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A Specific Inhibitory Action of Lithium on the 5-HT_{2c} Receptor Expressed in *Xenopus laevis* Oocytes

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SUMMARY

The effect of lithium on the phosphoinositide-signaling pathway was examined in 5-HT_{2C} receptors, which are involved in phospholipase C stimulation, expressed in *Xenopus laevis* oocytes by voltage-clamp recording and assay of intracellular Ca^{2+} concentrations. Treatment with lithium for 60 sec after the initial application of 5-HT reduced Ca^{2+}-dependent chloride currents in a dose-dependent manner (0.01–1 mM) and inhibited intracellular Ca^{2+} release, whereas pretreatment with lithium or injection into oocytes had no effect. Additionally, treatment with lithium for more than 24 hr reduced 5-HT-evoked currents to a much lesser extent. In contrast, the currents through other phosphoinositide-dependent receptors, such as endogenous "serum" and muscarinic ACh receptors, were not affected or less affected by a short term or long term treatment with lithium, respectively. These results indicate that lithium may have a specific blocking effect on the 5-HT_{2C} receptors and, in part, nonselectively act on the phosphoinositide metabolic pathway.

Lithium has been widely used as an effective drug for manic-depressive illness (1) as well as aggressive and self-mutilating behavior (2) or cluster headache (3). The mechanisms underlying the action of Li^+, however, largely remain unknown. There is a possibility that lithium interacts with inositol phosphate metabolism. Administration of Li^+ decreases the level of inositol in the brain (4), although it has no effect on the peripheral cells. Inositol is supplied via three pathways: uptake from the plasma, synthesis through glucose, and recycling and resynthesis from the cellular pool of phosphatidylinositol (5). In central neurons, most inositol is dependent on endogenous sources, because the blood-brain barrier is barely permeable to inositol (6–8), and the enzyme responsible for the conversion of glucose-6-phosphate to *myo*-inositol-1-phosphate is restricted to the cerebral vasculare (9). Thus, lithium seems to block endogenous inositol production in the central nervous system.

5-HT receptors are thought to exert diverse actions on psychiatric disorders such as anxiety, manic-depression, obsessive-compulsive disorder, schizophrenia, suicidal behavior, and alcoholism (10). The receptors are classified into 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, 5-HT_7, and others (11). Of these groups, the 5-HT2 receptors are further subdivided into 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} and are coupled to the stimulation of phospholipase C (11). The 5-HT_{2C} receptor, which is preferentially expressed in the brain, is proposed to be involved in pathogenesis of manic-depressive illness, as is the 5-HT_{2A} receptor (12). There is speculation that lithium improves manic-depressive psychosis by normalizing 5-HT_2 receptor-mediated phosphoinositide signaling. The present study investigated the effects of lithium on the 5-HT_{2C} receptors expressed in *Xenopus laevis* oocytes by assaying Ca^{2+}-dependent chloride currents and intracellular Ca^{2+} mobilization. Previous reports demonstrate that lithium has little effect when phosphoinositide-dependent receptors are operating normally, which leads to the conclusion that Li^+ is an uncompetitive inhibitor of inositol phosphate metabolism (5). The results of the present study suggest, however, that lithium inhibits phosphoinositide signaling by its specific action on the 5-HT_{2C} receptors.

Materials and Methods

In vitro transcription and translation in *X. laevis* oocytes

mRNA coding for the rat brain 5-HT_{2C} receptor was provided Dr. K. Sumikawa (University of California, Irvine, CA). *X. laevis* oocytes were surgically removed from female frogs and manually separated from the ovary. The isolated oocytes were incubated in Barth's solution: 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_2)_2$, 0.41 mM $CaCl_2$, and 7.5 mM Tris, pH 7.6. Collagenase (0.5 mg/ml) treatment of oocytes was carried out to remove the follicular cell layer 1 day before microinjection. Oocytes were injected (approximately 40 nl) with 5-HT_{2C} receptor mRNA and incubated at 18°.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); ACh, acetylcholine; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, tetrapotassium salt, hydrate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Electrophysiology

The injected oocytes were transferred to the recording chamber 24–48 hr after incubation and continuously superfused at room temperature (20–22°) in a standard frog Ringer's solution: 115 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.0). 5-HT-, blood serum-, and ACh-activated currents were recorded using two-electrode voltage-clamp techniques with a GeneClamp-500 amplifier (Axon Instruments, Foster City, CA) as previously described (13). The currents were digitized at 2 kHz, stored on a computer disk, and analyzed on a laboratory computer using pClamp software (version 6; Axon Instruments).

Co-assay of intracellular Ca^{2+} and membrane current

Oocytes were injected with Calcium Green-1 (Molecular Probes, Eugene, OR) (40 nl, 200 μM, approximately 15 μM final concentration) and were incubated at 18° for 30–60 min. The oocytes were transferred in a recording chamber onto the stage of a Nikon DIAPHOT 300 microscope (Nikon, Tokyo, Japan) and continuously superfused at room temperature (20–22°) in frog Ringer's solution: 115 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.0). The oocytes were viewed with a 4× UV fluor Nikon objective lens, and the images were acquired at 2-sec intervals with a xenon confocal laser-scanning microscope (Nikon) attached to an intensified charge-coupled device camera (ARGUS-50/CA; Hamamatsu Photonics, Tokyo, Japan). The Calcium Green signal was long pass-filtered (490 nm). Images were analyzed with ARGUS-50/CA software (version 3.0). Simultaneously, a two-electrode voltage-clamp recording was carried out with a GeneClamp-500 amplifier, and the currents obtained were analyzed by the same method as described above.

Results and Discussion

X. laevis oocytes have endogenous Ca^{2+}-sensitive chloride channels (14) that are activated through the phosphoinositide-dependent receptors, including 5-HT_2 receptors. Application of 5-HT (1 μM) to the oocytes expressing 5-HT_{2C} receptors induced inward currents with a latency of 30–40 sec at a holding potential of −60 mV (Fig. 1A). The currents reversed at approximately −15 mV and were blocked by injection of BAPTA (final concentration 10 mM) in the oocytes (data not shown), which indicates that the endogenous Ca^{2+}-dependent chloride channel was activated through the 5-HT_{2C} receptors. Treatment with Li^+ (0.1 mM) for 60 sec after the initial activation of the 5-HT_{2C} receptors reduced the currents to 20%, and, afterward, the currents were recovered with washing (Fig. 1A, *upper column*). The currents were inhibited by lithium (0.01–1 mM) in a dose-dependent manner (Fig. 1B). In contrast, pretreatment with Li^+ (0.1 mM) for 10 min had no effect on the currents (Fig. 1A, *lower column*). Additionally, injection of Li^+ (0.1 mM and 1 mM, final conc.) before or after the initial application of 5-HT never inhibited the currents (Fig. 2). These results suggest that lithium reduced Ca^{2+}-dependent chloride currents by a specific action on the 5-HT_{2C} receptors during or after activation of the receptors but not by an inhibition of the intracellular inositol pathway. The data also may indicate that lithium acted on the receptor outside the membrane.

X. laevis oocytes are known to have endogenous phosphoinositide-dependent receptors such as the "serum receptor" (15, 16) and muscarinic ACh receptor (17). Fetal bovine serum (0.5%, v/v) or ACh (100 μM) also produced Ca^{2+} -dependent chloride currents (Fig. 3). Unlike the 5-HT-induced response, these currents were not affected by treatment with Li^+ (0.1 mM) after the first application of serum or ACh (Fig. 3A) or pretreatment with Li^+ (Fig. 3B). 5-HT (1 μM)-induced currents in the same oocyte, however, were inhibited by treatment with Li^+ after the first application of 5-HT, although they were not affected by pretreatment with Li^+ (Fig. 3A). These results suggest that lithium did not act on the nonselective phosphoinositide-dependent receptors but selectively modified the 5-HT_{2C} receptors. The data also suggest

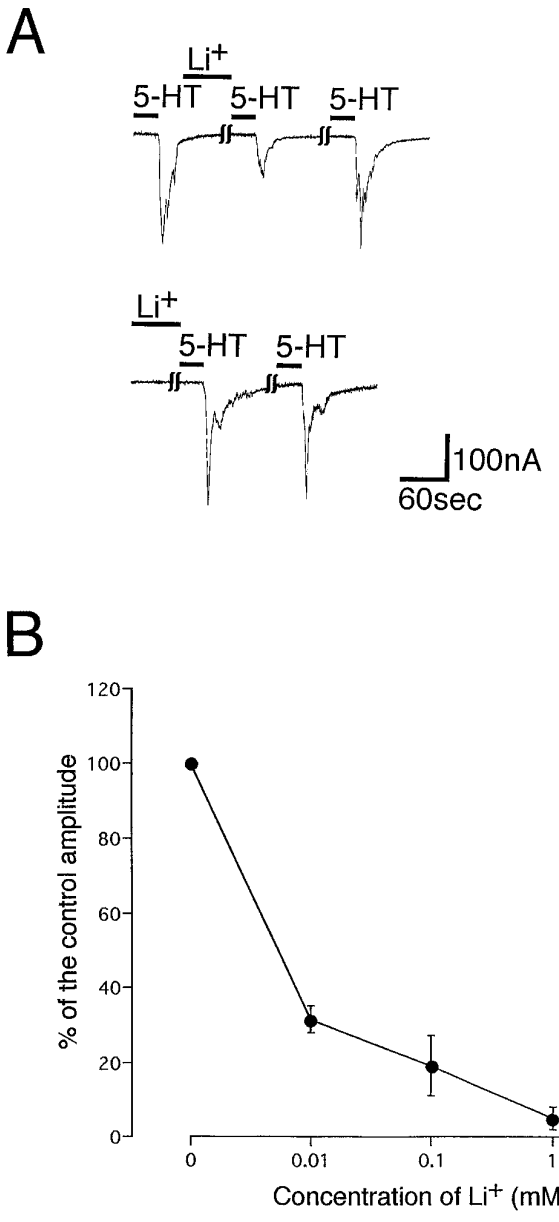

Fig. 1. Effect of a short term treatment with lithium on 5-HT-induced currents. 5-HT (1 μM) was repetitively applied to a single oocyte expressing the 5-HT_{2C} receptors at 15-min intervals. A, An oocyte was treated with lithium (0.1 mM) for 60 sec after the first application of 5-HT (n = 20) (*upper column*). In another case, an oocyte was pretreated with lithium (0.1 mM) for 10 min, and, afterward, 5-HT was applied to the oocyte (n = 15) (*lower column*). The holding potential was −60 mV. In this and other figures, inward currents correspond to downward deflections. B, The dose-response effect of lithium on 5-HT-evoked currents are demonstrated. 5-HT (1 μM) was applied to a single oocyte before and after 60-sec treatment with lithium in order of concentrations at 0.01 mM, 0.1 mM, and 1 mM, or in the opposite order. The current amplitude recorded in an oocyte without lithium treatment was regarded as 100%. *Points,* average from 15–20 oocytes; *bars,* mean ± standard deviation.

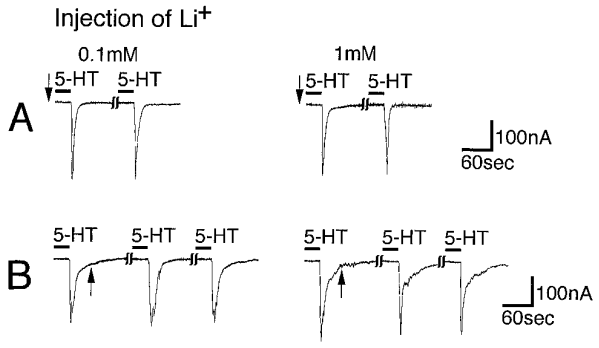

Fig. 2. Effects of injection of lithium on 5-HT$_{2C}$ receptor-induced currents. Two electrodes for voltage-clamp were placed, and, subsequently, an injection needle was inserted in the oocytes. Lithium (0.1 mM and 1 mM, final concentrations) was injected in an oocyte expressing 5-HT$_{2C}$ receptors 5 min before (A) and immediately after (B) the initial application of 5-HT (1 μM) (*arrows*). Each trial was carried out in 15 oocytes. The holding potential was −60 mV.

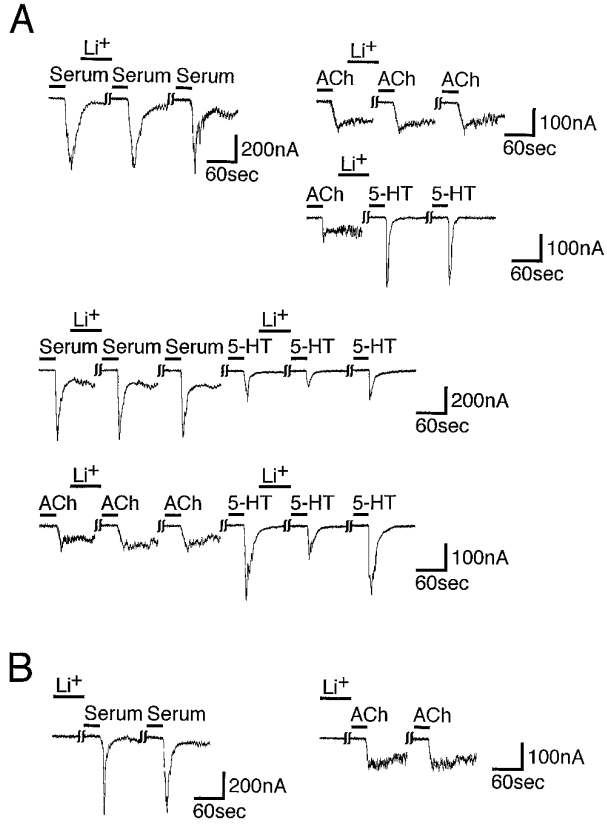

Fig. 3. Effects of a short term treatment with lithium on the currents induced by serum or ACh. A, Oocytes were treated with lithium (0.1 mM) for 60 sec after the first application of fetal bovine serum (0.5%, v/v) or ACh (100 μM) (n = 15). Afterward, 5-HT (0.1 mM) was applied to the same oocytes before and after the second treatment with lithium in some cases (n = 5). B, fetal bovine serum or ACh was applied to an oocyte after pretreatment with lithium for 10 min (n = 15). The holding potential was −60 mV.

that a prior 5-HT$_{2C}$ receptor activation was needed for the Li$^+$ inhibition of subsequent 5-HT$_{2C}$ receptor-mediated response.

To ensure that depression of 5-HT-evoked currents induced by lithium is caused by inactivation of the Ca^{2+}-activated chloride channels or by decrease of intracellular Ca^{2+} release, intracellular Ca^{2+} concentrations and membrane currents were co-assayed. 5-HT (1 μM) enhanced intracellular Ca^{2+} concentrations and evoked the currents (Fig. 4A), which indicates that the 5-HT$_{2C}$ receptor was involved in phosphatidylinositol hydrolysis, followed by production of inositol-1,4,5-trisphosphate, to release Ca^{2+} from intracellular calcium stores. Subsequent treatment with Li$^+$ (0.1 mM) inhibited both enhancement in intracellular Ca^{2+} concentrations and currents induced by 5-HT (Fig. 4A). Otherwise, injection of Li$^+$ (final concentration 0.1 mM) had no effect on the intracellular Ca^{2+} concentrations and currents (Fig. 4B), which further supports the idea that lithium inhibits 5-HT$_{2C}$ receptor-mediated response outside the plasma membrane, presumably by modulation of the binding affinity of 5-HT to the 5-HT$_{2C}$ receptor or competitive binding to the receptor.

The effect of Li$^+$ on the phosphoinositide-dependent receptors was further examined by a long term treatment. 5-HT-evoked currents were abolished by co-treatment with Li$^+$ (0.1 mM) and 5-HT (1 μM) for more than 24 hr and reduced to 76% by treatment with Li$^+$ alone, whereas a long term treatment with 5-HT alone had no effect (Fig. 5A). In contrast, the currents induced by serum (0.5%, v/v) or ACh (100 μM) were decreased to 67% or 83%, respectively, by a long term co-treatment with Li$^+$ (0.1 mM) and serum or ACh, to 71% or 85% by treatment with lithium alone, respectively, but they were not affected by a long term treatment with serum or ACh (Fig. 5B, C). These results suggest that lithium might serve as a blocker of intracellular inositol metabolic pathways only in a small part.

Previous studies demonstrate that lithium reduces the supply phosphatidylinositol-4,5-bisphosphate for signaling by blocking inositolpolyphosphate-1-phosphatase, which converts inositol-1,3,4-trisphosphate or inositol-1,4-bisphosphate to inositol-3,4-bisphosphate or inositol-4-monophosphate, respectively, and by blocking inositol monophosphate phosphatase, which converts inositol monophosphates to inositol in a process called "inositol depletion hypothesis" (5). Indeed, the finding that a long term co-treatment with lithium and 5-HT completely inhibited 5-HT-induced currents may support this hypothesis. However, 5-HT-evoked currents were decreased to 20% by a short term treatment with lithium and were not blocked by injection of lithium in oocytes, which indicates that inhibition of 5-HT-evoked currents by lithium is mainly the result of a modulation of the 5-HT$_{2C}$ receptor. The finding that serum- and ACh-evoked currents were not inhibited or little inhibited by a short term or long term treatment with lithium may provide further evidence that lithium has a less functional role in the inositol signaling pathways.

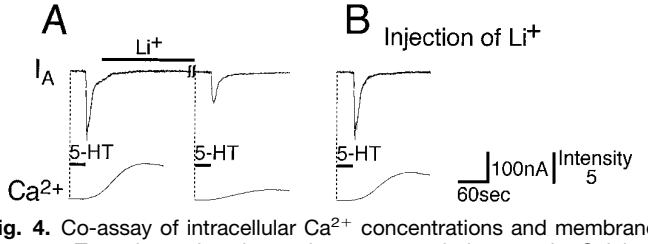

Fig. 4. Co-assay of intracellular Ca^{2+} concentrations and membrane currents. Two-electrode voltage-clamp was carried out on the Calcium Green-loaded oocytes. The oocytes were treated with lithium (0.1 mM) (n = 5) (A) or injected with Li$^+$ (final concentration 0.1 mM) (n = 5) (B) after the initial application of 5-HT (1 μM). The currents (I_A) and Ca^{2+} signals in the confocal section of the oocytes (Ca^{2+}) are illustrated in the same *trace*. The holding potential was −60 mV.

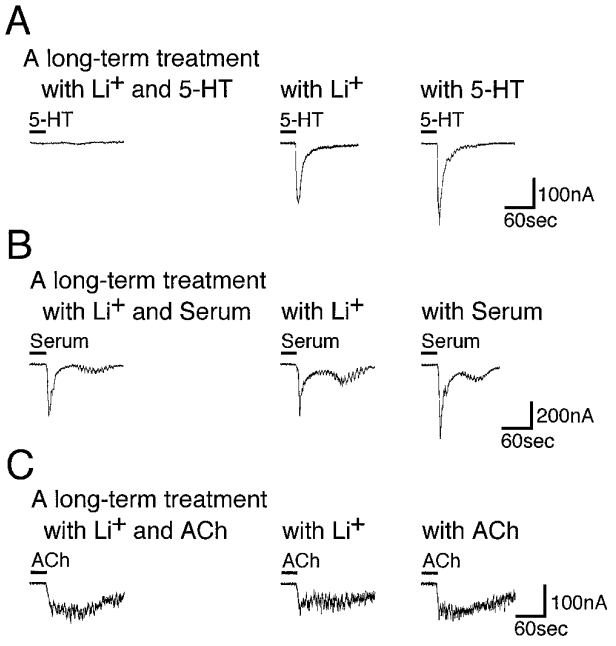

Fig. 5. Effects of a long term treatment with lithium on 5-HT-, serum-, and ACh-induced currents. A, The oocytes expressing 5-HT_{2C} receptors were treated with both lithium (0.1 mM) and 5-HT (1 μM), lithium alone, or 5-HT alone. B, The oocytes were treated with both lithium (0.1 mM) and fetal bovine serum (0.5%, v/v), lithium alone, or fetal bovine serum alone. C, The oocytes were treated with both lithium (0.1 mM) and ACh (100 μM), lithium alone, or ACh alone. More than 24 hr later, 5-HT (1 μM), serum (0.5%, v/v), or ACh (100 μM) was applied to the oocytes. The holding potential was −60 mV. The current amplitude evoked by 5-HT, fetal bovine serum, or ACh before these treatments was regarded as 100%. Each experiment was performed in 30 oocytes.

Lines of evidence suggest that manic-depressive illness is effectively controlled by Li^+ at concentrations of approximately 1 mM in the blood serum (5). A plausible explanation is that lithium modifies a downstream pathway to reestablish normal responses to the 5-HT_{2C} receptor, which is proposed to be one of the receptors responsible for manic-depressive illness, perhaps by interaction with the phosphoinositide metabolic pathways. The current results, however, indicate that lithium may control manic-depressive psychosis by acting selectively on the 5-HT_{2C} receptor in large part rather than by the nonselective effect on the phosphoinositide metabolic pathways.

In conclusion, we show herein that lithium inhibits inositol signaling mainly by its specific effect on the 5-HT_{2C} receptor and that it serves as an inhibitor of inositol phosphate metabolism only in small part.

References

1. Rosenthal, N. E., and F. K. Goodwin. The role of the lithium ion in medicine. *Annu. Rev. Med.* **33:**555–568 (1982).
2. Wickham, E. A., and J. V. Reed. Lithium for the control of aggressive and self-mutilating behaviour. *Int. Clin. Psychopharmacol.* **2:**181–190 (1987).
3. Ekbom, K. Lithium for cluster headache. *Headache* **21:**132–139 (1981).
4. Allison, J. H., and M. A. Stewart. Reduced brain inositol in lithium treated rats. *Nat. New Biol.* **233:**267–268 (1971).
5. Berridge, M. J., C. P. Downes, and M. R. Handley. Neural and developmental actions of lithium: a unifying hypothesis. *Cell* **59:**411–419 (1989).
6. Margolis, R. V., R. Press, N. Altszuler, and M. A. Stewart. Inositol production by the brain in normal and alloxan-diabetic dogs. *Brain Res.* **28:**535–539 (1971).
7. Lewin, L. M., Y. Yanna, S. Sulimovici, and P. F. Kraicer. Studies on the metabolic role of *myo*-inositol: distribution of radioactive *myo*-inositol in the male rat. *Biochem. J.* **156:**375–380 (1976).
8. Barkai, A. I. *myo*-Inositol turnover in the intact rat brain: increased production after d-amphetamine. *J. Neurochem.* **36:**1485–1491 (1981).
9. Wong, Y.-H. H., S. J. Kalmbach, B. K. Hartman, and W. R. Sherman. Immunohistochemical staining and enzyme activity measurements show *myo*-inositol-phosphate synthase to be localized in the vasculature of brain. *J. Neurochem.* **48:**1434–1442 (1987).
10. Zifa, E., and G. Filion. 5-hydroxytryptamine receptors. *Pharmacol. Rev.* **44:**401–458 (1992).
11. Branchek, T. More serotonin receptors? *Curr. Biol.* **3:**315–317 (1993).
12. Pandey, S. C., J. M. Davis, and G. N. Pandey. Phosphoinositide system-linked serotonin receptor subtypes and their pharmacological properties and clinical correlates. *J. Psychiatry Neurosci.* **20:**215–225 (1995).
13. Nishizaki, T., and Y. Ikeuchi. Activation of endogenous protein kinase C enhances currents through α1 and α2 glycine receptor channels. *Brain Res.* **687:**214–216 (1995).
14. Miledi, R., and I. Parker. Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol.* **357:**173–183 (1984).
15. Tigyi, G., D. Dyer, C. Matute, and R. Miledi. A serum factor that activates the phosphatidylinositol phosphate signaling in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **87:**1521–1525 (1990).
16. Tygi, G., A. Henschen, and R. Miledi. A factor that activates oscillatory chloride currents in *Xenopus* oocytes copurifies with a subfraction of serum albumin. *J. Biol. Chem.* **266:**20602–20609 (1991).
17. Kusano, K., R. Miledi, and J. Stinnakre. Acetylcholine receptors in the oocyte membrane. *Nature (Lond.)* **270:**739–741 (1977).

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