$[Ca^{2+}]$ spike that is larger than those which occurred in our experiments (*1*). Yet, the first and second flashes that theoretically produced large $[Ca^{2+}]$ spikes did not trigger channel activity.

Experimental conditions were then set to minimize the amplitude of the $[Ca^{2+}]$ spike (Fig. 2). The resting free $[Ca^{2+}]$ was adjusted to 1 μM so that the free [nitrophen] was extremely low. The pre-flash P_O was about 0.25, as one would predict from the steady-state Ca^{2+} dependence of the channel (*1*). One high-intensity laser flash was applied to elevate the free $[Ca^{2+}]$ to 10 μM. According to the interpretation of Lamb *et al.*, the fast $[Ca^{2+}]$ spike would be essentially absent under these conditions. Yet, in the absence of $[Ca^{2+}]$ spikes, photolysis induced fast activation followed by a slow decay in channel activity.

Finally, our interpretation of the data led to the hypothesis that single channels adapt to a Ca^{2+} stimulus (*1*). This complex single channel behavior correlates well with phenomena reported in other studies. For example, the self-adjusting mechanism described for mechanosensitive channels (*5*) is phenomenologically similar to adaptation. The "incremental" or "quantal" activation described in populations of IP_3 receptor channels (*6*) is also consistent with the existence of single channel adaptation.

Thus, in the absence of experimental data to the contrary, we have confidence in the interpretation of our results and in the existence of single channel adaptation.

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REFERENCES

1. S. Györke and M. Fill, *Science* **260**, 807 (1993).
2. J. H. Kaplan and G. C. R. Ellis-Davies, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6571 (1988).
3. R. S. Zucker, *Cell Calcium* **14**, 87 (1993).
4. J. Vergara and A. Escobar, *Biophys. J.* **64**, a37 (1993).
5. O. P. Hamill and D. W. Mcbride, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7462 (1992).
6. S. Muallem, S. J. Pandol, T. G. Beeker, *J. Biol. Chem.* **264**, 205 (1989); C. Taylor and B. V. L. Potter, *Biochem. J.* **266**, 189 (1990); I. Parker and I. Ivorra, *Science* **250**, 977 (1990); T. Meyer and L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3841 (1990); C. D. Ferris *et al.*, *Nature* **356**, 350 (1992).

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Fertilization and Ion Channels

We would like to point out flaws in the experimental design of a recent study by Kupitz and Atlas (*1*). The first part of the report describes a Na^+ channel in the immature oocyte that is activated by external concentrations of 0.2 to 2.0 mM of adenosine 5′-triphosphate (ATP) (*2*). Kupitz and Atlas then suggest that this ATP-activated Na^+ channel might be involved in fertilization of the mature egg. There are major differences, however, between an immature oocyte and a mature egg. Many changes in the plasma membrane occur during maturation, greatly decreasing the ion channel density and membrane permeability (*3*). Therefore, before suggesting that the sperm might activate a Na^+ channel in the mature egg, Kupitz and Atlas should have first determined if mature eggs also exhibited ATP-sensitive channels. In addition, they implied in their opening paragraph that an increase in Na^+ permeability normally occurs at fertilization, but this does not occur in the frog egg, where the sperm activates a Ca^{2+}-gated Cl^- efflux that depolarizes the mature egg and provides an electrical block to polyspermy (*4*).

The second flaw in their report is the proposal that the high amounts of ATP inside the sperm could activate the putative ATP-sensitive channel on the exterior surface of the egg's plasma membrane. This would require that the sperm secrete millimolar amounts of ATP during a period in which it is in need of this energy source to swim through the 0.5-mm-thick jelly layer surrounding the egg. While implausible, this idea could have been investigated directly, but was not. Instead, Kupitz and Atlas designed experiments in which sperm, jellied eggs, and various compounds were added together in the same dish and egg cleavage was used as an indirect indicator of fertilization. Many compounds will not easily diffuse through the thick egg jelly layer, so these conditions do not guarantee that the applied concentration of each compound was present at the membrane surface. Moreover, a more likely target for these compounds is the sperm. If any of the applied compounds interfered with sperm activation or motility, a similar absence of normal development could result. Yet Kupitz and Atlas do not describe control experiments that would test for the effects of the various compounds on the ability of the sperm to fertilize unexposed eggs.

These flaws in experimental design make it impossible to judge the reliability of this report.

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REFERENCES AND NOTES

1. Y. Kupitz and D. Atlas, *Science* **261**, 484 (1993).
2. This current-voltage response is similar to that reported by I. Lotan *et al.* [*Nature* **298**, 572 (1982)] for ATP-induced currents in the immature *Xenopus* oocyte, in which it was concluded that Cl^- was the main current carrier.
3. T. Maeno, *J. Gen. Physiol.* **43**, 139 (1959); M. E. Barish, *J. Physiol. (London)* **342**, 309 (1983); D. J. Webb and R. Nuccitelli, *Dev. Biol.* **107**, 395 (1985); J. L. Rodcau and J. P. Vilain, *ibid.* **120**, 481 (1987); L. C. Schlichter, in *Mechanisms of Egg Activation*, R. Nuccitelli, G. N. Cherr, W. H. Clark Jr., Eds. (Plenum, New York, 1989), p. 89.
4. R. D. Grey, M. J. Bastiani, D. J. Webb, E. R. Schertel, *Dev. Biol.* **89**, 475 (1982); D. J. Webb and R. Nuccitelli, *ibid.* **107**, 395 (1985).

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Response: In our report, we suggested that the involvement of ATP-activated channel in fertilization is suggested on the basis of analogy with oocytes.

1) It is clear that membranes of eggs and oocytes are absolutely different from each other; however, as different as they are, we found a strong correlation between inhibition of fertilization and inhibition of ATP-induced current in oocytes by guanosine 5′-triphosphate (GTP), guanosine .5′-[β,γ-imido]triphosphate (GppNHp), and amiloride.

2) ATP-activated current can be upstream to the Ca^{2+}-gated Cl-efflux.

3) High ATP concentration inside the sperm was mentioned in our report as a possible source for the ATP that acts at the membrane. We agree that the actual mechanism should be worked out.

4) Neither GTP, GppNHp, or amiloride had an effect on the mobility of the sperm in our study. More rigorous tests should be applied, as suggested in the comment.

5) Applying the antagonists to the jellied eggs at concentrations mentioned in our report would not reflect their actual concentration at the membrane. Nevertheless, the three compounds, at those concentrations, were effective at inhibiting sperm-induced fertilization.

A direct electrophysiological recording from mature eggs, compared with our oocytes recording, would help to confirm the indirect approach we took in comparing inhibitory action of various ligands at oocytes and mature eggs.

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