

Novel Cl⁻ Currents Elicited by Follicle Stimulating Hormone and Acetylcholine in Follicle-enclosed *Xenopus* Oocytes

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ABSTRACT Voltage-clamp techniques were used to study the membrane currents elicited by follicle stimulating hormone (FSH) and acetylcholine (ACh) in follicle-enclosed oocytes of *Xenopus laevis* (follicles). Both agonists caused complex responses that were more evident when the follicles were in hypotonic Ringer solution (HR; 190.4 mosM). In this medium, currents activated by FSH regularly showed three phases whereas currents activated by ACh displayed three to six phases. At a holding potential of -60 mV, FSH, and ACh responses involved combinations of inward and outward currents. Both FSH and ACh responses included a slow smooth inward component that was associated with an increase in membrane conductance, mainly to Cl⁻ (S_{in}). This current was strongly dependent on the osmolarity of the external solution: an increase in osmolarity of the HR solution of 18–20 mosM caused a 50% decrease in S_{in} . In contrast, a fast and transient Cl⁻ current (F_{in}) specifically elicited by ACh was not dependent on osmolarity. Both, F_{in} and S_{in} currents required the presence of follicular cells, since defolliculation using three different methods abolished all the response to FSH and at least four components of the ACh responses. The membrane channels carrying F_{in} and oscillatory Cl⁻ currents elicited by stimulation of ACh or serum receptors, were much more permeable to I⁻ and Br⁻ than Cl⁻, whereas S_{in} channels were equally permeable to these anions. Unlike the oscillatory Cl⁻ currents generated in the oocyte itself, S_{in} and F_{in} currents in follicle-enclosed oocytes were not abolished by chelation of intracellular Ca²⁺, either with EGTA or BAPTA, which suggests that intracellular Ca²⁺ does not play a critical role in the activation of these currents. Our experiments show that S_{in} and F_{in} currents are quite distinct from the previously characterized oscillatory Cl⁻ responses of oocytes. Moreover, the results strongly suggest that the FSH and ACh receptors, the Cl⁻ channels mediating the F_{in} and S_{in} currents, together with the necessary elements for their activation, are all located in the follicular cells and not in the oocyte.

Many aspects of follicular cell physiology in *Xenopus laevis*, and other species, are regulated by hormones and neurotransmitters, including FSH and ACh. The follicular Cl⁻ currents described in this paper may play an important role in the follicular cell-oocyte development.

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INTRODUCTION

Xenopus ovarian follicles are a very useful model system to study neurotransmitter and hormone receptors, ionic channels, and the intracellular mechanisms that operate them. A great variety of transmitters and hormones elicit slow membrane current responses due to activation of native receptors and channels in the follicle-enclosed oocytes (Kusano, Miledi, and Stinnakre, 1977, 1982; Woodward and Miledi, 1987*a, b*; Miledi and Woodward, 1989*a, b*). Moreover, several exogenous receptors and channels can be expressed in the membrane of the oocyte by microinjection of messenger RNAs from different sources (e.g., Miledi, Parker, and Sumikawa, 1989*a*).

The follicle is a cellular complex in which the oocyte and the surrounding follicular cells are electrically, and metabolically, communicated through gap junctions (Browne, Wiley, and Dumont, 1979; Browne and Werner, 1984; van den Hoef, Dictus, Hage, and Bluemink, 1984). Due to this intercellular coupling the currents being monitored from within an oocyte enclosed by its follicular cells are actually a mixture of the currents generated in the oocyte membrane itself and currents arising in membranes of the follicular cells. Elucidating the origin of the different endogenous electrical responses, and their mechanisms of activation, is fundamental to the understanding of oocyte-follicular cell physiology, and is also important when using oocytes to express foreign receptors and channels.

Native follicles have been shown to respond to numerous neurotransmitters and hormones, e.g., catecholamines (Kusano et al., 1977, 1982), purinergic agonists (Lotan, Dascal, Cohen, and Lass, 1982), gonadotropins, vasoactive intestinal peptide, atrial natriuretic factor, and prostaglandins (Woodward and Miledi, 1987*a, b*; Miledi and Woodward, 1989*a, b*). All these agonists appear to act on distinct populations of receptors, located in the membrane of the follicular cells, all positively coupled to adenylate cyclase. Activation of these receptors stimulates the synthesis of adenosine 3':5'-cyclic monophosphate (cAMP) and activates a K^+ current via intermediate steps still not well characterized. Previous studies have strongly suggested that all the elements involved in this type of follicle response are in the follicular cell compartment and not in the oocyte (Woodward and Miledi, 1987*a*; Miledi and Woodward, 1989*a*).

In addition, native follicles respond also to acetylcholine, with a characteristic oscillatory Cl^- current (Kusano et al., 1977, 1982). In contrast to the K^+ current elicited by the agonists listed above, the oscillatory Cl^- currents clearly arise in the oocyte itself and are due to mobilization of intracellular Ca^{2+} by inositolpolyphosphates and activation of Ca^{2+} -gated channels present in the oocyte membrane (Miledi, 1982; Miledi and Parker, 1984; Oron, Dascal, Nadler, and Lupu, 1985; Parker and Miledi, 1986; Takahashi, Neher, and Sakman, 1987). However, the current responses elicited by ACh in the follicles are often multiphasic (Kusano et al., 1977, 1982; Dascal and Landau, 1980), with outward and inward smooth components in addition to the oscillatory currents. Some of these phases are depressed, or may even be fully lost, after defolliculation. This has been specially clear for two components of ACh responses: (*a*) the ACh induced K^+ current (Kusano et al., 1982; Dascal and Cohen, 1987; Miledi and Woodward, 1989*a, b*) and (*b*) a transient Cl^- current with short latency (Miledi and Woodward, 1989*b*). Furthermore, it has also

been proposed (Miledi and Woodward, 1989a) that the muscarinic receptors involved in the attenuation of cyclic nucleotide-activated K⁺ currents are located in the membrane of the follicular cells (Dascal, Lotan, Gillo, Lester, and Lass, 1985; Van Renterghen, Penit-Soria, and Stinnakre, 1985; Stinnakre and Van Renterghen, 1986; Woodward and Miledi, 1991). Taken together, these studies clearly indicate that some phases of the ACh responses in follicle-enclosed oocytes arise in the follicular cells and not in the oocyte.

In the present paper we show that in follicles, FSH and ACh can also elicit slow, smooth Cl⁻ currents with characteristics that are very different from those of the typical oscillatory Cl⁻ currents activated in the oocyte. Our results strongly suggest that the follicular cell compartment has the FSH and ACh receptors, and the Cl⁻ channels that are involved in the generation of these novel currents.

MATERIALS AND METHODS

Follicles at stages V and VI (Dumont, 1972) were dissected from the ovaries of adult *Xenopus laevis* obtained from Xenopus I (Ann Arbor, MI) or Nasco (Fort Atkinson, WI). The follicles were normally used as epithelium removed follicles, in which the inner ovarian epithelium was removed leaving the thecal basement membrane for protection of the follicular cells. Follicles were incubated in sterile Barth's solution (containing [in mM]: 88 NaCl, 1 KCl, 2.4 NaHCO₃, 0.33 Ca(NO₃)₂, 0.41 CaCl₂, 0.82 MgSO₄, 5 HEPES, pH 7.4, supplemented with gentamycin 70 μg ml⁻¹) at 18°C, and used at least 5 h after dissection. In some cases, the follicles were incubated at 18°C in modified sterile Barth's solution (in mM: 88 NaCl, 0.2 KCl, 2.4 NaHCO₃, 0.33 Ca(NO₃)₂, 0.41 CaCl₂, 0.82 MgSO₄, 0.88 KH₂PO₄, 2.7 Na₂HPO₄, 5 glucose, pH 7.4, supplemented with gentamycin 70 μg ml⁻¹ and 0.1% FBS), in this medium the follicular current responses to the agonists were maintained for 3–6 d after dissection; and their characteristics were essentially the same as in fresh follicles. In different experiments oocytes were defolliculated using one of the following methods: (a) collagenase treatment (Sigma type I or Boehringer type b; 1–2 mg ml⁻¹) in frog normal Ringer (NR) solution (containing [in mM]: 115 NaCl, 2 KCl, 1.8 CaCl₂, 5 HEPES, pH 7.0) at room temperature for 1–2 h. After the treatment the oocytes were shaken, washed, and incubated in Barth's solution at 18°C. (b) Follicles with the inner ovarian epithelium and thecal layers removed manually were rolled over poly-L-lysine coated glass slides in Barth's or zero Ca²⁺ Ringer (containing [in mM]: 115 NaCl, 2 KCl, 10 MgCl₂, 1 EGTA, 5 HEPES, pH 7.0), washed and incubated for 1–2 h (rolled oocytes; Miledi and Woodward, 1989b); and (c) follicles with the inner ovarian epithelium and thecal layers removed manually and incubated in sterile Barth's solution at 18°C for three or more days, conditions in which the follicles lost the follicular cell layer or it could be easily removed using forceps.

In contrast to previous studies the membrane current responses were usually monitored first in a hypotonic Ringer (HR) solution containing (in mM): 88 NaCl, 2 KCl, 1.8 CaCl₂, 5 HEPES, pH 7.0, using a conventional two microelectrodes voltage-clamp technique (Miledi, 1982). Unless otherwise stated, oocytes and follicles were voltage clamped at -60 mV and drugs were applied via the superfusing fluid. Solutions with higher osmolarity were made by adding sucrose or NaCl. The NaI-HR and NaBr-HR solutions were made by substituting the corresponding salt for NaCl. In some cases 50% of the NaCl in HR was substituted by TRIS-HCl, or 10 mM NaCl by KCl. The low Cl⁻ (34 mM) HR solution contained (in mM): 28 NaCl, 30 Na₂SO₄, 30 sucrose, 2 KCl, 1.8 CaCl₂, 5 HEPES, pH 7.0.

Injection of ethylene glycol-bis(β-aminoethylether)*N,N,N',N'*-tetraacetic acid (EGTA, 200–400 mM) and 1,2-bis(2-aminophenoxy)ethane *N,N,N',N'*-tetraacetic acid (BAPTA, 400 mM)

in the follicles was made by pneumatic pressure ejection from micropipettes (Miledi and Parker, 1984), the solution containing 5 mM HEPES was adjusted to pH 7.0 with KOH.

FSH from porcine pituitary was purchased from Calbiochem (La Jolla, CA). ACh, atropine, pirenzepine, 4-(*N*-[3-chlorophenyl] carbamoyloxy)-2-butyryl trimethylammonium chloride (McN A-343), tubocurarine, quinidine, verapamil, EGTA and BAPTA were from Sigma Chemical Co. (St. Louis, MO).

RESULTS

Components of the Membrane Currents Elicited by FSH and ACh in Follicle-enclosed Oocytes

Using HR solution, the average resting potential in 255 follicle-enclosed oocytes (27 frogs) was -32 ± 7 mV (mean \pm SD). With the follicles voltage clamped at -60 mV and equilibrated in HR the typical responses to FSH showed three components. The first (1 in Fig. 1 *A*) was an outward current (FSH- K^+), associated with an increase in K^+ conductance caused by an intracellular rise in cAMP (Woodward and Miledi, 1987*a*). This first component was frequently cut short by the development of a second component (2 in Fig. 1 *A*) a slow and smooth inward current (FSH- S_{in}), also associated with an increase in membrane conductance, and the third (3 in Fig. 1 *A*) was a slow and long lasting outward membrane current that was associated with a decrease in membrane conductance. In the first example of follicle currents illustrated in Fig. 1 *A* this third component was unusually large, but in general it was less noticeable.

Follicle responses to ACh typically showed four components (Fig. 1 *B*): (*a*) an early, and very common, small transient chloride current (F_{in} ; Miledi and Woodward, 1989*a*); (*b*) a larger slow, smooth inward current (ACh- S_{in}) which resembles in many respects the second component of the current elicited by FSH; (*c*) the previously described outward current associated with an increase in membrane conductance to K^+ (ACh- K^+ current); and (*d*) an outward current associated with a decrease in the membrane conductance which, in general, was not very long lasting. In some frogs, the responses to ACh showed more complex characteristics and included the oscillatory currents (Kusano et al., 1977, 1982; see also Fig. 8, *B-F*). The outward currents associated with a decrease in conductance were not systematically studied here, but it appears that these currents depend on the presence of an underlying "resting" current which is reduced upon application of the agonists.

The different phases of the responses to FSH and ACh varied in size and normally overlapped causing the shape of the overall current to differ between different follicles (see examples in Fig. 1). Nevertheless, besides the K^+ currents, the most common component of the follicle responses to FSH and ACh was the slow smooth inward current, which had the characteristics described below.

Ionic Basis of S_{in} Currents

Follicles with large inward current responses to FSH and ACh were used to study the ionic basis of the slow smooth current. Because the more characteristic inward current response in *Xenopus* oocytes is the Ca^{2+} dependent Cl^- current, we were

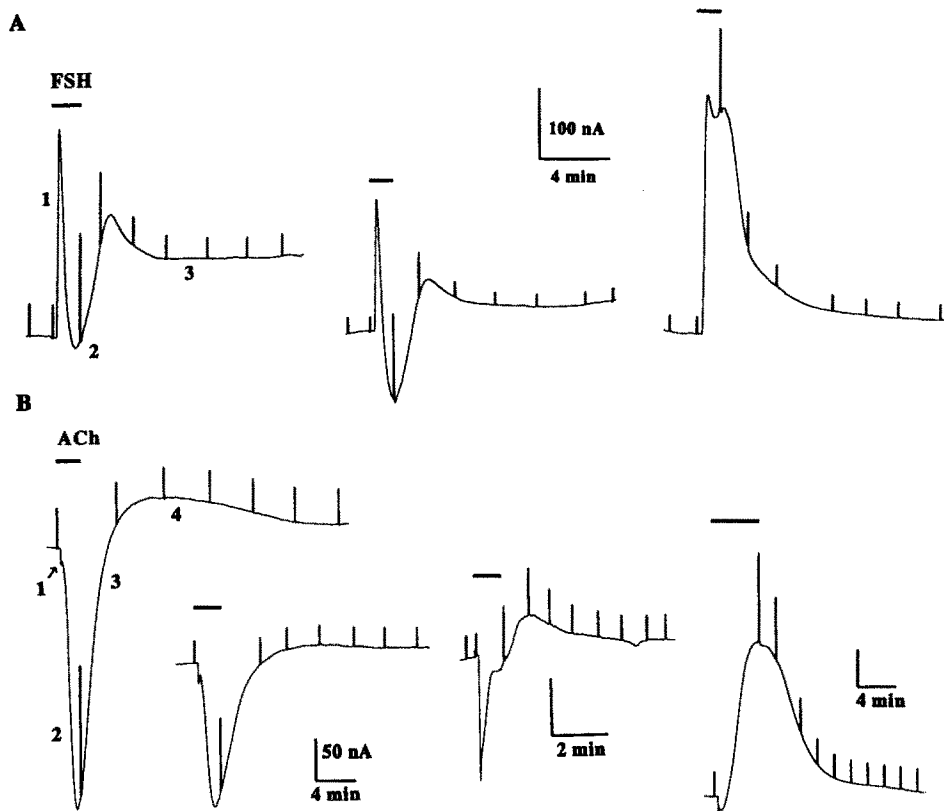


FIGURE 1. Native follicle responses to FSH and ACh in HR solution. (A) Currents elicited by $1 \mu\text{g ml}^{-1}$ FSH in three follicles (from different frogs) in HR solution (unless otherwise specified, in this, and following figures, the follicles were clamped at -60 mV , and the drugs were applied during the time indicated by the bars). The records illustrate the different components of the responses (numbered in the first record). In the first and second follicles the FSH- K^+ currents (phase 1) were cut short by the smooth slow inward current (S_{in} , phase 2), which was small in the third follicle. During the wash an outward current was disclosed (phase 3). This was accompanied by a decrease in conductance as evidenced by the amplitude of periodic $+10 \text{ mV}$ steps (3–5 s). In the first follicle, the third component was unusually large. (B) Native responses to $10 \mu\text{M}$ ACh in four follicles (from different frogs) showing four current components: a fast and transient inward current (F_{in} , phase 1); a slow and smooth inward current (S_{in} , phase 2); an outward current associated with an increase in K^+ membrane conductance (phase 3); and an outward current associated with a decrease in conductance (phase 4). The first two records illustrate the more usual responses in follicles.

interested to find out if the follicle slow current was generated through the same mechanism.

Current-voltage (I/V) relations for S_{in} currents were obtained in follicles that were usually clamped at -60 mV and superfused with HR to which BaCl_2 (1–2 mM) was added to block the outward K^+ currents frequently elicited by FSH and ACh. Voltage steps, with duration of 2 to 4 s and variable amplitude, were applied before exposing

the follicle to agonist and again at the peak of the current. The pulse current during the peak was subtracted from the current before application of the agonist, and the data plotted as in Fig. 2A. Similar I/V relations were obtained by measuring the currents after clamping the follicles at different holding potentials (Fig. 2B). The I/V relations for the oscillatory current were made using this latter method.

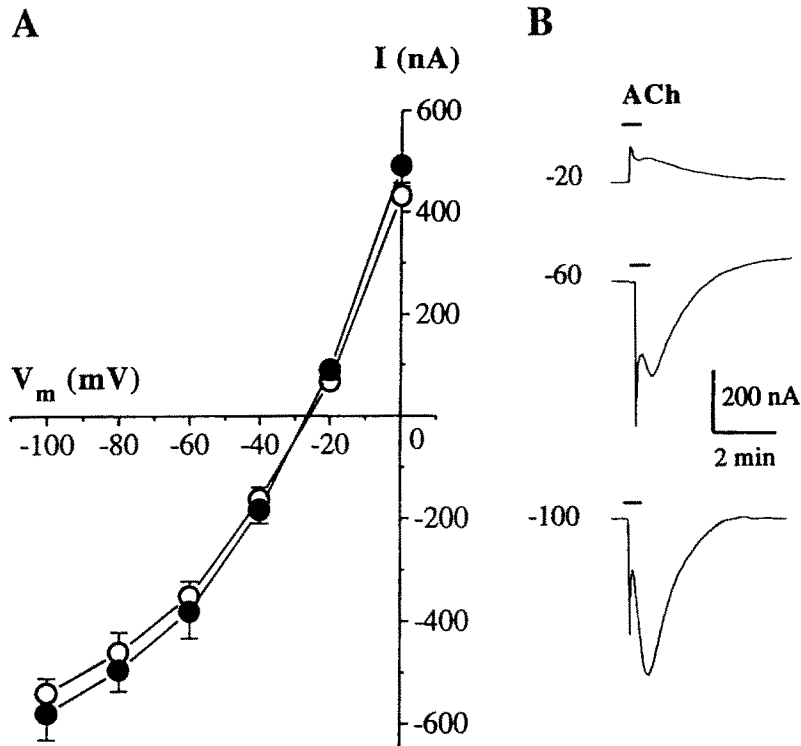


FIGURE 2. Current-voltage relations of S_{in} currents elicited by FSH and ACh. (A) I/V relation for FSH ($1 \mu\text{g ml}^{-1}$, ●) and ACh ($10 \mu\text{M}$, ○) determined in follicles in HR with 2 mM BaCl_2 to block K^+ currents, and with 2–4-s pulses to various potentials before and during the drug application. Each point represents the mean (\pm S.D.) of 11–12 follicles. Note that the reversal potential for both curves had a similar value of $-27 \pm 2.2 \text{ mV}$. (B) Currents elicited by ACh ($10 \mu\text{M}$) in a follicle maintained at the indicated potentials. In this follicle, S_{in} and F_{in} currents had a similar reversal potential, $\sim -28 \text{ mV}$.

The slow smooth inward current showed the same reversal potential (Fig. 2A), whether it was elicited by FSH (11 follicles, three frogs) or by ACh (12 follicles, three frogs), with an average of $-27 \pm 2.2 \text{ mV}$, corresponding to the equilibrium potential for chloride ions in *Xenopus* oocytes (Kusano et al., 1982). The currents showed a weak rectification at potentials more negative than about -60 mV . This was not due to the presence of Ba^{2+} in the external medium, because in follicles tested with ACh in HR without Ba^{2+} almost the same rectification was obtained (Fig. 3); although the

reversal potential was shifted to a more negative potential (-30 ± 2 mV, 10 follicles, three frogs) suggesting that the outward K^+ current was contributing to the total currents in these oocytes. If ACh was applied after reducing the HR concentration of chloride to 34 mM, using SO_4 as principal anion (Fig. 3), the slow smooth currents decreased in amplitude and their reversal potential shifted to more positive potentials, -17 ± 2 mV (eight follicles, two frogs; vs -6 mV value expected from Nernst equation) even in the presence of small ACh- K^+ currents. Moreover, when slow smooth currents elicited by ACh were tested in HR with 50% of the NaCl substituted by Tris-HCl, or 10 mM of NaCl by KCl, there were not significant changes in the

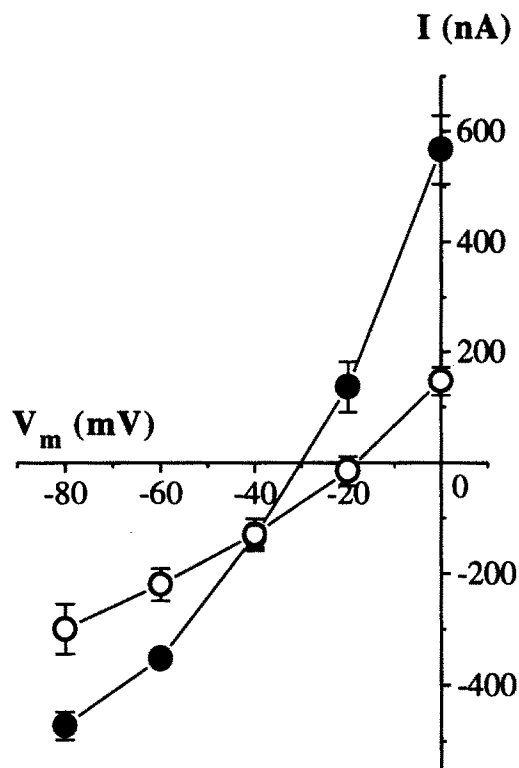


FIGURE 3. Effect of reducing external Cl^- on I/V relation of S_{in} . I/V relations were obtained by giving pulses to different potentials before and during S_{in} elicited by ACh ($10 \mu M$) in HR without Ba^{2+} with 94 mM Cl^- (●) or 34 mM Cl^- (○). The reversal potential in HR was of -30 ± 2 mV (10 follicles, 3 frogs) and in HR low Cl^- was of -17 ± 2 mV (eight follicles, two frogs).

reversal potential. All these experiments strongly suggest that the membrane channels mediating the slow smooth inward currents are permeable mainly to Cl^- .

Effects of I^- and Br^- on S_{in} and F_{in} Currents

The permeability of the channels responsible for the S_{in} and F_{in} to other anions was tested by equilibrating the follicles in solutions in which Cl^- was replaced by I^- and Br^- and then exposing the follicles to ACh or FSH. Fig. 4A shows typical I/V curves for the ACh- S_{in} current in follicles bathed in HR solution (no Ba^{2+} added) and in HR solution in which all the NaCl was substituted by NaI (NaI-HR). The amplitude of the

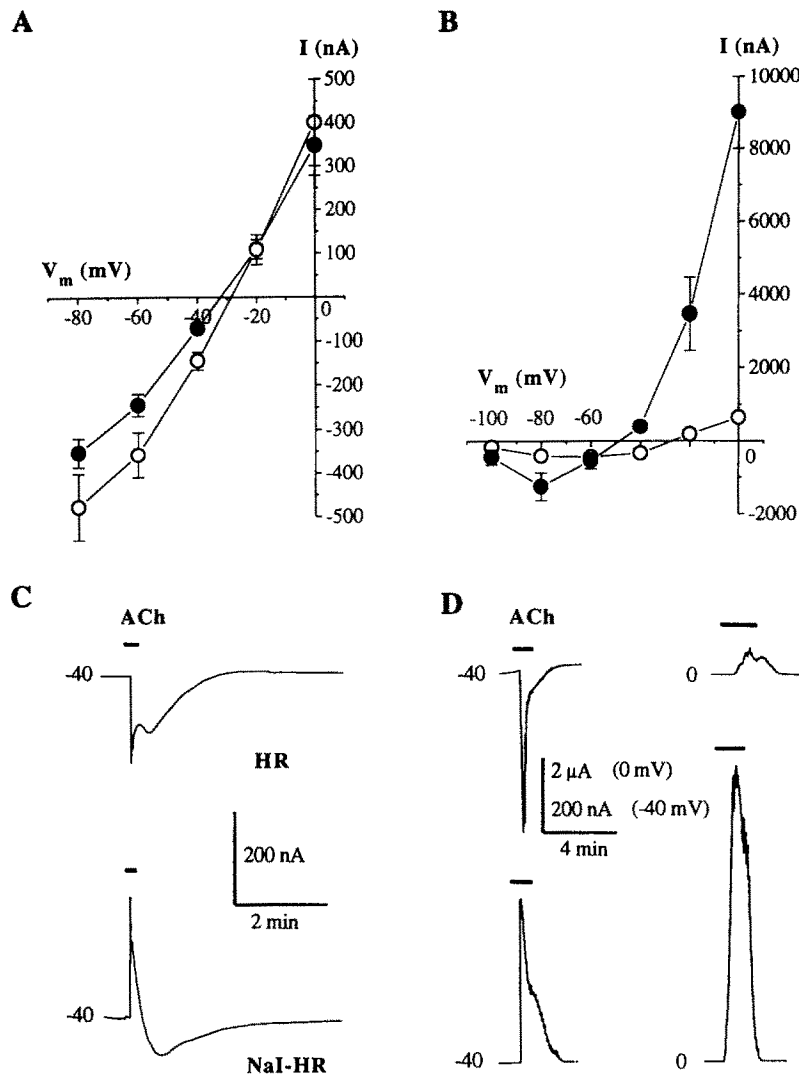


FIGURE 4. Effect of replacing Cl^- by I^- on follicle enclosed and defolliculated oocyte chloride currents. I/V relationships for the S_{in} currents (A) and oscillatory Cl^- currents (B) elicited by ACh ($10 \mu M$) in HR (○) and NaI-HR (●) solutions. S_{in} currents had nearly the same reversal potential in HR and NaI-HR (six follicles, two frogs). The reversal potential for the oscillatory current was -26 mV in HR and -48 mV in NaI-HR (three to four oocytes in each point). The records show sample currents elicited by ACh ($10 \mu M$) in a follicle clamped at -40 mV (C) and in a defolliculated oocyte clamped at -40 mV and 0 mV (D). In HR and at -40 mV the responses were inward currents (top traces); but in NaI-HR solution F_{in} , and the oscillatory Cl^- response developed as outward currents while S_{in} did not change in polarity.

S_{in} current was slightly reduced, and the reversal potential was not greatly altered, suggesting that the channels carrying the S_{in} current are nearly as permeable to iodide as to Cl^- . Similar results were obtained when the S_{in} current was tested using Br^- as the principal anion in the HR solution, and there was no difference if FSH (2 mM $BaCl_2$ added) was used in place of ACh to elicit the responses.

In contrast to the relatively small effect of substituting Cl^- for I^- on the S_{in} current, the oscillatory current responses elicited by stimulation of native muscarinic ACh receptors in HR and NaI-HR showed a great increase in current and a large shift in the reversal potential, which changed from -26 mV in HR to -48 mV in NaI-HR (Fig. 4, *B* and *D*), suggesting that in this case I^- was more permeable than Cl^- through the channel.

The F_{in} current seems to show permeability characteristics similar to those of the oscillatory response because its reversal potential was also shifted to negative values when tested in NaI-HR or NaBr-HR solutions. Fig. 4 *C* shows an example of F_{in} and S_{in} current responses to ACh in HR and in NaI-HR solutions, in a follicle clamped at -40 mV. Note that in the HR solution both S_{in} and F_{in} were inward currents; but when the same follicle was tested in the NaI-HR solution, the F_{in} current was outward whereas the S_{in} current did not change in polarity. These results, as well as the distinct osmolarity dependence (see below), suggest that S_{in} and F_{in} currents are mediated by the operation of different membrane channels.

Osmolarity Dependence of the Follicle-enclosed Oocyte Currents

The amplitude of the S_{in} current was strongly dependent on the osmolarity of the external solution. When follicles with large S_{in} currents in HR solution were also tested in NR (more than 32 follicles, four frogs) the size of the S_{in} currents decreased to $<5\%$ of the response in HR (Figs. 5, *A-B* and 6, *A-B*). In NR, the largest component of these responses were the outward K^+ current for FSH and the F_{in} current for ACh, both of which were not osmolarity dependent. Similar results were obtained if the follicles were tested first in NR and then equilibrated and tested in HR. The decrease in S_{in} current following an increase in osmolarity was a genuine decrease in S_{in} and not an artificial unmasking effect caused by changes in other response components.

A further effect of osmolarity was seen when the follicles were first equilibrated in NR, voltage clamped at -60 mV and then exposed to HR. After a delay of 0.5–4 min, outward and inward currents developed slowly (Fig. 7 *A*). These currents appeared to be associated with increases in conductance, principally to K^+ and Cl^- , as suggested by the equilibrium potentials of the currents (not shown) and by the blocking effect of Ba^{2+} on the outward component (Fig. 7 *B*). The amplitude and the relative sizes of the currents were very variable among follicles from different frogs, showing values from only a few nA to nearly a μA . These currents were completely reversible (Fig. 7, *B* and *D*) when NR (or HR + 54 mM sucrose) was reapplied, and they could be elicited several times in the same follicle (Fig. 7, *B* and *D*). As with the follicular responses to ACh and FSH these currents were completely eliminated by defolliculation of the oocytes (see below). When the follicles were maintained in HR, the inward currents inactivated slowly, returning to the NR basal levels of membrane conductance values in 20–35 min. (Fig. 7 *C*). In many follicles the hypotonic induced

outward current was still active after this time (Fig. 7 *A*), but follicles of the same frogs incubated for 5 h in HR (or Barth's medium, solution with similar HR osmolarity) also showed inactivation of this current.

As yet we do not know the relation between the hyposmotic medium induced currents and the currents elicited by the agonists. However, we have observed that full development of the currents during the hyposmotic shock did not prevent the generation of the complex currents elicited by FSH and ACh. Moreover, when the agonists responses were elicited during the peak of the inward current induced by HR their amplitudes appeared simply to add. This suggests that they may be

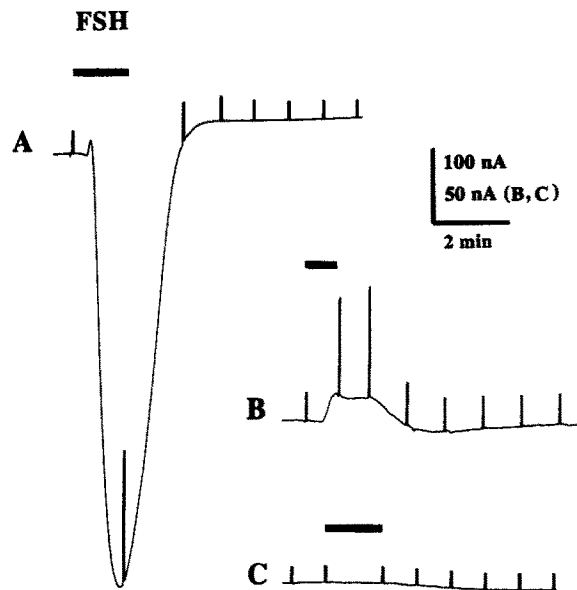


FIGURE 5. Effects of increased osmolarity and defolliculation on FSH elicited current responses. (*A*) Native responses to FSH ($1 \mu\text{g ml}^{-1}$) in a follicle clamped at -95 mV . At this potential the FSH-K^+ was almost completely masked by the S_{in} current. (*B*) Effect of increasing osmolarity. The same follicle was superfused for 30 min in NR and then re-exposed to the same concentration of FSH in NR. The main component of the current response was FSH-K^+ ; and the net S_{in} current was reduced to $<5\%$. (*C*) Effect of defolliculation. The follicular cells were removed by rolling the follicle over a poly-L-lysine coated slide, and after 1 h the defolliculated oocyte was re-exposed to FSH in HR (and in NR, not shown), all the responses previously elicited by the hormone were abolished.

generated through different mechanisms or by activation of different populations of channels. Activation of membrane currents by hyposmotic stress has been reported in other preparations, where it has been associated with regulation of cellular volume (Grinstein, Rothstein, Sarkadi, and Gelfand, 1984; Falke and Misler, 1989; McCann, Li, and Welsh, 1989). However, quinidine ($100 \mu\text{M}$, four follicles) and verapamil ($100 \mu\text{M}$, three follicles), which are known to be blockers of volume sensitive Cl^- channels (e.g., Valverde, Diaz, Sepulveda, Gill, Hyde, and Higgins, 1992) did not show effects on the currents generated in follicles by the hyposmotic solution (Fig. 7 *D*).

Role of the Follicular Cells in the Generation of the Responses to FSH and ACh

The various components of the responses elicited by FSH and ACh (and the currents generated by hyposmotic stress) were strictly dependent on the presence of the follicular cell layer electrically coupled to the oocyte. With the exception of the oscillatory Cl^- currents activated by ACh, defolliculation completely eliminated all the components of the responses to both agonists (Figs. 5 C and 6 C). This was the case with all three methods used to defolliculate the oocytes: (a) collagenase treatment for 1 h typically eliminated the responses of the follicles (16, two frogs). In two oocytes where $\sim 10\%$ of the S_{in} and F_{in} responses persisted after the treatment

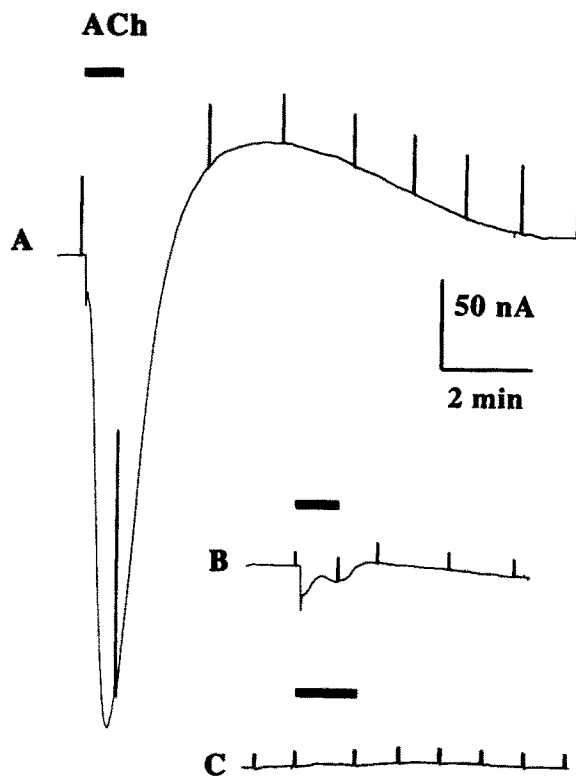


FIGURE 6. Effects of increased osmolarity and defolliculation on ACh elicited current responses. (A) Native responses elicited by ACh ($10 \mu\text{M}$) in a follicle clamped at -60 mV in HR solution. (B) Effect of increased osmolarity. The same follicle was again tested to ACh in NR after an equilibration period of 20 min; the ACh- S_{in} current was reduced to less than 5%, F_{in} did not show significant changes, and the net outward current was slightly reduced. (C) Effect of defolliculation. The oocyte was defolliculated (as in Fig. 5 C) and exposed to ACh in HR (and NR, not shown) solution 1 h later; all the currents previously elicited by ACh were abolished.

with collagenase, it was also observed that the treatment did not eliminate all the FSH- K^+ current in the same oocytes, implying incomplete uncoupling and removal of follicular cells. (b) The manual removal of the external layers and rolling of the follicles over poly-L-lysine-treated slides consistently eliminated the FSH and ACh responses (21, six frogs). (c) Manual removal of the epithelial and thecal layers and incubation of the follicles (25, six frogs) for 2 or 3 d in Barth's solution normally eliminated all the responses. During any stage of incubation, the oocytes could be rolled and residual responses were completely eliminated. In addition, follicles incubated for 5–7 d in Barth's solution lost their capability to generate S_{in} and F_{in}

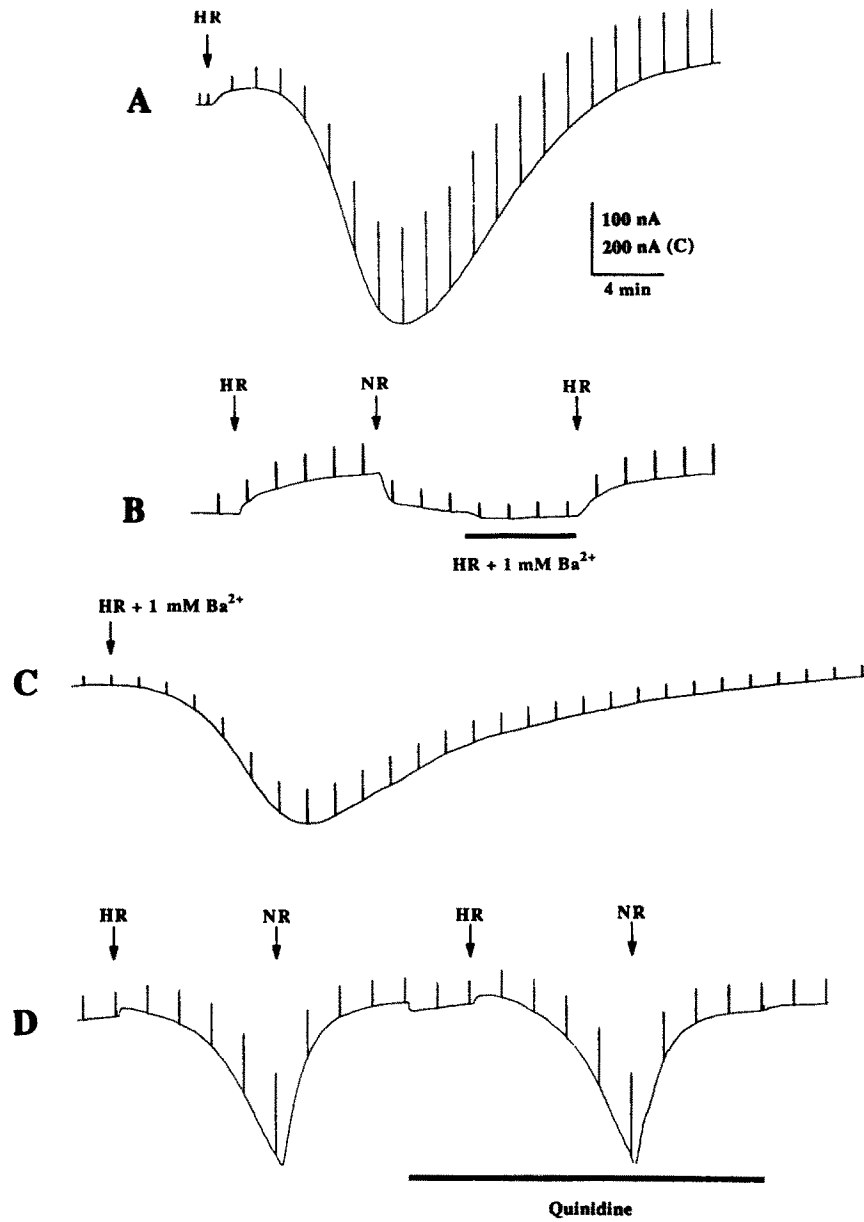


FIGURE 7. Effects of hyposmotic shock on follicle-enclosed oocytes. The four follicles (voltage clamped at -60 mV, pulses to -50 mV) were first equilibrated in NR (NR + 1 mM BaCl₂ in C), and at the arrows the external medium was changed to HR, or back to NR. (A) An example of currents generated by the application of HR. Note the multiphasic development of the current and disappearance of net inward current. (B) An example of a follicle in which HR generated mainly an outward current, the current was associated with an increase in conductance and was

currents upon the application of ACh and FSH, at a similar rate as FSH-K⁺ currents (Woodward and Miledi, 1987a; Miledi and Woodward, 1989a); however, all these currents were better preserved when the follicles were incubated in the modified Barth's medium. All these experiments strongly indicate that the receptors for FSH and ACh involved in the generation of these particular responses are located in the membrane of the follicular cells.

Relation Between Osmolarity and S_{in} Current Amplitude

The quantitative dependence of the S_{in} current on the osmolarity of the external medium was studied by testing the responses to ACh in Ringer solution with different osmolarity. For most of these experiments ACh (10 μM) was used to elicit the S_{in} current because, in contrast to FSH, the follicle responses to ACh showed consistently less desensitization and there was also less activation of the outward K⁺ currents. Furthermore, the F_{in} current elicited by ACh did not show a dependence on osmolarity (Figs. 6 B and 8, B–E), thus serving as a good control of the stimulation of ACh receptors, and of follicular coupling to the oocyte.

The osmolarity of the HR solution, 190.4 mosM, was used as the starting point and was increased by adding sucrose or NaCl (an increase of 54 mosM corresponded to the osmolarity of the NR solution), both of which gave similar results. Averaged peak currents for three to six follicles (two frogs) at each osmolarity were normalized with respect to the current responses obtained in HR and plotted as a function of the increase in osmolarity (Fig. 8 A). The S_{in} currents showed an exponential decrease to 50% of the normal response when 18–20 mosM were added to HR.

Fig. 8, B–F shows examples of the responses obtained in the two extreme conditions of osmolarity, HR and NR, for different follicles, to illustrate the large variability in proportions of the various components of the follicular cell-dependent currents and of the oscillatory Cl⁻ currents generated in the oocyte membrane by ACh (10 μM). In the first case (Fig. 8 B) the responses were totally dependent on the follicular cell layer, because they disappeared completely when the follicular cells were removed (see also Fig. 6, A–C). S_{in} decreased more than 95% after changing the bathing medium from HR to NR, but F_{in} showed no changes. The records in Fig. 8, C and D, show follicles with current responses of mixed origin: S_{in} and F_{in} from the follicular cells along with the characteristic oscillatory Cl⁻ current generated in the oocyte membrane. Once again S_{in} was greatly reduced in NR compared to the value obtained in HR, while F_{in} and the oscillatory current were practically unchanged, although in some cases the oscillatory component was slightly increased after changing to NR.

Follicles with oscillatory responses, like that illustrated in Fig. 8 D, developed a slow increase in current which was not the S_{in} current because it was not dependent on the osmolarity of external medium and was not eliminated by defolliculation (four

potently blocked by 1 mM Ba²⁺ added to HR (solid bar). (C) Temporal course of the follicle inward current generated by application of HR in the presence of 1 mM Ba²⁺. (D) Quinidine (100 μM) did not block the inward and outward currents elicited by hyposmotic shock. Note the fast closing of currents elicited by hyposmotic medium when NR was reapplied; and note also that the currents generated by HR could be elicited repeatedly.

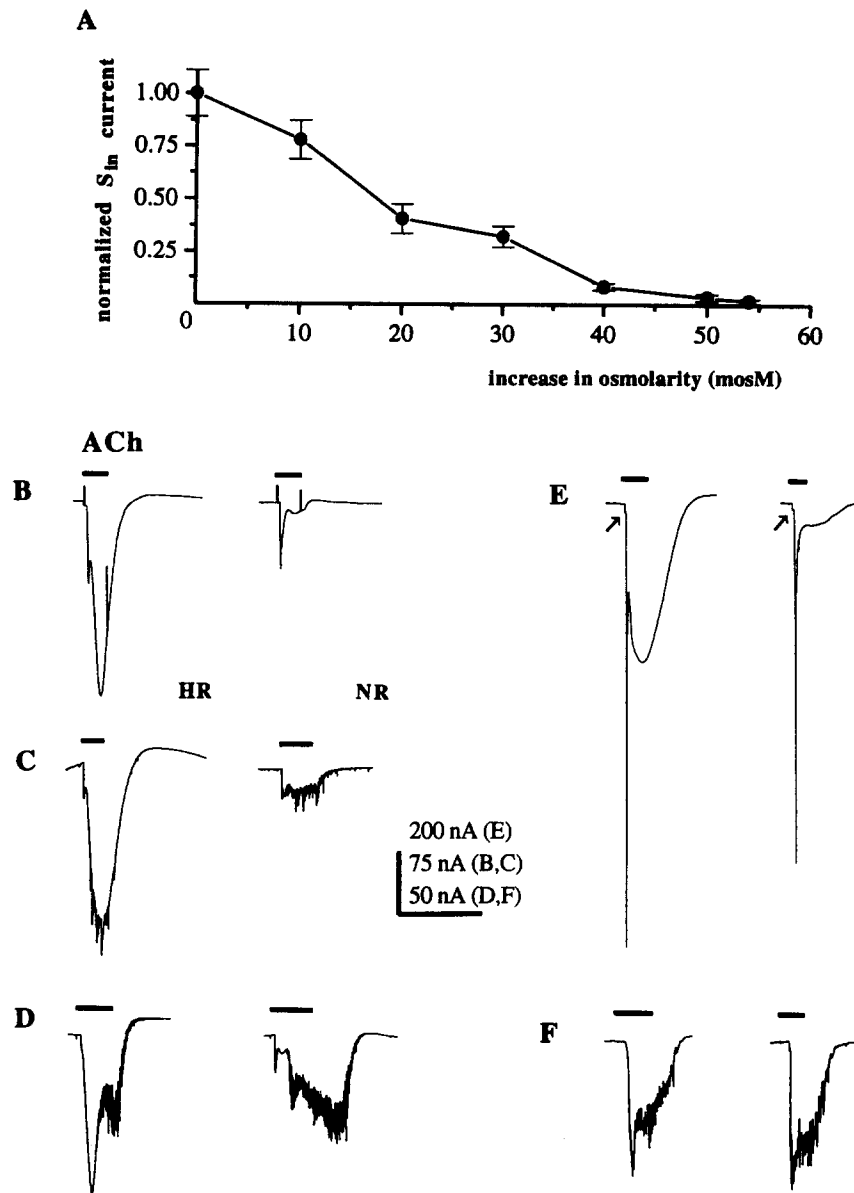


FIGURE 8. Relationship between the ACh- S_{in} current and changes in osmolarity of the external solution. (A) The point at zero corresponds to the current response to ACh ($10 \mu\text{M}$) in HR solution (190.4 mosM), and the peak values of the response in the different osmolarities after adding sucrose were normalized to that point. Each point is the mean \pm SD for 3–6 follicles tested from two frogs. The amplitude of S_{in} currents decreased 50% upon addition of 18–20 mosM. The records are examples of different responses to ACh ($10 \mu\text{M}$) in follicles clamped at -60 mV in the two extreme conditions of osmolarity, HR (first record in each pair) and NR. (B) The F_{in} current was not affected by the change in osmolarity, S_{in} was greatly reduced. (C and D) Examples of responses with a mixture of the follicle F_{in} and S_{in} currents,

follicles, two frogs). An example of this is shown in Figure 8 *F*, obtained from a batch of defolliculated oocytes (rolled oocytes) that showed responses like those in Fig. 8 *D* before the removal of the follicular cells. As expected, the defolliculation eliminated ACh- S_{in} and F_{in} ; but the oscillatory responses were preserved and similar in both HR or NR solution.

In still a further type of follicle response to ACh, there was a large inward current spike (Fig. 8 *E*), common in oocytes with very high sensitivity to the agonist. Sometimes, this spike developed after F_{in} ; it was not affected by defolliculation and was not appreciably altered by changes in osmolarity (six follicles, two frogs). In the example shown, most of the subsequent slow current corresponded to S_{in} , because it was osmolarity dependent and was eliminated by defolliculation.

In some follicles all the current components illustrated in Figure 8B–F were observed. Furthermore, ACh also elicited a slow smooth current that clearly differed from S_{in} because it was not osmolarity-dependent, it developed 2–3 times slower and it did not require the presence of the follicular cell layer. This current is presumably the one described previously (Parker, Gundersen, and Miledi, 1985a; Parker and Miledi, 1987), and is generated in the oocyte membrane.

Dose-response Relations and Pharmacology

All the FSH and ACh components of the responses showed a clear dose dependence. Follicles with a large sensitivity to FSH had a threshold, for S_{in} , at concentrations around $0.1 \mu\text{g ml}^{-1}$ and a maximum current response was reached with 2 to $2.5 \mu\text{g ml}^{-1}$. The concentration required to elicit half-maximal response was around 7.5 nM, taking 36000 as the FSH molecular weight (Fig. 9 *A*), the dose response curve showed a steep slope, thus suggesting nonlinearities in receptor-channel coupling mechanism. In all cases the S_{in} current elicited by FSH desensitized strongly upon repeated application of agonist. For example, three to four applications of FSH, at 15–20-min intervals, reduced S_{in} amplitude to 10–15% of the original response, even when low concentrations of FSH were used. The threshold concentration for ACh currents was usually between 1 to 2.5 nM, but in some follicles F_{in} required around 100 nM. The maximal S_{in} and F_{in} responses to ACh were reached with concentrations of 2.5 to 5 μM ACh, and the half-maximal response for ACh- S_{in} occurred with ~ 260 nM (Fig. 9 *A*).

As already stated, there was considerable variation in the maximal current responses among different follicles from different frogs. Out of 340 follicles, from 36 frogs only three follicles, all from the same frog, failed to give any response to FSH or ACh; and in six follicles from another frog the response was mainly the ACh outward

and the oocyte membrane oscillatory Cl^- currents. In both cases S_{in} currents decreased when the follicles were in NR, but F_{in} and the oscillatory currents did not show significant changes. (*E*) Responses in follicles in which ACh generated a large spike current, besides the follicular F_{in} (arrows) and S_{in} responses in HR solution. Again, in NR the S_{in} current was drastically reduced. The large spike was not eliminated by defolliculation. (*F*) Defolliculated oocyte from the same frog as used for (*D*) showing the oscillatory Cl^- current preserved and similar in HR and NR. Horizontal calibrations: for *B* and *D* 3 min, for *C*, *E* and *F*, 2.5 min.

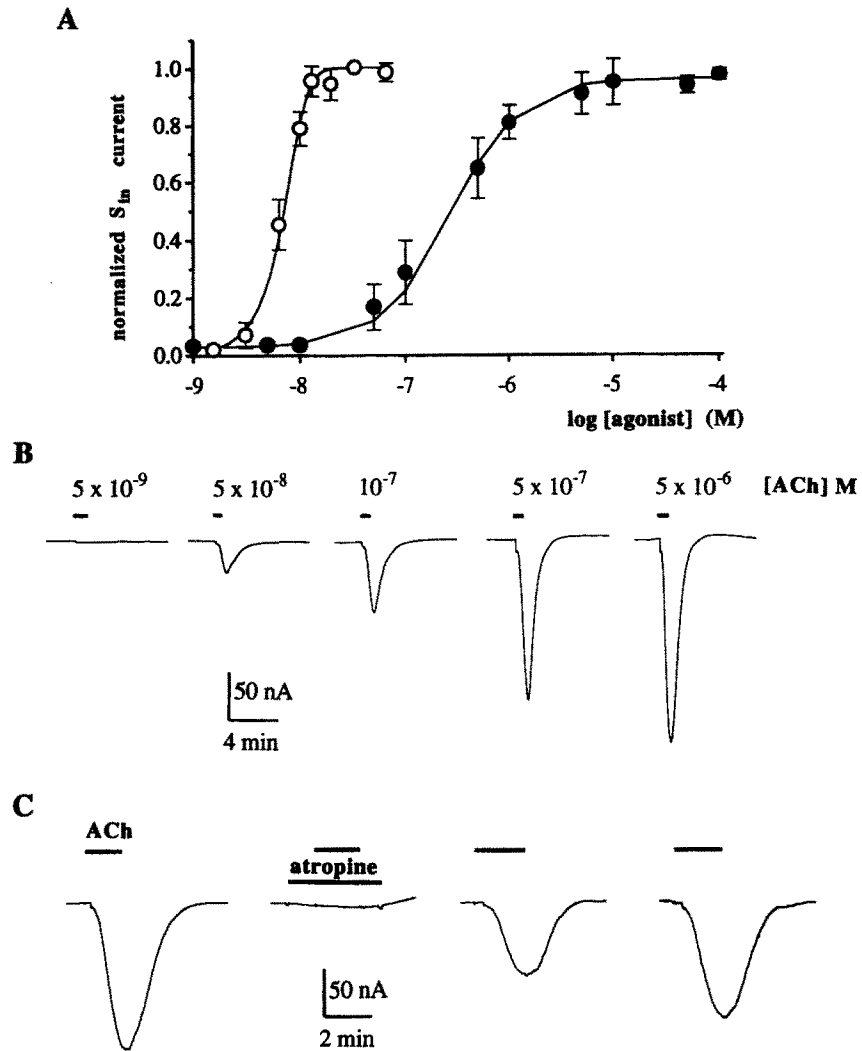


FIGURE 9. Dose-response relation of S_{in} currents and effect of atropine on ACh elicited currents. (A) Normalized S_{in} currents vs concentration of FSH (○) or ACh (●), the half-maximal response occurred with 7.5 and 260 nM, respectively. Each point represents the normalized mean current (\pm SD) elicited by the first application of the agonist in three to five follicles. The records in B show an example of follicle responses to different concentrations of ACh, note the short delay in the onset of responses even at low agonist concentrations. (C) The records show consecutive applications of ACh (10 μ M) on the same follicle with wash periods of 15 min. Note that atropine (10 nM) was a potent and reversible blocker of the follicular F_{in} and S_{in} currents elicited by ACh.

K⁺ current. So far we have not discerned any clear relation between the amplitudes of the responses elicited by FSH and ACh in the same follicles, either of the two agonists could be the most effective. For example, in three follicles from one frog, tested in HR solution with 2 mM BaCl₂ and at a holding potential of -60 mV, the FSH-S_{in} response had a mean of 37 ± 15 nA, and after a wash period of 20 min, the same follicles gave a S_{in} current response to ACh with a mean of 287 ± 65 nA. In follicles from other frogs, the relation was inverted, for example, in one case (four follicles) the FSH-S_{in} current had a mean of 240 ± 76 nA while the ACh-S_{in} was 83 ± 30 nA. There were also frogs with follicles that had the same high, or low, sensitivity to both agonists.

The F_{in} current was even more variable, and in 20% of the follicles there were no appreciable F_{in} responses, whilst in the other 80%, the mean amplitude in 63 follicles was 58 ± 40 nA. This does not include data from one frog (five follicles) in which the F_{in} had an unusually large amplitude with a mean of 540 ± 96 nA.

An important characteristic observed from the dose-response experiments was that ACh-F_{in} and ACh-S_{in} current responses showed comparatively short latencies (Fig. 9 B), and these latencies were not greatly increased when the currents were elicited by low agonist concentrations. This contrasts with the oscillatory current responses to ACh (Kusano et al., 1977, 1982; Miledi and Parker, 1989), serotonin (Gundersen, Miledi, and Parker, 1983), serum (Tigyi, Dyer, Matute, and Miledi, 1990) and divalent cations (Miledi et al., 1989b) all of which may show onset delays of several minutes when they are triggered near the threshold agonist concentration.

Similar to the oscillatory Cl⁻ current of the oocyte membrane, the follicular ACh-F_{in} and ACh-S_{in} currents were elicited through the activation of muscarinic receptors. The antagonists atropine (10 nM, Fig. 9 C) and pirenzepine (1 μM) strongly blocked the responses elicited by 10 μM of ACh (five follicles, two frogs), whilst tubocurarine had no effect on the same follicles. McN A-343 (10 μM) a selective agonist of M1 muscarinic receptor, did not elicit any response in the follicles (three follicles, two frogs). Both atropine (two follicles) and pirenzepine (two follicles) did not block the generation of the S_{in} current by FSH, indicating that the FSH response was not a secondary effect caused by the release of ACh from some intracellular source.

Intracellular Ca²⁺ and the Responses Elicited by ACh

It is well known that the Ca²⁺ dependent oscillatory Cl⁻ currents generated in the oocyte membrane are activated through the production of IP₃ and mobilization of intracellular calcium (Parker, Gundersen, and Miledi, 1985b; Parker and Miledi, 1986, 1987). Therefore, we examined whether the ACh-S_{in} and the F_{in} currents were also dependent on an increase in intracellular Ca²⁺. For these experiments we monitored the responses elicited by ACh in control follicles and in follicles after Ca²⁺ chelation by an intra-oocyte injection of EGTA (100 to 500 pmol) or BAPTA (100 to 200 pmol). Both substances had practically no effect on the S_{in} and F_{in} currents.

For these experiments, we followed two protocols: (a) the follicles (14 from two frogs) were first tested with ACh (10 μM). A few minutes later, the oocytes were injected with EGTA and after a further 15 or 30 min ACh was reapplied. The

amplitude of the control responses were compared with those obtained after EGTA. In a typical example of these experiments the F_{in} current showed a decrease from 112 ± 39 to 57 ± 24 nA and the S_{in} currents from 210 ± 50 to 174 ± 60 nA in nine oocytes injection with EGTA. Similar results were obtained after a second injection of EGTA. In some cases the voltage activated outward Cl^- current (T_{out}) was monitored with 3–5 s-depolarizing pulses from -100 to $+10$ mV. This outward currents has been shown to be Ca^{2+} dependent (Miledi, 1982) and was completely abolished within the first minutes after EGTA injection.

(b) To avoid the possibility of a decrease in current amplitude due to desensitization, we tested alternatively control follicles and follicles that had been injected with EGTA without prior exposure to ACh (41 follicles, three frogs, Fig. 10 A). Here again the responses to ACh persisted well after EGTA loading and had a mean amplitude of 30 ± 7 nA for F_{in} currents and 234 ± 40 nA for S_{in} currents, whilst the control

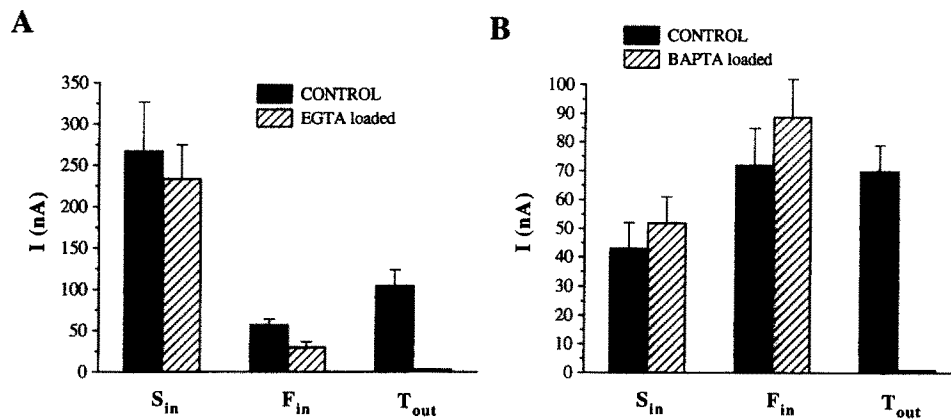


FIGURE 10. Effect of chelating intracellular Ca^{2+} on the S_{in} and F_{in} currents. (A) Average amplitudes (\pm SD) of S_{in} and F_{in} currents elicited by ACh (10μ M) and of the T_{out} currents in control follicles ($n = 10$) and in EGTA loaded follicles ($n = 10$). All the follicles were tested the same day at -60 mV in HR. (B) Similar experiment but in this case BAPTA was used to chelate intracellular Ca^{2+} and the currents were monitored in NR (nine follicles in each group).

follicle currents showed amplitudes of 57 ± 7 nA and 267 ± 60 nA for F_{in} and S_{in} , respectively. Here again, the T_{out} currents were monitored to test the effectiveness of the injection.

In another experiment we used the later protocol but loaded the oocytes with BAPTA (Fig. 10 B). This had even less effect than EGTA on the F_{in} and S_{in} currents and we could not detect any difference in the amplitude of the currents elicited by ACh in control and BAPTA injected oocytes; even though the T_{out} currents were completely abolished.

Thus, in no case were the responses elicited by ACh abolished after chelation of intracellular Ca^{2+} , and preliminary experiments suggest the same result for the FSH responses. Therefore, in contrast to the oscillatory and the T_{out} currents, the generation of the F_{in} and S_{in} currents is not strictly dependent on intracellular Ca^{2+} .

DISCUSSION

Novel Cl⁻ Currents in Xenopus Follicles

Our results show that, in follicle-enclosed *Xenopus* oocytes, FSH and ACh elicit fast and slow membrane currents, F_{in} and S_{in} , that are carried mainly by chloride ions, and suggest further that the S_{in} currents elicited by both agonists may be due to the opening of the same type of channels. Moreover, F_{in} and S_{in} currents showed very different characteristics from those of the classical oscillatory Ca^{2+} dependent Cl^- currents elicited in the native oocyte membrane by ACh, AII, serum factor and divalent cations (Kusano et al., 1977, 1982; Miledi et al., 1989b; Tigyi et al., 1990; Woodward and Miledi, 1991).

For example, (a) The I/V relation of S_{in} currents showed only a weak rectification at potentials more negative than ~ -60 mV, in contrast to the strong rectification of the oscillatory currents.

(b) During S_{in} currents elicited by either FSH and ACh the membrane permeability to I^- and Br^- was about the same as for Cl^- . In contrast, during the oscillatory and F_{in} currents the oocyte membrane was more permeable to I^- and Br^- , increasing the total currents and shifting their reversal potential to more negative values. The anion permeability of the channels involved in the S_{in} currents is interesting because most Cl^- channels described in other preparations display a higher permeability to I^- and Br^- than to Cl^- . Some notable exceptions are: (i) the Cl^- channel from the *Torpedo* electroplax membrane which is impermeable to I^- (Miller and White, 1980) but shows almost the same selectivity for Br^- and Cl^- ; (ii) the cAMP dependent- Cl^- channel of T84 cells and airway epithelial cells and which is defective in cystic fibrosis (Cliff and Frizell, 1990; Anderson and Welsh, 1991), which shows a permeability sequence $Cl^- > I^-$; (iii) the cystic fibrosis transmembrane regulator (CFTR) which, when expressed in HeLa cells (Anderson, Gregory, Thompson, Souza, Paul, Mulligan, Smith, and Welsh, 1991), generates cAMP dependent Cl^- currents that show a sequence permeability $Br^- \geq Cl^- > I^-$; and (d) CIC-2, a voltage gated Cl^- channel that is widely expressed in many kinds of cells with a permeability to $Cl^- \geq Br^- > I^-$. The nucleotide sequence of this channel is 50% identical to that of the *Torpedo* electroplax Cl^- channel (Thiemann, Gründer, Pusch, and Jentsch, 1992). Moreover, it has been demonstrated that the activity of the CIC-2 chloride channel can be increased in a hypotonic external solution (Gründer, Thiemann, Pusch, and Jentsch, 1992).

In our experiments, S_{in} currents elicited by ACh and FSH in NaI-HR did not show a substantial shift in reversal potential current, although the slope conductance of the currents was slightly decreased. It may be that iodide alters the channel's kinetics and/or it may be possible that the reversal potential is somewhat underestimated due to small K^+ currents during the responses. Even so, it is clear that I^- and Br^- ions were not more permeable than Cl^- . The simplest explanation of our results is that the Cl^- channels mediating the S_{in} currents and those mediating the oscillatory and F_{in} currents are intrinsically different.

(c) The S_{in} currents showed a strong dependence on the osmolarity of the external medium, increasing around 8–10 times with a decrease of 54 mosM in the normal

Ringer solution (from 244.4 to 190.4 mosM). In contrast the oscillatory Cl^- and F_{in} currents were not changed appreciably by the same change in osmolarity.

(d) In contrast to the oscillatory currents, the S_{in} and F_{in} follicular currents were not abolished after chelation of the intracellular Ca^{2+} by injection of EGTA or BAPTA into the oocyte, suggesting that channels and the receptor-channel coupling mechanism are not Ca^{2+} dependent.

(e) The S_{in} currents, the F_{in} transient current and the outward currents elicited by FSH and ACh all required the presence of follicular cells. The use of different methods of defolliculation, enzymatic and manual, reduces the possibility that the loss of the responses was a consequence of damage to the oocyte membrane, or to the receptor-channel coupling mechanism; and clearly indicates that the responses originate in the membrane of the follicular cells. In contrast to this, and in agreement with previous works (Kusano et al., 1982; Miledi and Woodward, 1989a; Miledi et al., 1989b), the oscillatory Cl^- responses were well preserved after the various procedures used to defolliculate the oocytes.

Receptors and Channels in Follicular Cells

It has previously been demonstrated that follicle-enclosed oocytes carry gonadotropin receptors (Browne et al., 1979; Adashi and Hsue, 1981), whose stimulation elicits an increase in cAMP levels (Greenfield, Hackett, and Linden, 1990) leading to activation of K^+ channels (Woodward and Miledi, 1987a). Several experiments indicate that the receptors to gonadotropins, as well as the K^+ channels, are all in the membrane of the follicular cells (Woodward and Miledi, 1987a; Miledi and Woodward, 1989a).

The presence of muscarinic ACh receptors on the surface membrane of *Xenopus* oocytes was first demonstrated by Kusano et al. (1977, 1982). More recently, Miledi and Woodward (1989b) proposed that there are also muscarinic receptors located in the membrane of the follicular cells, which are involved in the attenuation of the cyclic nucleotide-activate K^+ currents. In addition, they showed that the small and transient Cl^- current (named F_{in} in this paper) was completely abolished by the defolliculation, supporting the notion that muscarinic receptors are also present in the membrane of the follicular cells (Miledi and Woodward, 1989a).

The present experiments confirm the earlier studies and extend our knowledge of the effects of FSH and ACh on the membrane of follicular cells. Furthermore, our results raise the strong possibility that the Cl^- channels activated by these agonists are also in the follicular cell compartment. This is important because it means that our capacity to monitor the S_{in} and F_{in} chloride currents, as well as the outward K^+ currents, would depend on the degree of coupling of the follicular cells to the oocyte, and this coupling may be affected by the procedures used and may be even by the agonists themselves. If we were to place the Cl^- channels mediating the S_{in} and F_{in} currents in the oocyte membrane itself, there are two main possibilities of explaining our results. In one, the ACh and FSH would act on receptors located in the follicular cells and cause the release of some substance to the external medium. The oocyte membrane could then react to this factor and activate the Cl^- channels. In the second, ACh and FSH, acting on the follicular cells would stimulate the production of an intracellular second messenger that would cross the gap junctions and activate the channels presumed to be located in the oocyte membrane. This mechanism has been

proposed for the follicle responses to angiotensin II: the stimulation of angiotensin II receptors, located in the membrane of the follicular cells, promotes the synthesis of IP₃ which diffuses into the oocyte to produce intra-oocyte release of calcium and the generation of the oscillatory current in the oocyte membrane (Sandberg, Bor, Ji, Markwick, Millan, and Catt, 1990). Angiotensin II receptors are also known to be located in the oocyte membrane itself (Woodward and Miledi, 1991).

Because we were unable to obtain S_{in} and F_{in} currents after applying ACh and FSH to defolliculated oocytes, even when these gave large native responses to ACh, angiotensin II or serum factor, or in oocytes injected with mRNA and expressing receptors to serotonin, glutamic acid, substance *P* and *K*, it seems more likely that both the Cl⁻ channels and the receptors involved in the generation of the S_{in} and F_{in} currents are in the membrane of the follicular cells.

Osmotic Effects on Membrane Currents

It is known that in other cells changes in the osmolarity of the external medium alter the membrane conductance to Cl⁻, K⁺ and Na⁺. In some cases the changes in conductance were due to an increase in the activity of ionic channels, but the mechanisms involved are still not well understood. It has been proposed that the modulation of the channels is through a second messenger system that is altered by the change in volume (see for example Doroshenko and Neher, 1992). It has also been suggested that changes in the cytoskeleton can produce changes in the activity of the channels (Falke and Mislner, 1989; McCann et al., 1989), perhaps, by changing the affinity of their interaction with an inactivating component of the channel, as proposed for CIC-2 chloride channels (Gründer, Rothstein, Sarkadi, and Gelfand, 1992).

Some of our results resemble the modifications mentioned above. For instance, changing the bathing fluid from NR to HR produced slow currents (outward and inward) associated with an increase in membrane conductance. The mechanisms responsible for the generation of those currents are not known, but the inward currents were transient, returning to control levels within several minutes. Nevertheless, although the slow inward currents had disappeared whereas the follicles were maintained in HR, the S_{in} currents generated by FSH or ACh were greatly increased and remained so for days. One possible explanation of the osmotic effect on the S_{in} currents is that the follicle-enclosed oocyte volume increase produced by hyposmotic solutions could not be regulated, as in *Xenopus* oocytes (Fry and Shaw, 1988; Fischbarg, Kuang, Vera, Arant, Silverstem, Loike, and Rosen, 1990). This nonreversible change could inhibit an "inactivating" gate in a manner similar to that proposed for the CIC-2 chloride channel (Gründer et al., 1992), the removal of an inactivation process would then lead to the generation of larger currents by the agonists. It will be interesting to find out if there is volume regulation in follicle-enclosed oocytes, and its detailed relation to the osmotically regulated currents.

The Role of Intracellular Ca²⁺ in the Generation of S_{in} and F_{in} Currents

One important question arising from our results concerns the role of Ca²⁺ in generating the S_{in} and F_{in} responses. Because these currents were not abolished after buffering the intracellular calcium with EGTA or BAPTA, our results suggest that in

contrast to Ca^{2+} dependent Cl^- channels in the oocyte membrane, intracellular calcium does not play a principal role in the activation of the channels mediating these currents. The possibility still remains that the intraoocyte injection of EGTA (or BAPTA) was unable to buffer adequately the Ca^{2+} level within the follicular cells, perhaps due to low permeability of these substances through the follicular gap junctions. However, it is known that in several tissues, gap junction channels are permeable to substances even with greater molecular weights than those of EGTA and BAPTA (Lowenstein, 1981). For example, the fluorescent dye fura2 which is structurally related to BAPTA is able to cross hepatocyte gap junctions (Sáez, Connor, Spray, and Bennett, 1989), and there is also evidence that in *Xenopus* follicular cell-oocyte gap junctions allow the diffusion of molecules as carboxyfluorescein, cAMP and IP_3 (Browne et al., 1979; Miledi and Woodward, 1989a; Sandberg et al., 1990) substances which have similar molecular weights to EGTA and BAPTA.

Another important difference concerns to the short delay of the follicular currents elicited by low concentrations of ACh, as compare with the very long delays of the oscillatory currents due to the production of IP_3 (Miledi and Parker, 1989). Thus, suggesting that different mechanisms are involved in the generation of these responses to ACh.

It is known that FSH receptors coupled to the cAMP system in follicular cells, cause an increase in the cyclic nucleotide that activates the slow K^+ current (Woodward and Miledi, 1987a). It is possible that the S_{in} current is similarly caused by an intracellular increase in cAMP, in a process similar to the cAMP activation of Cl^- channels in several epithelial cells (Cliff and Frizzell, 1990; Tabcharani, Low, Elie, and Hanrahan, 1990; Anderson and Welsh, 1991) and cardiac cells (Bahinski, Nairn, Greengard, and Gadsby, 1989).

We do not know the physiological role of the S_{in} and F_{in} currents, but as in other tissues, the Cl^- channels may be involved in transport of nutrients, secretion of substances or regulation of the cellular volumes, functions that have been shown to occur in a very active way during the growth phase of the follicles and maturation of the oocyte (Eppig, 1979; Shultz, 1985; Buccione, Cecconoi, Tatone, Mangia, and Colonna, 1987; Buccione, Vanderhyden, Caron, and Eppig, 1990).

In conclusion, native follicle-enclosed *Xenopus* oocytes have receptors to FSH and ACh that trigger Cl^- currents, which have very different properties from those of the oscillatory Cl^- currents generated in the oocyte itself. Our results suggest strongly that the receptors and the channels involved in the generation of these currents are both located in the membrane of the follicular cells. The nature of the channels involved, and the mechanisms of their activation still remains to be elucidated. Nevertheless, our results show that the osmolarity of the external solution has very important effects on these channels, effects that should be taken into account when studying endogenous currents in follicle-enclosed oocytes.

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