Iₛ currents.

Application of 5 µM FSK to follicles (23 follicles, six frogs) frequently elicited slow and smooth inward currents (figure 1b) which had the following characteristics: (i) they required the presence of follicular cells: defolliculated oocytes lost their sensitivity to FSK (ten oocytes, three frogs; figure 1b); (ii) they were osmo-dependent, similar to FSH-Iₛ currents, the inward currents elicited by FSK were reduced to 5-10% in NR; and (iii) they were carried principally by Cl⁻ ions, as suggested by a reversal potential (£Eᵥₑᵥₑ) of -31±2 mV (mean±s.d.; figure 1c), estimated from the subtraction of current–voltage (£I–V) relations obtained at rest and during the peak of the responses to FSK. Importantly, the £I–V relations for FSH and FSK-Iₛ currents in follicles from the same frog were similar throughout the range tested (figure 1c). Thus the Cl⁻ currents elicited by FSK had characteristics similar to those we have described previously for both the FSH-Iₛ and the ACh-Iₛ currents (Arellano & Miledi 1993).

Moreover, in follicles incubated for 10 min in a medium containing a low concentration of FSK (0.3-0.5 µM), the FSH-Iₛ currents were amplified and lengthened in duration (figure 1d), measured as the time elapsed between the end of agonist applications and return to the basal level. For example, follicles of one frog developed FSH-Iₛ currents with an average amplitude of 263±73 nA and a duration of 1.8±0.5 min (five follicles; figure 1d(i)). Pre-incubation with 0.5 µM FSK resulted in FSH-Iₛ currents of 500±43 nA and duration of 5.25±1.5 min (12 follicles; figure 1d(ii)). The £Eᵥₑᵥₑ of the FSH-activated currents, in presence or absence of FSK, were not different, suggesting that other currents were not significantly increased.

In contrast to the potentiation of FSH-elicited currents, the ACh-activated chloride currents, both the fast chloride current (£Fᵢₑₑ) (Arellano & Miledi 1993) and Iₛᵦᵦ, were not enhanced by pre-incubation with FSK (figure 1d; 18 follicles from three frogs). For example, in follicles from one frog, ACh (0.5 µM) induced £Fᵢₑₑ currents of 104±11 nA and Iₛᵦᵦ currents of 327±28 nA (five follicles) under control conditions. These currents had similar amplitudes, 117±32 nA and 308±24 nA, respectively (five follicles), when ACh was applied to follicles pre-incubated for 10 min with FSK (0.5 µM); the duration of the ACh-Iₛᵦᵦ currents were also similar (1.8±0.33 min for the control group, and 1.92±0.34 min for the treated
CAMP-activated Cl currents in Xenopus follicles

R. O. Arellano and R. Miledi

Figure 1. Follicular currents induced by FSH, FSK or ACh. (a) Membrane current responses elicited by FSH (1 μg ml<sup>-1</sup>) in two follicles of the same frog held at −60 mV and superfused with HR, or HR containing 1 mM BaCl<sub>2</sub>. In this and following figures, depolarizing pulses of 10 mV (3–5 s) were applied periodically to monitor membrane conductance, and bars above each record show the duration of drug applications. (b) (i) Currents elicited by FSK (5 μM) and FSH (1 μg ml<sup>-1</sup>) in HR containing Ba<sup>2+</sup> (1 mM) in a follicle held at −60 mV. (ii) The same follicle was defolliculated and the oocyte exposed again to the agonists. (c) I-V relations of S<sub>in</sub> currents activated by FSK (5 μM; open circles) or FSH (1 μg ml<sup>-1</sup>; filled triangles). The points represent average current (5–8 follicles) normalized with respect to the peak current obtained at −80 mV. (d) Follicle membrane current responses to FSH (1 μg ml<sup>-1</sup>) and ACh (0.5 μM; arrows indicate Fin currents), (i) in control conditions and (ii) in a follicle (same frog) pre-incubated for 10 min in medium containing FSK (0.5 μM). All were recorded in HR (1 mM Ba<sup>2+</sup>) and voltage clamped at −80 mV.

Intra-oocyte injection of cAMP and S<sub>in</sub> currents

In follicle-enclosed oocytes, intra-oocyte injection of cAMP generated similar S<sub>in</sub> currents (more than 56 follicles from 11 frogs; figure 2a), but injections of cAMP failed to generate membrane currents in defolliculated oocytes (20 oocytes, eight frogs; figure 2b). Follicles injected with 0.5–5.0 pmol cAMP developed large smooth inward currents with characteristics similar to those of the FSH-S<sub>in</sub> currents. The average amplitude of the currents elicited by cAMP was 226 ± 150 nA, but showed high variability among follicles from different frogs. For example, in follicles of one frog, tested in HR (1 mM BaCl<sub>2</sub>) and held at −80 mV, the average for the cAMP-S<sub>in</sub> currents was 79.5 ± 30 nA (six follicles), whereas in follicles of another frog the average current was 467 ± 93 nA (five follicles).

The S<sub>in</sub> currents elicited by injection of cAMP were strongly dependent on the osmolarity of the external medium, and were not observed in follicles equilibrated in NR (1 mM BaCl<sub>2</sub>; five follicles, two frogs). Moreover, in follicles equilibrated in HR, the cAMP-S<sub>in</sub> currents were rapidly and reversibly reduced when the follicles were perfused briefly with NR (figure 2c; eight follicles, three frogs). It should be noted that the FSH and ACh-S<sub>in</sub> currents behave in a similar way (Arellano &
cAMP-activated Cl⁻ currents in Xenopus follicles

Miledi 1993). Follicles which were perfused briefly with NR, before cAMP injections, generated only small inward or outward currents of 17 ± 11 nA (three follicles, two frogs).

In follicles tested in TEA-HR (figure 3a), or in HR with 1 mM BaCl₂ added, cAMP-Sᵢᵣ currents had an average $E_{rev}$ of $-29 ± 3$ mV (12 follicles), similar to that of FSH and ACh-Sᵢᵣ currents in follicles of other donors, $-28 ± 3.5$ mV (22 follicles). Thus all these experiments suggest that the currents elicited by cAMP were carried mainly by chloride ions, whose equilibrium potential is close to this value in follicles (Kusano et al. 1982). This conclusion was supported further by results of ionic substitution experiments, where the $E_{rev}$ of the currents elicited by cAMP was largely unaltered by substituting 50% of the NaCl with Tris-HCl, or by increasing K⁺ to 10 mM in HR (ten follicles, two frogs). In contrast, in low Cl⁻-HR solution (figure 3a), the cAMP inward currents decreased in amplitude and their $E_{rev}$ was shifted to more positive values, from $-29$ mV to $-15 ± 2$ mV (five follicles). This 10–14 mV shift of $E_{rev}$ is lower than that expected from the Nernst equation (23 mV). The cause of this discrepancy is not well understood, but could be due, for example to the following: (i) changes in the intracellular concentration of Cl⁻ during follicular superfusion in low Cl⁻-HR, (ii) permeability of other ions, in addition to chloride, via the cAMP-activated channels; or (iii) cAMP-elicited potassium currents driving the $E_{rev}$ to more negative potentials. $E_{rev}$ of the cAMP-Sᵢᵣ currents in NaI-HR or NaBr-HR were $-31 ± 2$ mV and $-30 ± 2$ mV, respectively (five follicles each anion), and the peak Sᵢᵣ currents in these solutions were similar to the peak currents in HR (throughout the $-100$ mV to +20 mV range, not shown). All this suggests that the channels opened by cAMP, like those involved in generating the FSH and ACh-Sᵢᵣ currents, were also less permeable to iodide or bromide than the Ca²⁺-dependent chloride channels located in the oocyte membrane (Arellano & Miledi 1993).

The Sᵢᵣ currents evoked by cAMP showed strong desensitization, and a second injection of cAMP applied a few minutes later produced little or no current (figure 3b). Another important point is that the cAMP-Sᵢᵣ currents strongly cross-desensitized the FSH-Sᵢᵣ responses in all follicles tested, but had no effect on the ACh-Sᵢᵣ and Fᵢᵣ currents (figure 3b (ii), 15 follicles from four frogs). These results again suggest strongly that the FSH and cAMP-Sᵢᵣ currents involve activation of a common mechanism, and show that, although ACh-Sᵢᵣ and FSH-Sᵢᵣ currents seemed to be carried through similar channels, changes in cAMP levels within the follicles did not affect the currents induced by ACh.

In follicles that responded well to cAMP injections ($S_{iᵣ}$ of $370 ± 95$ nA; three follicles), similar intra-follicular injections of cGMP (0.5–20.0 pmol) elicited only very small inward currents ($10 ± 3$ nA; nine follicles, two frogs).

(c) The IP₃/diacylglycerol second messenger systems and $S_{iᵣ}$ and $F_{ᵣ}$ currents

It has been shown recently that All receptors are present in the membrane of the oocyte (Woodward & Miledi 1991) as well as in the membrane of the follicular cells (Sandberg et al. 1990). Stimulation of these receptors activates phospholipase C, and produces IP₃/diacylglycerol which raises the intracellular level of Ca²⁺ that triggers the Ca²⁺-dependent oscillatory chloride currents in the oocyte membrane itself. Furthermore, we proposed that the muscarinic receptors, as well as the channels, involved in the generation of the ACh-Sᵢᵣ and Fᵢᵣ currents are located in the membrane of the follicular cells (Arellano & Miledi 1993). We reasoned that if synthesis of IP₃/diacylglycerol is involved in generating either the ACh-Sᵢᵣ or Fᵢᵣ currents, then activation of All receptors located in the membrane of the follicular cells would be expected to elicit similar types of follicular currents. To investigate this question we examined the effects of All and ACh in follicles and defolliculated oocytes from several frogs, selecting only follicles in which the currents elicited by All were completely eliminated by defolliculation; that is, follicles in which the All receptors were presumably all located in the membrane of the follicular cells (figure 4).

As shown before (Kusano et al. 1977, 1982; Arellano...
cAMP-activated Cl⁻ currents in Xenopus follicles
R. O. Arellano and R. Miledi

Figure 3. Current–voltage relations and desensitization of the cAMP-Sᵢᵣ currents. (a) cAMP-Sᵢᵣ currents in follicles, from one frog, superfused with TEA-HR (94 mM Cl⁻; filled circles) or low-Cl⁻ TEA-HR (34 mM Cl⁻; open circles) solutions; the reversal potential shifted by about 10 mV. Sᵢᵣ currents normalized with respect to the peak currents obtained at the holding potential in TEA-HR. (b) Records from a follicle, held at −60 mV in HR (1 mM Ba²⁺), showing the strong desensitization of the cAMP current. Both traces are continuous in time. After the inward current elicited by the first injection of cAMP had recovered, (i) a double injection of cAMP (double arrow) or (ii) external application of FSH (1 ng ml⁻¹) failed to elicit follicular currents. However, both ACh-evoked currents (100 μM ACh), Fᵢᵣ, and Sᵢᵣ, were preserved.

Figure 4. Follicular inward membrane currents elicited by ACh or All. (a) Responses elicited by ACh (50 μM) and All (600 nM) in the same follicle-enclosed oocyte perfused in HR and held at −60 mV. (b) After defolliculation, agonists fail to elicit responses in a different oocyte from the same frog (rolled oocyte). Similar results were obtained in seven other follicles and eight oocytes from the same frog.

& Miledi 1993), ACh responses in follicles are very variable in the proportions of their various current components. However, in all the follicles selected, All (100 nM to 1 μM) elicited oscillatory oocyte membrane currents (ranging from 100 nA to 4 μA) but failed to elicit Sᵢᵣ or Fᵢᵣ follicular currents (more than 37 follicles from ten frogs). Furthermore, follicles from several frogs that gave large oscillatory currents to activation of follicular All receptors responded to injection of ACh with only Sᵢᵣ and Fᵢᵣ currents (figure 4). The absence of the oscillatory responses strongly suggests that, in these cases, ACh did not stimulate appreciably follicular or oocyte synthesis of IP₃/diacylglycerol.

4. DISCUSSION

One central finding in our work is that an increase in the intracellular concentration of cAMP in follicles elicits slow smooth Cl⁻ currents (Sᵢᵣ) that are similar to those activated by FSH, and that low concentrations of FSK potentiate the currents elicited by FSH. We also show that the Sᵢᵣ currents elicited by intra-oocyte injections of cAMP require the presence of the follicular cells. Taken together these results strongly suggest that stimulation of FSH receptors, located in the membrane of the follicular cells, promotes the synthesis of intracellular cAMP, which in turn opens osmo-
dependent Cl⁻ channels located in the membrane of the follicular cells.

The cAMP-activated inward currents were similar to the previously described FSH and ACh-Sₘ currents (Arellano & Miledi 1993). We do not know yet if the cAMP-Sₘ current is related to the chloride currents induced by hyposmotic stress in follicles (Arellano & Miledi 1993; Ackerman et al. 1994). However, some preliminary observations suggest that the two currents may be different, for example: (i) the amplitudes of the cAMP-Sₘ currents and those elicited by hyposmotic stress did not vary in parallel among different follicles; and (ii) the inward current induced by a hyposmotic solution was not altered by drugs that raise intracellular cAMP (R. O. Arellano & R. Miledi, unpublished results). Although more experiments are necessary to determine in detail any possible relation between these currents, our defolliculation experiments (using either manual or enzymic techniques) strongly suggest that the osmo-dependent channels gated by the FSH or ACh actions, as well as the channels activated by hyposmotic stress, were located in the membrane of the follicular cells. However, the possibility that defolliculation damages channels located in the membrane of the oocyte, or their activation mechanism, cannot yet be completely discarded.

Although cAMP-activated Cl⁻ currents have been previously described, for example, in epithelial cells and cardiac myocytes, much of that research has focused on chloride channels that belong to the ATP-binding cassette (ABC) protein family and are involved in cystic fibrosis disease (Welsh et al. 1992).

The cAMP-Sₘ currents that we describe here are strongly dependent on the osmolarity of the external medium, a characteristic not yet reported in other preparations. However, osmolarity-dependent Cl⁻ channels have been found in different tissues, but it appears that those channels are not activated by an increase in cAMP (McCann et al. 1989; Worrell et al. 1992). An osmolarity-dependent Cl⁻ current evoked by isoprenaline (Sorota 1992) was found in canine atrial cells, but it is not yet known if this current is related to the cAMP-dependent chloride channels in cardiac myocytes. Furthermore, another member of the ABC family, P-glycoprotein, coded by a multi-drug resistance gene, when permanently expressed in NIH3T3 and S₁ cell lines acts as an ATP-dependent transporter of cytotoxic substances and as a voltage-dependent chloride channel that is also strongly modulated by osmolarity (Valverde et al. 1992). However, so far there is no clear evidence for the existence of a similar ATP-dependent transporter system in the membrane of Xenopus follicular cells.

The pathways activated by the intracellular increase of cAMP, necessary to elicit the Sₘ current in Xenopus follicles, have not been fully characterized. However, our results suggest that they involve a process specific for cAMP, because CGMP was only a weak activator of these currents. The mechanisms that confer the current osmo-dependence remain unknown, but it is possible that protein molecules associated with cytoskeletal elements are involved (see, for example, Kravivinsky et al. 1994).

Muscarnic stimulation of the follicles did not appear to involve much production of cAMP, and it was clear that, although activation of AII receptors located in the membrane of the follicular cells did not evoke Sₘ or Iᵣ currents like those elicited by ACh, it did elicit oscillatory chloride membrane currents, almost certainly through stimulation of phospholipase C and production of IP₃. Furthermore, in many cases the responses to ACh did not include oscillatory currents, even when the follicles gave strong oscillatory currents in response to activation of follicular cell AII receptors. All this strongly suggests that the processes activated by follicular cell ACh receptors do not necessarily involve the production of IP₃. Thus it seems likely that the osmo-dependent Sₘ currents generated by ACh are mediated by other receptor–channel coupling pathways not yet characterized. More experiments will be necessary to distinguish among many possible explanations. For example, it could be that the receptors to ACh and AII are located in different subpopulations of follicular cells.

In conclusion, our results show that FSH activates osmo-dependent Cl⁻ channels, located in the membrane of Xenopus follicular cells, via a mechanism involving an increase in the intracellular level of cAMP. As in other cell systems, the ensuing chloride currents may play an important role in several physiological processes, particularly in follicular cell–oocyte volume regulation. Furthermore, our results raise the strong possibility that the Cl⁻ currents elicited by ACh are activated through a distinct, as yet uncharacterized pathways. The nature of the channels, and details of their activation and modulation, still remain to be elucidated. Nevertheless, the results presented should be taken into account when Xenopus oocytes are used to study expressed chloride channels, especially those which may be osmo-dependent.

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