J. Membrane Biol. 165, 161–174 (1998)

The Journal of
Membrane
Biology

© Springer-Verlag New York Inc. 1998

Effects of Mechano-Gated Cation Channel Blockers on *Xenopus* Oocyte Growth and Development

N.C. Wilkinson, F. Gao, O.P. Hamill

Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77555-0641, USA

Received: 27 March 1998/Revised: 10 June 1998

Abstract. The putative role(s) of a mechanically gated (MG) cation channel in *Xenopus* oocyte growth, maturation, fertilization and embryogenesis has been examined. Using a pharmacological approach, we have tested the effects of the MG channel blockers, gadolinium, gentamicin and amiloride on the above developmental events. Our results indicate that oocyte maturation, fertilization and early embryogenesis (up to the free-swimming stage 45) can proceed normally in the presence of concentrations of agents that either completely abolish (i.e., $\geqslant$10 μM Gd^{3+}) or partially block (i.e., 1 mM gentamicin) single MG channel activity as measured by patch-clamp recording. However, we also find that higher concentrations of Gd^{3+} ($\geqslant$50 μM) can lead to an increased percentage (>20%) of axis-perturbed embryos compared with control (<1%) and that amiloride (0.5 mM) reduces the success of fertilization (from 100% to <50%) and increases mortality (by ~75%) in developing embryos. Furthermore, we find that all three agents inhibit oocyte growth in vitro. However, their order of effectiveness (amiloride > gentamicin > Gd^{3+}) is opposite to their order for blocking MG channels ($Gd^{3+} \gg$ gentamicin > amiloride). These discrepancies indicated that the drugs effects occur by mechanisms other than, or in addition to, MG channel block. Our results provide no compelling evidence for the idea that MG channel activity is critical for development in *Xenopus.* This could mean that there are other mechanisms in the oocyte that can compensate when MG channel activity is blocked or that the protein that forms the channel can undergo additional interactions that result in a function insensitive to MG channel blockers.

Key words: Gadolinium — Gentamicin — Amiloride — Development — Mechanosensitive channels

Correspondence to: O.P. Hamill

Introduction

Since single mechanically gated (MG) channels were first observed in oocytes of *Xenopus laevis* (Methfessel et al., 1986) they have been recorded in oocytes or eggs of a number of other species, including frog (Taglietti & Toselli, 1988), fish (Medina & Bregestovski, 1988, 1991) and tunicate (Moody & Bosma, 1989). Patch-clamp studies of oocytes from both invertebrate and vertebrate species have demonstrated many similarities in MG channel properties. For example, the MG channels in both tunicate (*Boltenia villosa*) and frog (*Xenopus* and *Rana esculenta*) oocytes are expressed uniformly and at relatively high density (~2/μm^2) over the membrane surface (Methfessel et al., 1986; Taglietti & Toselli, 1988; Moody & Bosma, 1989). They are weakly cation selective allowing Na^+, K^+ and Ca^{2+} to permeate (Taglietti & Toselli, 1988; Moody & Bosma, 1989; Yang & Sachs, 1989; 1990). They show similar open/closed single channel kinetics and permeant ion channel block by divalent cations (Taglietti & Toselli, 1988; Moody & Bosma, 1989; Yang & Sachs, 1989; 1990; Lane et al., 1993; Wu et al., 1998) and they display similar rapid adaptation (i.e., channel closure) in response to sustained mechanical stimulation (Moody & Bosma, 1989; Hamill & McBride, 1992; Small & Morris, 1994).

Despite their detailed biophysical characterization and indications that oocyte MG channels are apparently conserved since they display similar properties in species spanning over 500 million years of evolution, there is no direct evidence for their physiological role. Nevertheless, there has been general speculation that MG channels play a fundamental role in oocyte development and/or maintenance (Sachs, 1988; Morris, 1990; Moody & Bosma, 1989; Medina & Bregestovski, 1989; Hamill & McBride, 1995; Sackin, 1996; but *see* Morris, 1992). These speculations are reasonable given that a number of events, including oocyte growth, maturation, fertilization, cell cleavage and morphogenesis involve mechani-

cal distortions and/or reorganization of the plasma membrane and its underlying cytoskeleton (CSK) (Hiramoto, 1974; Hara, Tudeman & Kircher, 1980; Odell et al., 1981; Usui & Yoneda, 1982; Beloussov, Lakirev & Naumidi, 1988; Sardet, McDougall & Houliston, 1994). Furthermore, external mechanical forces can clearly trigger or influence developmental events. For example, mechanical pricking of the egg with a needle can initiate the fertilization response (Andreuccetti et al., 1984; Kline & Nuccitelli, 1985; Peres & Manicinelli, 1985; *see also* Green & Purves, 1984) and gravitational rotation of the egg can influence development of the polarity of the embryo's body axis (Neff et al., 1983; Black & Gerhart, 1985, but *see* Souza et al., 1995). Given these mechanical events it would seem reasonable to propose that the MG channel, by its ability to sense and transduce mechanical forces, would be a plausible candidate to regulate or influence such processes.

The first attempt to investigate a developmental role for MG cation channels was carried out by Steffensen, Bates & Morris (1991) using a pharmacological approach. Specifically, they demonstrated that when prefertilized eggs of *Xenopus* or *Boltenia* were exposed to gadolinium (Gd^{3+}) concentrations, well above those known to completely block the MG channel (Yang & Sacks, 1989), *Xenopus* embryos and *Boltenia* larvae developed normally. Based on these observations they concluded that MG channel activity was not critical in the developmental events subsequent to fertilization (Steffensen et al., 1991).

In the present study, we extend the observations of Steffensen et al., (1991). We do this by first investigating the putative role of MG channels in developmental events preceding fertilization (i.e., oocyte growth and maturation), in fertilization itself, and in subsequent embryogenesis. Second, in addition to testing the effects of Gd^{3+} on these developmental events, we also test the effects of two clinically relevant drugs, gentamicin and amiloride, which have also been shown to block MG channels (Kroese, Das & Hudspeth, 1989; Jorgensen & Ohmori, 1988; Lane, McBride & Hamill, 1991; Ruesch, Kros & Richardson, 1994). Although none of the above three agents are highly selective in terms of only blocking MG channels, they do block the *Xenopus* MG channel with a characteristic order of efficacy (i.e., $Gd^{3+} \gg$ gentamicin > amiloride, for review *see* Hamill and McBride (1996*a*). The aim of this study was to determine whether the developmental events in *Xenopus* show sensitivity to these agents consistent with their known effects on MG channels.

Materials and Methods

Frog Care and Maintenance

Mature male and female frogs (*Xenopus laevis*) were obtained from either Xenopus I (Ann Arbor, MI) or Nasco Science (Fort Atkinson, WI). However, in fertilization experiments we used Nasco ''proven breeders'' since these frogs were superior in terms of their control fertilization rates (~100%). The frog care and handling was in accordance with NIH and Institutional guidelines. Specifically, to obtain oocytes for in vitro growth and maturation as well as patch clamp studies, frogs were anesthetized by being placed for approximately 20 min in a beaker containing 300 mg ethyl 3-aminobenzoate methanesulphonic acid (Aldrich) in 200 ml distilled water. Sterile surgical procedures were used in the removal of oocytes. Frogs underwent no more than two surgeries separated by at least 6 weeks.

Oocyte growth in vitro

In order to grow oocytes in vitro, we adopted the procedure developed by Wallace, Misulovin & Wiley, (1980). The basic features of their procedure involved manual dissection of oocytes from their outer follicular layers and incubation of the isolated oocytes in nutrient medium containing vitellogenin, the macromolecular precursor to yolk proteins. To accumulate vitellogenin in the blood of female frogs, they were injected with estrogen (4 mg estradiol-17 β dissolved in 0.4 ml propylene glycol per 100 grams of body weight). After two to three weeks, the frogs were anesthetized (*see above*) and exhaustively bled into centrifuge tubes according to the procedure of Anstall and Huntsman (1960). The blood was stored at room temperature for ~3 hr in order to form a clot that was then centrifuged. The serum was collected, divided into small aliquots and frozen at −20°C for up to 1 month for later use. The concentration of vitellogenin in the serum was determined according to the same procedure described by Wallace et al. (1980). The vitellogenin-rich serum was frozen and thawed only once and was discarded if it showed signs of cloudiness. The oocyte culture medium was Dublecco's modified Eagle's medium (DMEM) with 4.5 g/l of L-glutamine and glucose, without sodium phosphate and sodium bicarbonate, and buffered to pH 7.2 with 15 mM HEPES (Sigma). The DMEM was diluted to 50% with sterile distilled water and supplemented with insulin (1 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml) and the serum supplement (10%). The serum consisted of *Xenopus* and fetal bovine sera. The appropriate volume of *Xenopus* serum was added to provide a final vitellogenin concentration in the medium of 2.5 mg/ml and the remainder of the 10% was made up with fetal bovine serum. To grow oocytes, a portion of ovary was removed from the frog and initially placed in frog Ringer (in mM; NaCl 115, KCl 2.5, $CaCl_2$ 1.8, HEPES 10) (NaOH, pH 7.2). Although we initially attempted to grow and differentiate stage I, II, and III oocytes (Dumont, 1972), we found they showed no growth over a two-week period (*see also* Wallace et al., 1980). We therefore began with Stage IV oocytes of a defined size range (0.8 ± 0.05 mm in diameter) which were manually dissected (i.e., no collagenase treatment) from their follicles. Subsequently, groups of oocytes were transferred to the growth culture media with or without the appropriate concentration of MG channel blocking drugs. The oocyte growth was monitored for 15 days and fresh culture media was added every 3–4 days. The growth medial solutions were periodically examined under high magnification (400×) to ensure no precipitate with the agents formed. Any dying oocytes (1–10%, depending on the MG channel blocker), as judged by their loss of animal-vegetal polarity and swollen appearance, were discarded and not included in the growth analysis. Each treatment group consisted of 12–18 oocytes with subgroups of 2–3 oocytes being placed in a well of a 24-well culture plate (Corning) containing 0.5 ml medium. The concentrations of MG channel blockers tested were 10, 50, or 100 (μM) for Gd^{3+} (Aldrich) and 0.5, 1, or 2 (mM) each for gentamicin and amiloride (Sigma). Oocytes in culture were then maintained on a stationary platform at 20°C in a dark chamber with water saturated air. Oocyte diameters were measured with an ocular micrometer under a dissecting

microscope and measurements in two perpendicular directions were averaged to give a single value for the oocyte diameter.

Oocyte Maturation In Vitro

Surgically removed oocytes were matured in vitro by incubating frog Ringer (*see above*) containing 50 μg/ml progesterone with or without MG channel blockers. Maturation was judged by germinal vesicle breakdown (GVB) which could be seen as a white spot that developed on the dark animal pole and was monitored for up to 24 hr.

Fertilization

Female frogs were primed by injecting 50 I.U. of pregnant mare serum into the dorsal lymph sac. Three days later the frogs were injected with 500–700 I.U. human chorionic gonadotropin to induce ovulation which typically occurred about 12 hr later. After waiting about 1 hr from the first release of eggs, up to 200 eggs were manually squeezed from the frog and groups of 25 to 50 were transferred to the appropriate test solution. The test solution consisted of Ringer diluted to 30% with distilled water (adjusted to pH 7.8 with NaOH) and included the appropriate concentration of MG channel blocker. The eggs were then incubated for 10 min at room temperature in this solution before exposure to sperm. To obtain sperm, male frogs were anesthetized, pithed and dissected to remove the testes. A 2-mm piece of testes was macerated in 2 ml of the appropriate test solution (*see above*). Sperm were used within 30 min after isolation. The motility and viability of the sperm in the various test solutions were determined by direct visualization (at ×400) and trypan blue exclusion. No differences in sperm viability and motility were evident for sperm incubated in the 30% Ringer solutions with 100 μM Gd^{3+}, 1 mM amiloride or 1 mM gentamicin either after a 10-min incubation or after 24-hr incubation in these solutions at 4°C. In addition to the MG channel blockers we also tested incubations with BAPTA and BAPTA (AM) (Molecular Probes), tetrodotoxin, halothane or chlorobutanol (Sigma). After separate 10-min incubations, the eggs were added directly to the sperm for 5 min and then transferred to a petri dish with 20 ml of the appropriate fresh test solution. Eggs under the different test conditions were visualized for signs of fertilization which included an initial oocyte rotation (stage 1) resulting in the animal pole facing up and then subsequent cleavage and cell division (*see* Nieuwkoop & Faber, 1956). Typically, the first cleavage occurred (i.e., stage 2) after ~1.5 hr of mixing the sperm and individual eggs. Subsequent cell divisions could be followed for 3 to 4 hr. At 5 hr the blastula (stage 8) had formed and the next critical stages occurred at around 15 hr with the formation of the neural fold and neural plate (stage 13–14) and at 22 hr with the closure of the neural tube (stage 20). After 48 hr a distinct head and tail could be recognized (stage 35) and 3 days later the tadpoles (stage 40) showed active swimming responses. At ~stage 45 we discontinued their exposure to the MG channel blockers and allowed them to develop in small aerated aquaria filled with dechlorinated tap water, but we did not follow systematically their further development into frogs.

Removal of the Egg Jelly Coat and Preparation of Jelly Water

To remove the possibility that the egg jelly coat reduced or prevented access of the chemicals to MG channels in the plasma membrane, in some experiments, the jelly coat was removed before the eggs were incubated in the appropriate test solutions. Eggs were dejellied according to the procedure described in Lindsay and Hedrick (1989). They were first collected in DeBoers solution (DB) (in mM: 110 NaCl, 1.3 KCl, 1.3 $CaCl_2$, buffered to pH 7.2 with $NaHCO_3$), then dejellied by incubation for 1 to 2 min with 45 mM mercaptoethanol in Tris-buffered DB (i.e., 10 mM Tris-HCl, pH 8.9) and then washed extensively with DB. The dejellied eggs were washed gently with Ringer then placed in the appropriate 30% Ringer test solution. When the jelly coat is removed, it is necessary to prime the sperm with ''jelly water'' in order to ensure high (>90% fertilization) rates. The jelly water was prepared by incubating eggs with intact jelly in 30% Ringer solution (8 ml/3 g eggs) for 45 min at room temperature, followed by collection of the liquid and addition of Ficoll (400 DL, Sigma) to 10% (w/v) (*see* Lindsay & Hedrick, 1989). A testis was macerated in jelly water with the appropriate concentration of MG channel blocker and incubated for 10 min at room temperature and then the sperm suspension was added dropwise to the dejellied eggs and the fertilization procedure followed as described above.

Patch-Clamp Recording From Oocytes

Standard patch clamp recording techniques (Hamill et al., 1981) were used for recording from *Xenopus* oocytes (Methfessel et al., 1986). To activate MG channels, the pressure clamp technique (McBride & Hamill, 1992) was used to apply steps or ramps of suction to the patch pipette holder. For the experiments described in Fig. 1, the pipette solution was (in mM): 100 KCl, 5 HEPES (KOH, pH = 7.2), 5 mM EGTA (KOH) with and without 1 mM gentamicin and 1 mM amiloride. In the case of Gd^{3+}, EGTA was not added to avoid chelation (*see* Hamill & McBride, 1996*a*). To ensure that Gd^{3+} in the different composition test solutions (i.e., the actual media used in growing, maturing, and fertilizing oocytes/eggs) was still active in blocking MG channel activity, samples of these ''oocyte/egg conditioned'' solutions were tested as pipette solutions in cell-attached patch-clamp recordings. In all cases, Gd^{3+} concentrations of 10 μM and above abolished MG channel activity.

Results

Block of MG Channels by Gd^{3+}, Gentamicin and Amiloride

Figure 1 illustrates the actions of Gd^{3+}, gentamicin and amiloride on MG channel activity when measured under similar cell-attached patch recording conditions and in response to similar step changes in suction applied to membrane patches of stage VI oocytes. In *Xenopus* oocytes, a step change in suction (top trace) produces a rapid activation of MG channel currents (control traces) which then turn off over ~1 sec. This turnoff with maintained mechanical stimulation is referred to as adaptation (e.g., see Hamill & McBride, 1992). In Fig. 1*A*, the control current is larger than the control currents in Fig. 1*B* and *C* because larger tip pipettes were used to sample more membrane area. Figure 1*A* indicates that 1 μM Gd^{3+} partially blocks and 5 μM completely blocks MG channel activity. In a previous study of *Xenopus* oocytes, Yang and Sachs (1989) found that 10 μM Gd^{3+} was required for complete block of MG channels. However, they focally perfused Gd^{3+} solutions onto outside-

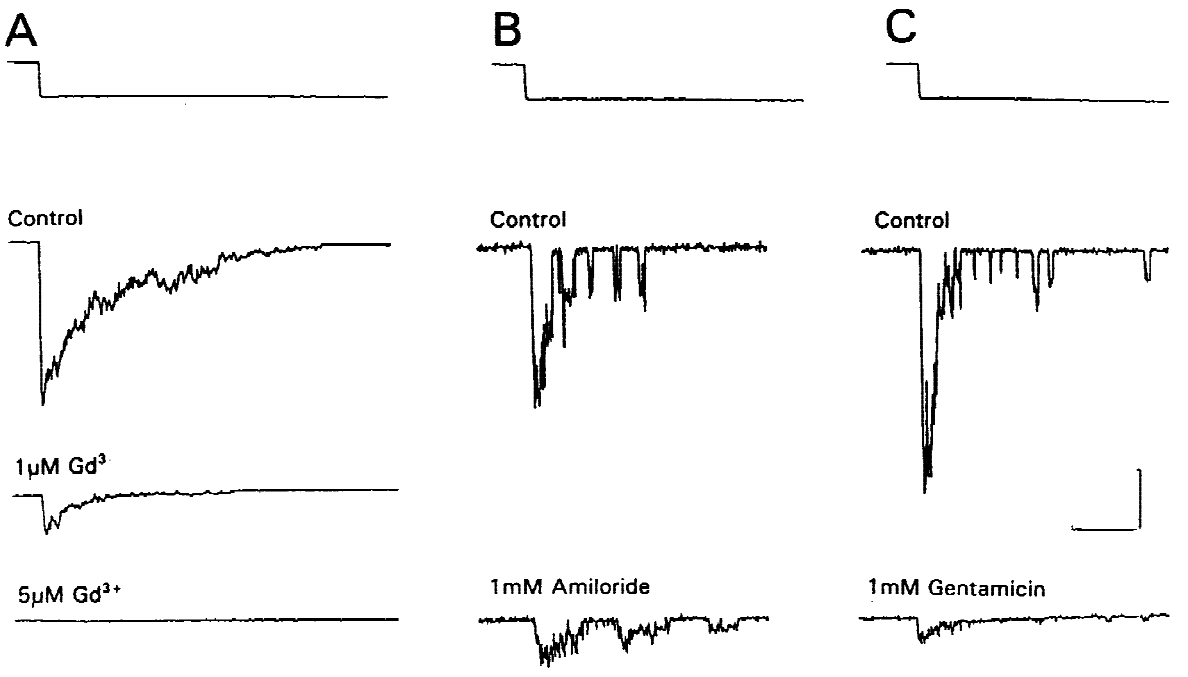

Fig. 1. Block of mechanically gated channels by Gd^{3+}, amiloride and gentamicin as measured in cell-attached patch-clamp recordings. In all panels, the top trace is the pressure step (10 mm Hg in *A*, 20 mm Hg in *B* and *C*) applied to the patch pipette. The lower traces are the current responses, all made at a membrane potential 100 mV more negative than the potential at which single channel currents reversed (i.e., the assumed zero voltage) of different patches with various concentrations of Gd^{3+} (*A*), amiloride (*B*) and gentamicin (*C*) in the pipette solution. The three patch pipettes used in A were all ~3 μm in tip diameter and the patch pipettes in *B* and *C* were paired pipettes (i.e., each half of a single pull) and had a tip diameter of ~2 μm. The vertical scale was 10 pA and the horizontal scale 500 msec.

side patches and as a consequence the Gd^{3+} solution was probably diluted before reaching the patch. Our studies indicate that the IC_{50} for Gd^{3+} is less than 1 μM and that complete block by 5 μM Gd^{3+} was voltage independent and could not be reversed by stronger steps or ramps of mechanical stimulation (*data not shown*).

Amiloride is known to cause an ''open channel'' block which is seen as a time averaged reduction in single MG channel current amplitude (Lane et al., 1991). In Fig. 1*B*, 1 mM amiloride reduced the single channel current from ~8 pA to ~2 pA. Previous analysis of amiloride's action in *Xenopus* indicates the IC_{50} for amiloride block is ~500 μM at −100 mV but increases with depolarization with the block being completely reversed with depolarizations to 50 mV or greater (Lane, McBride & Hamill, 1991, 1992, 1993). Figure 1*C* indicates 1 mM gentamicin caused a ~10-fold reduction in single MG channel current amplitude and thus was a more effective blocker than 1 mM amiloride. Previous studies of mechanoelectrical transduction in audiovestibular hair cells also indicate that gentamicin is more effective than amiloride in blocking MG currents with IC_{50} sec of 5 μM and 50 μM, respectively (Kroese et al., 1998; Jorgensen & Ohmori, 1988; Ruesch et al., 1994). In summary, the results of Fig. 1 are consistent with an order of efficacy for the three chemicals for blocking *Xenopus* MG channels of $Gd^{3+} \gg$ gentamicin > amiloride.

Oocyte Growth in the Presence of MG Channel Blockers

Because stage I to III oocytes show little growth under in vitro tissue culture conditions, even in the absence of MG channel blockers (*see also* Wallace et al., 1980), we studied the effects of the blockers on the growth of stage IV oocytes. Figure 2 shows oocyte growth in the absence and presence of MG channel blockers. Over a period of 15 days in control medium the stage IV oocytes grew in diameter with a daily growth rate of 0.027 mm/day to reach approximately 150% of their original size (~1.2 mm in diameter), which is the average diameter of stage VI oocytes. In the presence of 10 μM and 50 μM Gd^{3+}, the average growth rates were reduced by 10% (to 0.0244 mm/day) and 20% (to 0.0216 mm/day), respectively (*data not shown*). The graph in Fig. 1 indicates that even with 100 μM Gd^{3+} significant growth rates still occurred although it was reduced by 30% (to 0.019 mm/day). In comparison, 1 mM gentamicin and 1 mM amiloride, which are both less effective than 10 μM Gd^{3+} in blocking MG channels, reduced growth rates by 50%

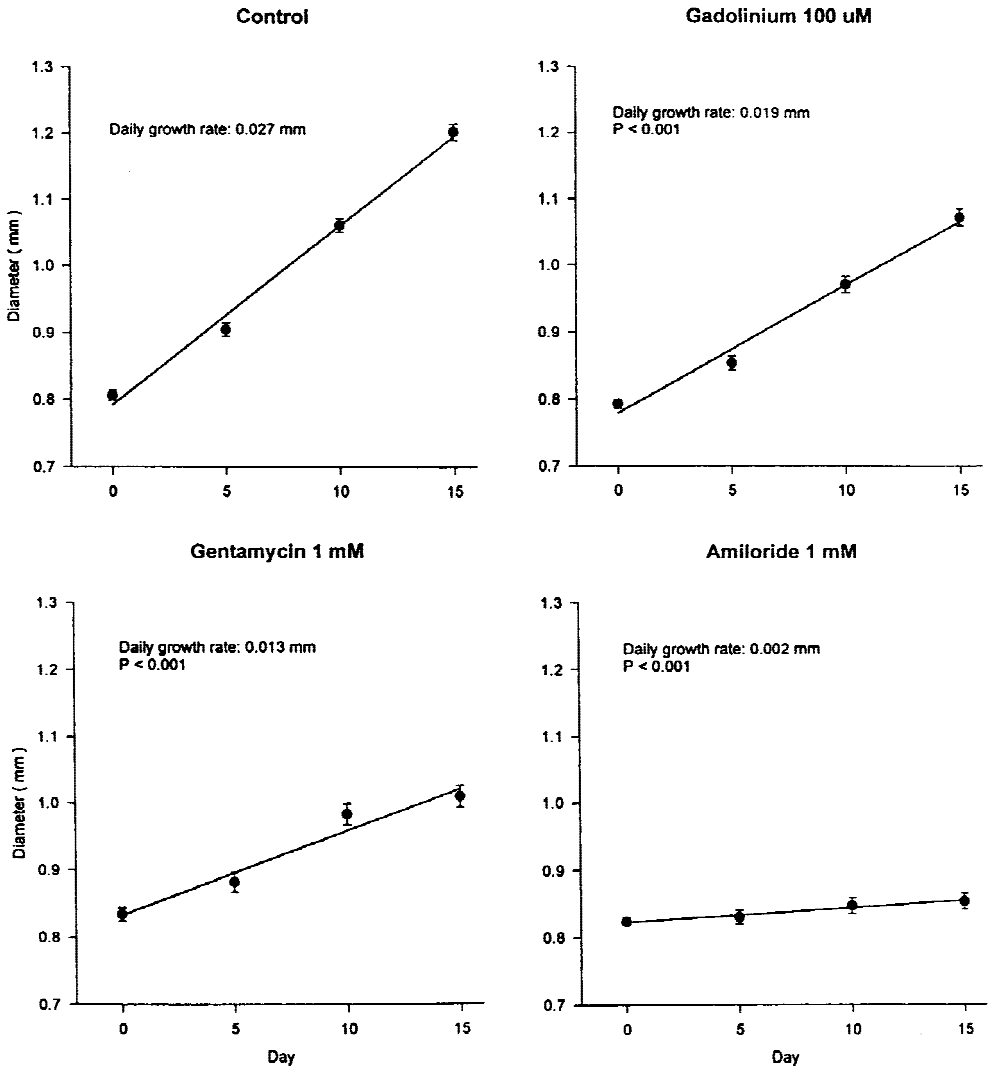

Fig. 2. Oocyte growth carried out in vitro in the absence and presence of MG channel blockers. In each panel the average diameter of oocytes (measured in two perpendicular dimensions) is plotted as a function of days in culture. The data points are the mean ±SD of ~100 oocytes from 4 different experiments using 20–25 oocytes from each of 4 different donor frogs. The slope of the regression fits was used to estimate the daily growth rate in terms of oocyte diameter. Only healthy oocytes were measured and oocytes that took on an unhealthy appearance (i.e., lost their distinct polarity) were discarded. In general, less than 10% of oocytes were discarded in any of the conditions over the course of 15 days.

(0.013 mm/day) and 90% (0.002 mm/day), respectively. Therefore, although all the MG channel blockers significantly reduced oocyte growth, their order of effectiveness (amiloride > gentamicin > Gd^{3+}) was not consistent with their order for blocking MG channels. These results indicate that the agents probably inhibit growth by a mechanism(s) other than, or in addition to, MG channel block. For example, amiloride is well recognized as also blocking the Na^+/H^+ and Na^+/Ca^{2+} exchangers (*see* Hamill & McBride, 1996*a* and refs. therein) which are presumably both important for normal growth. On the other hand, the effect of 10 μM Gd^{3+} indicates, that at most, only 10% of oocyte growth rate is dependent on MG channel activity. This percentage could be even less if 10 μM Gd^{3+} affects growth by other mechanisms. Finally, in the process of studying the effects of MG channel blockers on oocyte growth, we demonstrated that these agents do not inhibit the ability of oocytes to maintain their animal-vegetal polarity.

Oocyte Maturation in the Presence of MG Channel Blockers

Table 1 indicates that oocyte maturation induced by in vitro incubation of 50 μg/ml progesterone was insensitive to MG channel blockers. In fact, instead of inhibition we found that a higher percentage of oocytes ma-

Table I. MG channel blockers on in vitro maturation

Condition	Total number oocytes tested	Number matured	% matured
No Progesterone	178	0	0
50 μg/ml Progesterone	217	184	85
50 μg/ml Progesterone plus 100 μM Gd^{3+}	216	203	94
50 μmg/ml Progesterone plus 1 mM Gentamicin	149	131	88
50 μg/ml Progesterone plus 1 mM Amiloride	143	106	74

tured in 100 μM Gd^{3+} and 1 mM gentamicin (94% and 88%, respectively) compared with the control (85%). The potentiating effect of Gd^{3+} is consistent with a previous report that found that lanthanum in the absence of progesterone can initiate meiotic maturation in *Xenopus* (Schorderet-Slatkine, Schorderet & Baulieu, 1976). Only in the case of 1 mM amiloride was maturation reduced, but still the majority (75%) of oocytes matured. The maturation of oocytes is not critically dependent on extracellular Ca^{2+} since we observed germinal vesicle breakdown (GVB) in oocytes (~50%) incubated in the absence of extracellular Ca^{2+}. However, we also observed that prolonged incubation of oocytes in Ca^{2+} free media was toxic to oocytes, possibly due to the increase in a large, nonselective hemi-gap junctional conductance (Zhang, McBride & Hamill, 1998). Together, these results do not support the idea that MG channel activity or Ca^{2+} influx is critical for oocyte maturation.

Fertilization in the Presence of MG Channel Blockers

Successful fertilization was indicated by the initial cell cleavage, which typically occurred ~1.5 hr after mixing of sperm and eggs. Under control conditions, with the jelly coat intact, the fertilization success rate was ~100% and neither Gd^{3+} nor amiloride reduced this rate. Gentamicin produced a slight reduction but it still remained high at 87% (Table 2). In one experiment, we tested a cocktail of MG channel blockers (i.e., 100 μM Gd^{3+}, 1 mM amiloride, and 1 mM gentamicin) on fertilization. Under these extreme drug conditions, 95% of eggs were still fertilized. The presence of the jelly coat could not explain the lack of effect of 10 or 50 μM Gd^{3+} on fertilization. However, removal of the jelly coat did reveal inhibitory effects of 100 μM Gd^{3+} and 0.5 mM amiloride, in that fertilization success was reduced to 84% and 39%, respectively (Table 2). Whether the presence of the jelly coat blocks these inhibitory effects by reducing the effective drug concentration or protecting the egg against drug actions remains to be determined.

Table 2. MG channel blockers on fertilization with and without jelly coat

Condition	Total eggs	Number fertilized	% success
Jelly coat intact			
Control	97	96	99
Gd^{3+} 10 μM	78	77	99
Gd^{3+} 50 μM	63	61	97
Gd^{3+} 100 μM	99	99	100
Control	55	53	96
Gentamicin 1 mM	52	45	87
Control	69	69	100
Amiloride 1 mM	72	71	99
Amiloride 2 mM	96	95	99
MG blocker cocktail*	285	270	95
Jelly coat removed			
Control	147	145	99
Gd^{3+} 10 μM	131	131	100
Gd^{3+} 50 μM	133	132	99
Gd^{3+} 100 μM	140	118	84
Control	67	67	100
Gentamicin 0.5 mM	94	94	100
Gentamicin 1 mM	73	59	81
Amiloride 0.5 mM	106	41	39
Amiloride 1 mM	84	33	39

* MG channel cocktail included 100 μM Gd^{3+}, 1 mM amiloride and 1 mM gentamicin

One hypothesized role for the MG channel is that it provides a pathway for Ca^{2+} influx, which in turn influences the fertilization response. However, we found that Ca^{2+} influx was not critical for *Xenopus* fertilization since fertilization occurred in the presence of a 5 mM BAPTA/Ca^{2+}-free fertilization solution (Table 3). In contrast, fertilization is highly sensitive to agents known to buffer or inhibit Ca^{2+} release from internal stores (e.g., BAPTA (AM), halothane, and chlorobutanol; *see* Table 3).

In summary, fertilization can occur when both sperm and eggs are exposed to MG channel blocker concentrations that completely abolish (50 μM Gd^{3+}) or partially block (1 mM amiloride or gentamicin) MG channel activity. This result indicates that either MG channel activity is not critical for fertilization or that the sperm/eggs have mechanisms that can compensate in its absence.

Embryogenesis in the Presence of MG Channel Blockers

After the eggs described in Table 2 underwent successful fertilization, they were observed for subsequent development and embryogenesis in the same test solutions. Figure 3 illustrates the different stages of development in a single egg that was fertilized in the presence of 100 μM Gd^{3+}. In this example, the morphological appearance and timing of the different stages of development were

indistinguishable from those seen under control conditions (Niewkoop & Faber, 1956; *see* Materials and Methods). Figure 4 shows photographs of tadpoles that had developed in the absence (*A*) or presence (*B*) of 100 μM Gd^{3+}. The abnormal phenotypes (Fig. 5*A*) were characterized by either a highly twisted spinal column and/or the presence of swollen abdomens or ascites (i.e., swelling due to fluid accumulation). These abnormal phenotypes are similar to the axis perturbed phenotypes that can be produced by a variety of treatments, including the prevention of initial egg rotation after fertilization (Neff et al., 1983), cooling and exposing the egg to elevated hydrostatic pressure or ultraviolet light (Scharf & Gerhart, 1983). Occasionally, control tadpoles showed such abnormalities (~1%) and this percentage increased (~5%) in eggs which had their jelly coat removed (Table 4). As indicated in Table 4, 10 μM Gd^{3+} did not increase the percentage of *abnormal phenotypes* over control, but higher concentrations of Gd^{3+} did produce a dose-dependent increase to 8% and 13%, in 50 μM and 100 μM Gd^{3+}, respectively. An even higher percentage of *abnormal phenotypes* were noted in embryos that developed from jelly-free eggs exposed to Gd^{3+}. The frequency of *abnormal phenotypes* was not increased by exposure to gentamicin. In amiloride, all the embryos died around stage 35 without showing any morphological abnormalities. Amiloride, is presumably toxic to the embryos because it blocks other transporters in addition to MG channels (*see* Hamill & McBride, 1996).

In summary, normal embryogenesis can occur in the large majority (~80%) of fertilized eggs (with or without the jelly coat) exposed to 100 μM Gd^{3+}. High concentrations of Gd^{3+} increase the proportion of axis perturbed *abnormal phenotypes* (from <1 to ~20%) which is similar to the *abnormal phenotypes* produced by a variety of nonspecific treatments not obviously related to block of MG channel activity. Amiloride, and to a lesser effect gentamicin, are toxic to embryos.

Table 3. Effects of other agents on fertilization

Condition	Total eggs	Number fertilized	% success
Jelly coat intact			
BAPTA 5 mM (Ca^{2+}-free)	72	71	99
BAPTA (AM) 3 μM	36	0	0
Tetrodotoxin 1 μM	42	40	95
Halothane 1 mM	73	0	0
Chlorobutanol 10 mM	66	0	0

Discussion

There are a number of general issues related to MG channel function that will be considered before discussing the specific results of this study. To begin with, there are four broad mechanisms by which MG channel activity may produce effects on the oocyte or the egg. These include: (i) Ca^{2+} influx, which could serve to refill depleted internal Ca^{2+} stores and/or activate Ca^{2+}-sensitive channels/enzymes, (ii) net influx or efflux of osmotically active particles, which could mediate volume regulatory responses by causing cell swelling or shrinking, respectively, (iii) membrane potential changes, which could affect the activity of voltage-sensitive channels/processes and (iv) mechanical/structural effects (i.e., independent of ion movements) on the cytoskeleton or internal membrane channels via mechanical links with the MG channel. The direction or polarity of the effects mediated by mechanisms i–iii will depend upon the external ionic environment bathing the oocyte or egg. For example, the oocyte *in situ* is bathed in a relatively high Na^+/Ca^{2+} solution (i.e., ~115 mM/2 mM) such that MG channel opening should lead to Na^+ and Ca^{2+} influx, membrane depolarization and a tendency to cause the oocyte to swell. In contrast, the mature egg may be released into an extremely low ionic strength solution with ~0 mM Ca^{2+}. In this case, MG channel opening would result in mainly K^+ efflux, membrane hyperpolarization and a tendency to shrink the oocyte.

The magnitude of the effects mediated by the four mechanisms will depend upon the overall MG channel density. Stage I–VI oocytes express MG channel activities with similar densities (i.e., several channels activateable on every patch). However, stage VI oocytes matured in vitro and eggs matured *in situ* express a significantly lower density of MG channel activity (i.e., >50% of patches display no activity) (O.P. Hamill, *unpublished observations*). This developmental down regulation of MG channel activity is similar to the suppression of other membrane conductances seen during maturation (Taglietti et al., 1984; Zhang et al., 1998) and presumably serves to increase the membrane sensitivity (i.e., by increasing input resistance) to fertilization-induced conductance changes (Jaffe, 1996). The suppression of MG channel activity implies that it plays a reduced role in the mature egg. Indeed, we have excluded the specific idea that MG channels provide a critical pathway for Ca^{2+} influx during fertilization and early embryogenesis since these processes proceed normally in solutions with zero Ca^{2+} (Table 3). Apparently, the *Xenopus* egg has sufficient internal Ca^{2+} to allow it to function independent of the extracellular [Ca^{2+}] and Ca^{2+} influx. This does not appear to be the case for some marine eggs, which depend upon voltage-sensitive Ca^{2+} influx to refill internal Ca^{2+} stores depleted after fertilization (Arnoult, Grunwald & Villaz, 1996).

Another fundamental issue concerning the role of MG channels goes beyond the oocyte and relates to many other cells that have been shown, through patch-

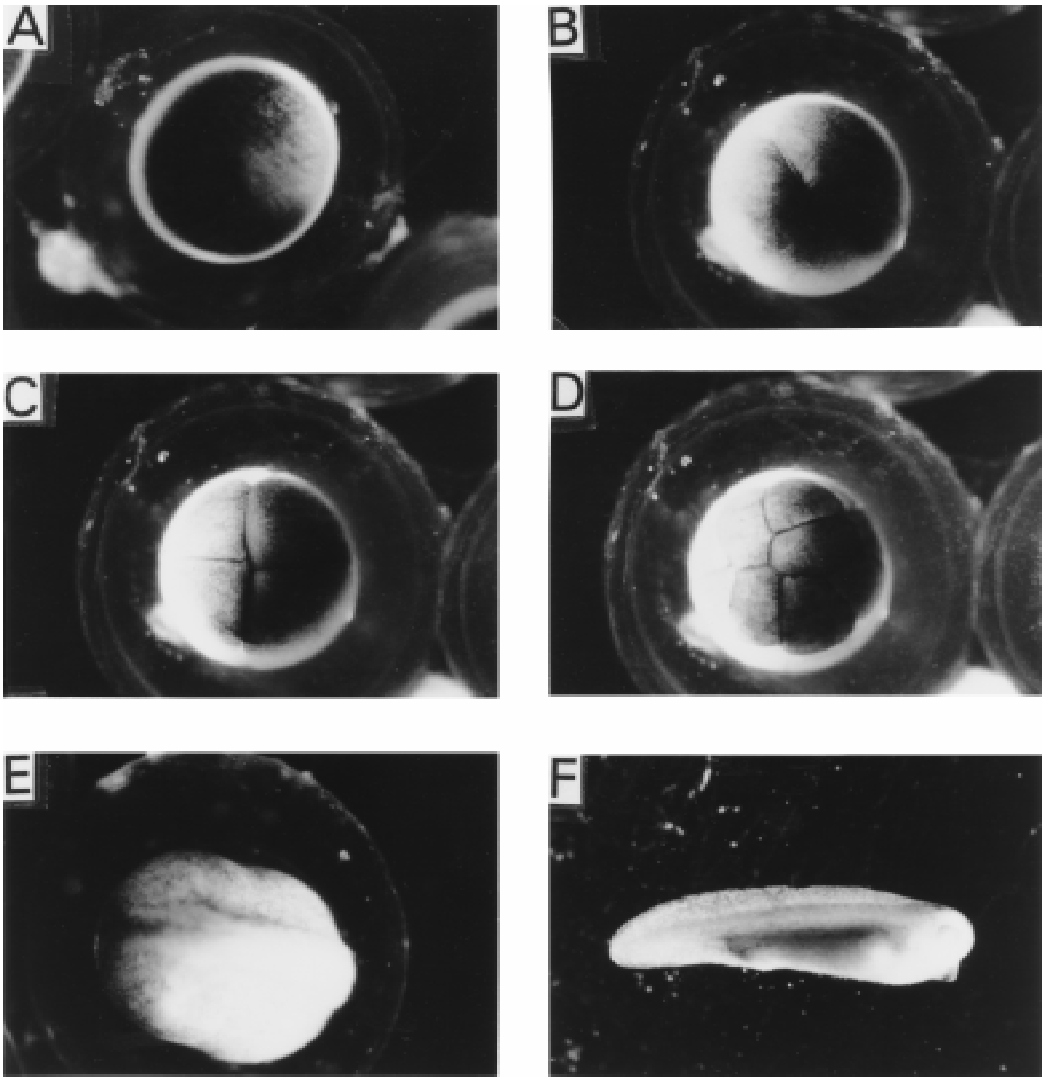

Fig. 3. Different stages of embryogenesis measured in the presence of 100 μM Gd^{3+}. The egg with its jelly coat intact was incubated for 10 min in a solution containing 100 μM Gd^{3+} prior to being mixed with sperm that had also been incubated in Gd^{3+}. Based on morphological criteria the stages shown in this figure were indistinguishable from the same stages seen in eggs developing under control conditions. (*A*) The first evidence of fertilization was when the egg rotated (stage 1) so that its animal pole faced upwards. Neither Gd^{3+}, amiloride or gentamicin inhibited this rotation. (*B*) Evidence of the first cell division (stage 2) occurred 1 hr and 10 min after sperm mixing, and the cell cleavage showed the same appearance and time course in test solutions as seen in control eggs. (*C*) and (*D*) Show subsequent cell divisions that also appeared normal in Gd^{3+}. Over the next 3 to 5 hr, the morula and blastula stages could be observed but are not shown here. (*E*) Stage 20, approximately 21 hr after fertilization, with the neural plate and closed neural tube evident. (*F*) Stage 35, approximately 48 hr after fertilization (note the magnification has been changed for this photo). At this stage the embryo could make swimming motions when the dish was shaken or disturbed.

clamp recording, to express stretch-activated channels. This issue relates to the physiological stimulus that activates the MG channel in the whole cell, as opposed to the patched membrane. At this time, all that is known about the oocyte MG channel has been derived from experiments in which the channel is activated by pressure or suction applied to the membrane patch. Various attempts by us and others to activate the MG channel in the whole oocyte by osmotic swelling, cell inflation or fluid shear stress fail to produce a current that can be unequivocally related to MG channel activity. Although resolving this issue is a continuing project in our laboratory, it could mean, as originally proposed by Morris and Horn (1991), that the so-called stretch-activated channel is an artifact of patch-clamp recording. In this case, the channel in the whole cell may not be mechanosensitive but instead gated by another stimulus, such as voltage changes (e.g., Hamill & McBride, 1996*b*) or transmitters/2nd messengers (Vandorpe et al., 1994). Contrary to this idea, we find that mechanical stresses

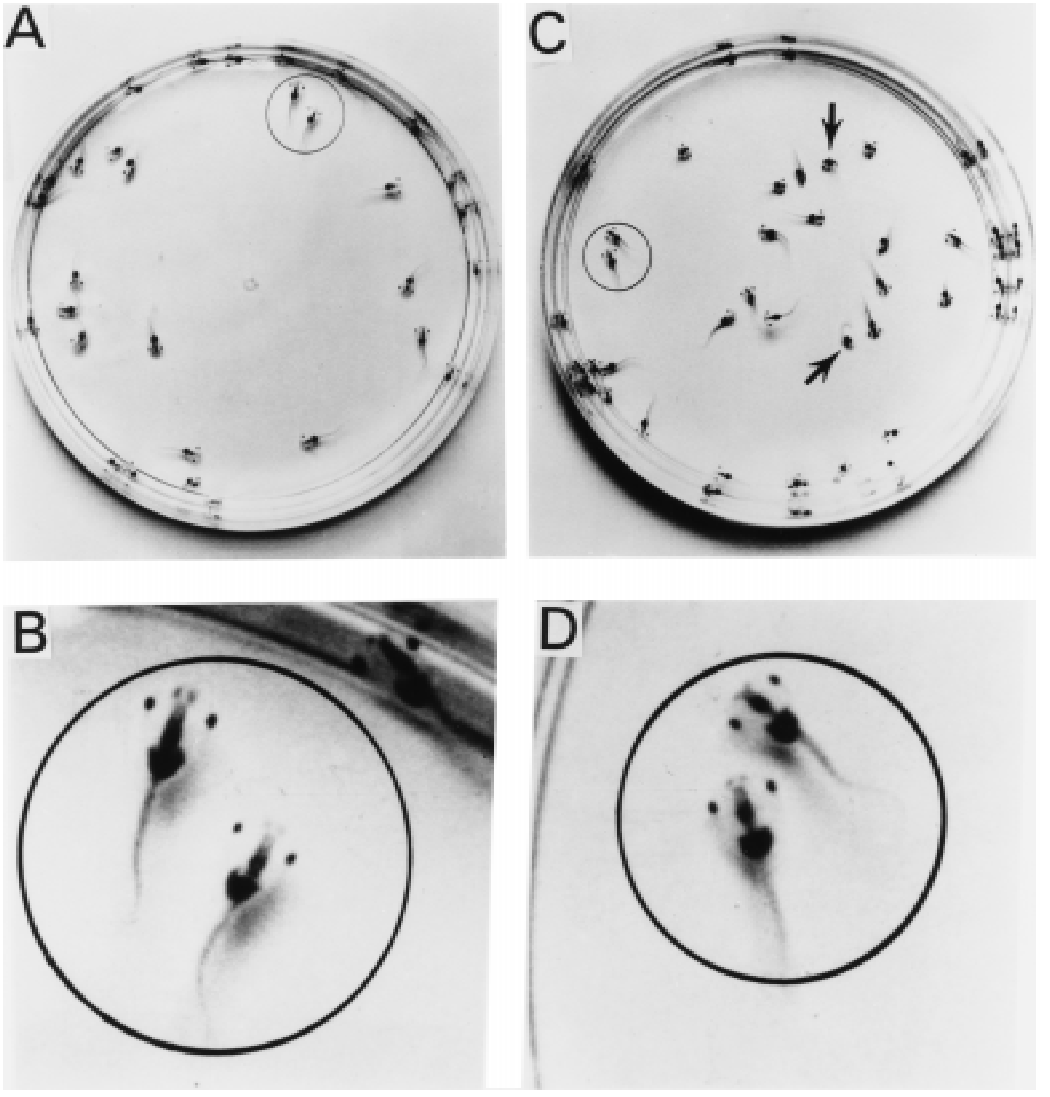

Fig. 4. Tadpoles that were grown in the absence (*A* and *B*) or continual presence (*C* and *D*) of 100 μM Gd^{3+}. The photographs were taken around 4 days after fertilization and represent stage 45 embryos. For each condition, the lower panels (*B* and *D*) represent enlargements of upper photos (*A* and *B*) and show the morphology of tadpoles under the two conditions were indistinguishable. At this stage the tadpoles were quite active and swimming continually, but no difference was noted in the swimming ability of the control and the majority of Gd^{3+}-treated tadpoles. In the control group, all 32 tadpoles were judged normal according to morphological criteria as were the majority (37 out of 39) of tadpoles in Gd^{3+}. However, 2 out of 39 were abnormal and had permanently bent tails and tended to swim in circles.

associated with patch recordings decrease rather than increase mechanosensitivity in the oocyte, so one would expect the channel to be even more mechanosensitive in the whole cell (Hamill & McBride, 1997). Regardless of the physiological stimulus, one still has to explain the functional role of this type of gated channel activity. From the point of view of the present study, the actual gating stimulus is probably not critical for the action of the three MG channel blockers, which can inhibit channels gated by chemical transmitter, second messenger, membrane voltage changes or membrane tension (Hamill & McBride, 1996*a*).

MG Channel Blockers Rapidly Block Currents in Whole Oocytes

The basic hypothesis tested in this study was that MG channels, which are expressed at a relatively high uniform density over the immature oocyte membrane surface (i.e., $>10^7$ copies per *Xenopus* oocyte), play some role in oocyte/egg development. In the absence of information on the gene that encodes the MG channel and the ability to form transgenic knockouts, we used three MG channel blockers to test the putative role(s) of MG channel activity in developmental events. Although none of

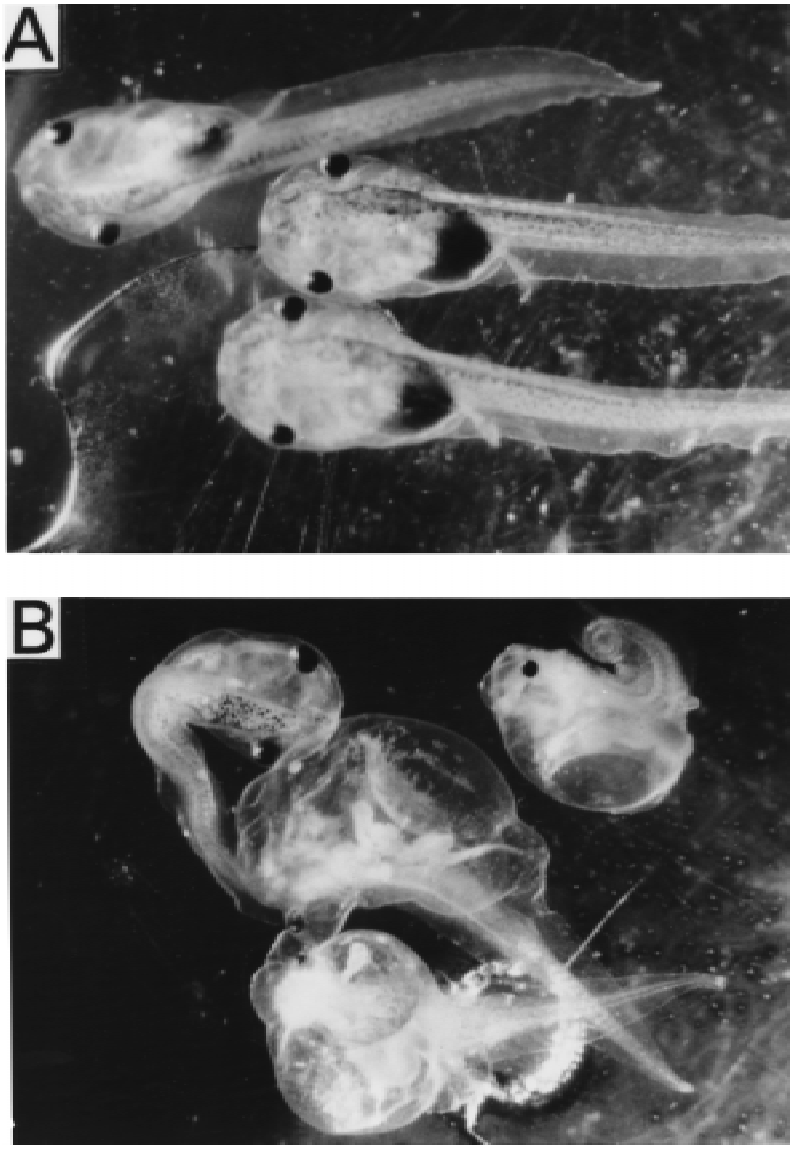

Fig. 5. Comparison of the morphology of normal and axis perturbed phenotypes that could be recognized among tadpoles that had been exposed to 100 μM Gd^{3+}. (*A*) The predominant phenotype which was seen in ~90% of animals that had been exposed to 100 μM Gd^{3+}. (*B*) Selected examples of the axis-perturbed phenotype, which was seen in as many as 10% of animals that had developed in 100 μM Gd^{3+}. The abnormal phenotypes were characterized by highly twisted spinal columns in some cases, and in other cases, the presence of swollen abdomens or ascites (i.e., swelling due to fluid accumulation). Normally tadpoles were highly active with the abnormal phenotypes often swimming in continual circles. To take these photographs, the animals were cooled in the freezer for ~5 min and afterwards allowed to recover by warming to room temperature.

the agents block MG channels with high selectivity/specificity, at least Gd^{3+} (i.e., $\geqslant$10 μM is well recognized to abolish single MG channel activity in the *Xenopus* oocyte (Fig. 1; Yang & Sachs, 1989; Hamill & McBride, 1996*a*). Nevertheless, an initial concern in this study was whether the MG channel blockers, which are effective when applied directly to patch clamped membranes, would also be effective when applied to whole oocytes that are enclosed in follicular and vitelline membranes, or eggs that are surrounded by an additional jelly coat. Specifically, we were concerned that the additional cellular layers and membranes might act as passive or active barriers and prevent the MG blockers reaching their effective blocking concentrations at the plasma membrane. This was a particular concern for Gd^{3+}, which can be precipitated out by phosphate and bicarbonate, and may be subject to uptake and chelation mechanisms (*see* Hamill & McBride, 1996*a*). For these reasons we were careful to select solutions that did not interact with Gd^{3+} (*see* Materials and Methods). Furthermore, to rule out the possibility that chemicals released from oocytes/eggs interfered with Gd^{3+}, we directly confirmed that ''oocyte conditioned'' Gd^{3+} solutions retained their ability to abolish MG channel activity in patch clamp experiments. Other direct experimental evidence arguing against access problems is that the presence of follicular and vitelline layers does not prevent the rapid block (<1 min) by the same three agents of an endogenous Ca^{2+}-inactivated current (I_c) in *Xenopus* oocytes. I_c can be recorded with conventional sharp microelectrodes without removing the follicular and vitelline membranes and is blocked completely by 1 μM Gd^{3+} (Arellano, Woodward & Miledi, 1995; Zhang et al., 1998) and partially by 200 μM or 200 μM amiloride (Zhang et al., 1998). De-

spite their similar pharmacology, I_c channels are not MG cation channels, but instead nonselective hemi-gap-junctional channels (Ebihara, 1996; Zhang et al., 1998). Even so, the fact that MG channel blockers can rapidly block I_c, indicates that these agents are freely permeable through the follicular and vitelline membranes and should be capable of blocking any MG channel activity. In the case of the jelly coat, we found its removal did not alter the ineffectiveness of 100 μM Gd^{3+} in blocking fertilization and embryogenesis.

MG Channel Activity Does Not Appear Critical for Many Developmental Events in Xenopus

Given that drug access is not a problem, the results of this study can provide no compelling evidence that MG channel activity is critical for many developmental events in *Xenopus.* The developmental events that have been examined (either directly or indirectly) for MG channel blocker sensitivity is impressive and includes oocyte growth, maintenance of the animal-vegetal polarity, progesterone-induced oocyte maturation, sperm motility and the ability of sperm to fuse with the egg, the fertilization response (including egg rotation, fast polyspermy block, exocytosis and formation of the fertilization envelope), cell cleavage and mitosis, blastulation, gastrulation, tail bud formation and presumably any other morphogenetic events that lead to generation of free-swimming stage 45 tadpoles. We have found that all of these events can apparently proceed under conditions in which MG channel activity should be abolished or at least severely inhibited. In cases where we did see inhibition, it was either incomplete, (i.e., some oocytes or eggs were unaffected and developed normally) or the inhibition observed was inconsistent with the agent's order of potency for blocking MG channels.

However, there were several developmental events we did not study. For example, we were unable to test MG channel blockers on early oocyte differentiation. This was because the in vitro tissue culture conditions that allow the growth of stage IV oocytes do not support, even in the absence of MG channel blockers, the differentiation and growth of stage I–III oocytes (*see also* Wallace et al., 1980). During early differentiation, oocytes develop their distinct animal-vegetal axis/polarity and MG channel activity could presumably participate in establishing this polarity. However, Robinson (1979) has recorded an animal-vegetal current in growing *Xenopus* oocytes and suggested that this current may be involved in establishing the polarity of the oocyte. The current appears to be a Ca^{2+}-sensitive Cl^- current which is not sensitive to block by 1 mM amiloride and is initially stimulated by 1 mM La^{3+} (Robinson, 1979). These pharmacological properties tend to argue against the idea that Ca^{2+} influx through the MG channels activates the Ca^{2+}-sensitive Cl^- current. Furthermore, our observation that oocytes (i.e., stage IV–VI), grown in the presence of MG channel blockers, retain their animal-vegetal polarity argues against a critical role for MG channel at least in polarity maintenance. Another process not directly examined here was the meiotic progression following GVB. Although GVB does not depend on external Ca^{2+} influx, evidence indicates, Ca^{2+} influx is required for progression past metaphase I, at least in pig oocytes (Kaufman & Homa, 1993).

Since there is no evidence at this time to indicate that mechanically gated channel activity is involved in many developmental events, it would seem worthwhile to reexamine the basic idea that external mechanical forces can trigger or regulate developmental processes (i.e., *see* refs. in Introduction). For example, regarding the possibility that gravity is a critical cue in development, it has recently been demonstrated that *Xenopus* eggs can be fertilized and undergo apparently normal embryogenesis and development in the microgravity of earth's orbit (Souza, Black & Wassersug, 1995). In the case of the mechanically induced (i.e., pricking) fertilization response, this may occur because of Ca^{2+} leakage through damaged areas of the membrane rather than through specific MG channels. On the other hand, Jaffe, Kado & Muncy, (1985) has described an outward current

Table 4. MG channel blockers and other agents on embryogenesis

Condition	Number of eggs fertilized	Tadpoles stage 35 (% success)	Number of axis-perturbed tadpoles
Jelly coat intact			
Control	96	96 (99)	1
Gd^{3+} 10 μM	77	76 (99)	1
Gd^{3+} 50 μM	61	61 (100)	5
Gd^{3+} 100 μM	99	99 (100)	13
Control	53	53 (100)	0
Gentamicin 1 mM	45	45 (100)	0
Control	69	69 (100)	0
Amiloride 1 mM	71	60[t] (84)	—
Amiloride 2 mM	95	70[t] (73)	—
BAPTA 5 mM	71	71* (100)	0
Tetrodotoxin 1 μM	40	40* (100)	0
Jelly coat removed			
Control	144	116 (80)	6
Gd^{3+} 10 μM	131	127 (97)	5
Gd^{3+} 50 μM	132	113 (85)	13
Gd^{3+} 100 μM	118	105 (88)	22
Control	67	47 (70)	3
Gentamicin 0.5 mM	94	68 (72)	1
Gentamicin 1 mM	59	41 (69)	2
Amiloride 0.5 mM	41	11[t] (26)	—
Amiloride 1 mM	33	0 (0)	—

[t] All embryos that reached ~stage 35 died suddenly without showing morphological abnormalities.

* All embryos died around stage 45.

associated with artificial activation in the frog egg (*Rana pipiens*) which was proposed to underlie fast polyspermy block. Furthermore, the single channel activity recorded in membrane patches, correlated with the fertilization current, was consistent (under recording the conditions) with K^+ efflux through a cation nonselective channel. Even more intriguing, Jaffe et al. (1985) reported that the frequency of this channel's opening was sensitive to mechanical disturbances of the patch. The mechanosensitivity of this channel and its sensitivity to MG channel blockers needs to be established. However, at least our results indicate that neither external Ca^{2+} nor a Gd^{3+}-sensitive current are involved in the fast polyspermy block in *Xenopus* (Grey et al., 1982). Finally, in the case of cell cleavage, which clearly involves bending and development of tension in the plasma membrane, there is a wave of elevated internal Ca^{2+} associated with the cleavage furrow, but this wave also develops in the absence of external Ca^{2+} (Keating, Cork & Robinson, 1994; Muto et al., 1996). This *indicates* that if any MG channel is involved it must mediate release of Ca^{2+} from internal membrane stores.

Although the major focus of this discussion has been on the idea that any functions of the MG channel *arise* only from ionic movements through the open channel. There also exists the possibility that the protein that forms the channel has functions independent of ion fluxes. Two possibilities come to mind, one function could be analogous to the voltage-dependent gating by the dihydropyridine (DHP) receptor of the ryanodine receptor (i.e., Ca^{2+} release channel) in the sarcoplasmic reticulum of skeletal muscle. Although the DHP receptor is a Ca^{2+} channel, the release channel is gated through direct mechanical coupling between the proteins and is independent of Ca^{2+} influx through the channel. In the specific case of the MG channel, there is evidence of its coupling to the CSK and that membrane potential can influence this channel's activity as long as this coupling is intact (Hamill & McBride, 1992, 1996*b*). It remains to be determined whether this coupling could have functional effects on other internal stores such as the Ca^{2+} release channels in the endoplasmic reticulum and whether such effects are insensitive to MG channel blockers. The second possible function could arise because the MG channel protein is a precursor which must be modified or interact with other proteins to form a new functional molecule. This scenario would be analogous to the situation with the hemi-gap-junctional channels which are expressed in the immature oocyte (Ebihara, 1996; Zhang et al., 1998). These channels are normally kept closed by *extracellular Ca^{2+} concentrations* and presumably make no contribution to the oocyte conductance (Zhang et al., 1998) but instead serve as the protein precursors for formation of the developmentally critical gap junctions between the oocyte and follicular cells (e.g., *see* Simon et al., 1997). Interestingly, while the hemi-gap conductance is blocked by external Gd^{3+}, amiloride or gentamicin, the gap-junctional channel would be expected to be insensitive, at least when the agents were applied to the external solution.

Conclusion

At this time a physiological role for the MG channel in *Xenopus* remains a mystery. The current failure to detect such a role in development may indicate that our methods of observations of all-or-none events, in some cases in isolation of one another, are not subtle enough to detect a role in which there are redundant mechanisms that can compensate in one another's absence. Given the critical importance of these events in the preservation of the species, it is not surprising that the mature egg has a high resistance to block by chemicals that could be natural contaminates of the external environment. On the other hand, we have not been able to study all developmental events in the immature and maturing oocyte, which may turn out to depend on MG channel activity. For these reasons, we tend to believe that MG channels are neither a recording artifact nor a protein without a function. It will be important to confirm our pharmacological results once the MG channel gene is cloned and the development of transgenics lacking this channel protein can be examined.

Our research is supported by grants from the National Institute of Arthritis and Musculoskeletal and Skin Diseases, Grant RO1-AR42782 and the Muscular Dystrophy Association.

References

Andreccetti, P., Denis-Donini, S., Burrini, A.G., Campanella, C. 1984. Calcium ultrastructural localization in *Xenopus laevis* eggs following activation by pricking or by calcium ionophore A23187. *J. Exp. Zool.* **229:**295–308

Anstall, H.B., Huntsman, R.G. 1960. Influence of temperature upon blood coagulation in a cold and warm-blooded animal. *Nature* **186:**726

Arellano, R.O., Woodward, R.M., Miledi, R. 1995. A monovalent cationic conductance that is blocked by extracellular divalent cations in *Xenopus* oocytes. *J. Physiol.* **484(3):**593–604

Arnoult C, Grunwald, D., Villaz, M. 1996. Novel postfertilization inward Ca^{2+} current in *Ascidian* eggs ensuring a calcium entry throughout meiosis. *Dev. Biol.* **174:**322–334

Beloussov, L.V., Lakirev, A.V., Naumidi, I.I. 1988. The role of external tensions in differentiation of *Xenopus laevis* embryonic tissues. *Cell. Diff. Devel.* **25:**165–176

Black, S.D., Gerhardt, J.C. 1985. Experimental control of embryonic axis formation in *Xenopus laevis* eggs centrifuged before first cleavage. *Dev. Biol.* **108:**310–324

Dumont, J.N. 1972. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* **136:**153–180

Ebihara, L. 1996. *Xenopus* connexin 38 forms hemi-gap-junctional

channels in the nonjunctional plasma membrane of *Xenopus* oocytes. *Biophys. J.* **71:**742–748

Green, D.P.L., Purves, R.D. 1984. Mechanical hypothesis of sperm penetration. *Biophys. J.* **45:**659–662

Grey, R.D., Bastiani, M.J., Webb, D.J., Schertel, E.R. 1982. An electrical block required to prevent polyspermy in eggs fertilized by natural mating of *Xenopus laevis*. *Develop. Biol.* **89(2):**475–484

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cell and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hamill, O.P., McBride, D.W., Jr. 1992. Rapid adaptation of the mechanosensitive channel in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **89:**7462–7466

Hamill, O.P., McBride, D.W., Jr. 1995. Mechanoreceptive Membrane Ion Channels. *American Scientist* **83(1):**30–37

Hamill, O.P., McBride, D.W., Jr. 1996*a*. The pharmacology of mechanogated membrane ion channels. *Pharmacol. Rev.* **48:**231–252

Hamill, O.P., McBride, D.W., Jr. 1996*b*. Voltage and tension sensitivity of a mechanogated channel. *Biophys. J.* **70:**A348

Hamill, O.P., McBride, D.W., Jr. 1997. Induced membrane hypo-/hypermechanosensitivity: a limitation of patch clamp recording. *Ann. Rev. Physiol.* **59:**621–631

Hara, K., Tudeman, P., Kircher, M. 1980. A cytoplamic clock with the same period as the division cycle in *Xenopus* eggs. *Proc. Natl. Acad. USA* **77:**462–466

Hiramoto, Y. 1974. Mechanical properties of the surface of the sea urchin egg at fertilization and during cleavage. *Exp. Cell Res.* **89:**320–326

Jaffe, L.A. 1996. Egg membranes during fertilization. In: *Molecular Biology of Membrane Transport Disorders.* S.G. Schultz et al., Editors. pp. 367–378 Plenum Press, New York

Jaffe, L.A., Kado, R.T., Muncy, L. 1985. Propagating potassium and chloride conductances during activation and fertilization of the egg of the frog. *Rana pipens. J. Physiol.* **368:**227–242

Jorgensen, F., Ohmori, H. 1988. Amiloride blocks the mechano-electrical transduction channels of hair cells of the chick. *J. Physiol.* **403:**577–578

Kaufmann, M.L., Homa, S.T. 1993. Defining a role for calcium in the resumption and progression of meiosis in the pig oocyte. *J. Exper. Zool.* **265:**69–76

Keating, T.J., Cork, R.J., Robinson, K.R. 1994. Intracellular free calcium oscillations in normal and cleavage-blocked embryos and artificially activated eggs of *Xenopus laevis*. *J. Cell Science* **107:**2229–2237

Kline, D., Nuccitelli, R. 1985. The activation wave in the *Xenopus* egg. *Dev. Biol.* **111:**471–487

Kroese, A.B.A., Das, A., Hudspeth, A.J. 1989. Blockage of the transduction channels of hair cells in the bullfrog's sacculus by aminoglycoside antibiotics. *Hearing Res.* **37:**203–218

Lane, J.W., McBride, D.W., Jr., Hamill, O.P. 1991. Amiloride block of the mechanosensitive cation channel in *Xenopus* oocytes. *J. Physiol.* **441:**347–366

Lane, J.W., McBride, D.W., Jr., Hamill, O.P. 1992. Structure-activity relations of amiloride and its analogues in blocking the mechanosensitive channel in *Xenopus* oocytes. *Brit. J. Pharmacol.* **106(2):**283–286

Lane, J.W., McBride, D.W., Jr., Hamill, O.P. 1993. Ionic effects on amiloride block of the mechanosensitive channel in *Xenopus* oocytes. *Brit. J. Pharmacol.* **108:**116–119

Lindsay, L.L., Hedrick, J.L. 1989. Proteases released by *Xenopus laevis* eggs at activation and their role in envelope development. *Dev. Biol.* **135:**202–211

McBride, D.W., Jr., Hamill, O.P. 1992. Pressure-clamp: a method for rapid step perturbation of mechanosensitive channels. *Pfluegers Arch.* **421:**606–612

Medina, I.R., Bregestovski, P.D. 1988. Stretch-activated ion channels modulate the resting membrane potential during early embryogenesis. *Proc. Roy. Soc. Lond. B* **235:**95–102

Medina, I.R., Bregestovski, P.D. 1991. Sensitivity of stretch-activated K^+ channel changes during cell-cleavage cycle and may be regulated by cAMP-dependent protein kinase. *Proc. R. Soc. Lond. B.* **245:**159–164

Methfessel, C., Witzemann, V., Takahashi, T., Mishina, M., Numa, S., Sakmann, B. 1986. Patch clamp measurements on *Xenopus laevis* oocytes: currents through endogenous channels and implanted acetylcholine receptor and sodium channels. *Pfluegers Arch.* **407:**577–588

Moody, W.J., Bosma, M.M. 1989. A nonselective cation channel activated by membrane deformation in oocytes of the ascidian *Boltenia villosa. J. Membrane Biol.* **107:**179–188

Morris, C.E. 1990. Mechanosensitive ion channels. *J. Membrane Biol.* **113:**93–107

Morris, C.E. 1992. Are stretch-sensitive channels in molluscan cells and elsewhere physiological mechanotransducers? *Experientia* **48:**852–858

Morris, C.E., Horn, R. 1991. Failure to elicit neuronal macroscopic mechanosensitive currents anticipated by single channel studies. *Science* **251:**1246–1249

Muto, A., Kume, S., Inoue, T., Okano, H., Mikoshiba, K. 1996. Calcium waves along the cleavage furrows in cleavage-stage *Xenopus* embryos and its inhibition by heparin. *J. Cell Biol.* **135:**181–190

Neff, A.W., Malacinski, G.M., Wakahara, M., Jurand, A. 1983. Pattern formation in amphibian embryos prevented from undergoing the classical ''rotation response'' to egg activation. *Dev. Biol.* **97:**103–112

Nieuwkoop, P.D., Faber, J. 1956. Normal Tables of *Xenopus laevis* (Daudin). North Holland Publishing, Amsterdam

Odell, G.M., Oster, G., Alberch, P., Burnside, B. 1981. The mechanical basis of morphogenesis. I. Epithelial folding and invagination. *Dev. Biol.* **85:**446–462

Peres, A., Mancinelli, E. 1985. Sodium conductance and the activation potential in *Xenopus laevis* eggs. *Pfluegers Arch.* **405:**29–36

Robinson, K.R. 1979. Electrical currents through full-grown and maturing *Xenopus* oocytes. *Proc. Natl. Acad. USA* **76:**837–841

Ruesch, A., Kros, C.J., Richardson, G.P. 1994. Block by amiloride and its derivatives of mechano-electrical transduction in outer hair cells of mouse cochlear cultures. *J. Physiol* **474(1):**75–86

Sachs, F. 1988. Mechanical transduction in biological systems. *CRC Crit. Rev. Biomed. Eng.* **16:**141–169

Sackin, H. 1996. Mechanosensitive channels. *Ann. Rev. Physiol.* **57:**333–353

Sardet, C., McDougall, A., Houliston, E. 1994. Cytoplasmic domains in eggs. *Trends Cell Biol.* **4:**166–172

Scharf, S.R., Gerhart, J.C. 1983. Axis determination in eggs of *Xenopus laevis:* a critical period before first cleavage, identified by the common effects of cold, pressure, and ultraviolet irradiation. *Dev. Biol.* **99:**75–87

Schorderet-Slatkine, S., Schorderet, M., Baulieu, E.E. 1976. Initiation of meiotic maturation in *Xenopus laevis* oocytes by lanthanum. *Nature* **262:**289–290

Simon, A.M., Goodenough, D.A., Li, E., Paul, D.L. 1997. Female infertility in mice lacking connexin 37. *Nature* **385:**525–528

Small, D.L., Morris, C.E. 1994. Delayed activation of single mechanosensitive channels in *Lymnaea* neurons. *Am. J. Physiol.* **267:**C598–C606

Souza, K.A., Black, S.D., Wassersug, R.J. 1995. Amphibian develop-

ment in the virtual absence of gravity. *Proc. Natl. Acad. Sci. USA* **92:**1975–1978

Steffensen, I., Bates, W.R., Morris, C.E. 1991. Embryogenesis in the presence of blockers of mechanosensitive ion channels. *Dev. Growth and Diffn.* **33(5):**427–442

Taglietti, V., Tanzi, F., Romero, R., Simoncini, L. 1984. Maturation involved suppression of voltage-gated currents in frog oocyte. *J. Cell Physiol.* **121:**576–588

Taglietti, V., Toselli, M. 1988. A study of stretch activated ion channels in frog oocytes: interaction with Ca^{2+} ions. *J. Physiol.* **407:**311–328

Usui, N., Yoneda, M. 1982. Ultrastructural basis of the tension increase in sea urchin eggs prior to cytokinesis. *Dev. Growth Diffn.* **24:**453–465

Vandorpe, D.H., Small, D.L., Dabrowski, A.R., Morris, C.E. 1994. FMRFamide and membrane stretch as activators of the *Aplysia* S-channel. *Biophys. J.* **66:**46–58

Wallace, R.A., Misulovin, A., Wiley, H.S. 1980. Growth of anuran oocytes in serum-supplemented medium. *Reprod. Nutr. Develop.* **20(3A):**699–708

Wu, G., McBride, D.W., Jr., Hamill, O.P. 1998. Mg^{2+} block and inward rectification of mechanosensitive channels in *Xenopus* oocytes. *Pfluegers Arch.* **435:**572–574

Yang, X.C., Sachs, F. 1989. Block of stretch-activated ion channels in *Xenopus* oocytes by gadolinium and calcium ions. *Science* **242:**1068–1071

Yang, X.C., Sachs, F. 1990. Characterization of stretch-activated ion channels in *Xenopus* oocytes. *J. Physiol.* **431:**103–122

Zhang, Y., McBride, D.W., Jr., Hamill, O.P. 1998. The ion selectivity of a membrane conductance inactivated by extracellular calcium in *Xenopus* oocytes. *J. Physiol.* **508:**763–776