Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5464–5468, August 1987
Neurobiology

Expression of an ω-conotoxin-sensitive calcium channel in *Xenopus* oocytes injected with mRNA from *Torpedo* electric lobe

(*in vivo* translation/voltage clamp/barium currents)

JOY A. UMBACH AND CAMERON B. GUNDERSEN

Department of Pharmacology and The Jerry Lewis Neuromuscular Research Center, University of California at Los Angeles School of Medicine, Los Angeles, CA 90024-1735

Communicated by S. Hagiwara, April 2, 1987

ABSTRACT ***Xenopus laevis* oocytes were injected with poly(A)$^+$ RNA isolated from the electric lobe of *Torpedo californica*. Six to nine days after mRNA injection of the oocytes a cadmium-sensitive inward current could be detected in oocytes bathed in a calcium- and chloride-free solution containing 40 mM barium. This inward current could be distinguished from the native barium current of control oocytes by its high sensitivity to blockade by cadmium ions and its inhibition by ω-conotoxin, a peptide neurotoxin from *Conus geographicus*. Neither the current of control cells nor that of injected cells was detectably affected by nisoldipine (1 μM) or nitrendipine (1 μM). However, the barium current of control oocytes showed appreciably more inactivation (in the barium solution used for recording) than the ω-conotoxin-sensitive current that develops in mRNA-injected oocytes. Culturing of mRNA-injected oocytes in medium containing actinomycin D failed to prevent the appearance of the ω-conotoxin-sensitive current. These results support the conclusion that mRNA from *Torpedo* electric lobe is translated to produce an additional calcium channel in *Xenopus* oocytes. The features of this channel suggest that it may be the same type of calcium channel that controls transmitter release at nerve endings in *Torpedo* electroplax.**

Voltage-gated calcium channels mediate the cellular influx of calcium ions that triggers such events as neurotransmitter release and the contraction of ventricular myocardium (1–3). Excitable cells of vertebrates often possess two types of voltage-sensitive calcium channels (4–7), while derivatives of the neural crest appear to have a third distinguishable form of calcium channel (8). These different calcium channels have been designated N, L, and T (8) on the basis of physiological parameters (e.g., single-channel conductance and activation properties) and sensitivity to pharmacological agents (especially dihydropyridines and cadmium ions). If similar criteria are used to classify the calcium channels of invertebrate cells, at least two additional groupings of calcium channels may be needed to encompass the observed spectrum of channel properties (9, 10). It would be of considerable interest to resolve the extent to which this multiplicity of calcium channels is attributable to separate genes coding for each type of channel.

Recently, Dascal, Leonard, and colleagues (11, 12) reported the appearance of novel divalent-cation currents in *Xenopus laevis* oocytes injected with mRNA from rat brain or heart. The present investigation involves similar experiments in which oocytes express a calcium channel with characteristics unlike those observed previously in oocytes (11–13). Specifically, the divalent-cation current is insensitive to blockade by nitrendipine or nisoldipine (1 μM). However, inhibition is seen with submicromolar concentrations of cadmium ions or with ω-conotoxin (ωCgTx), a peptide toxin (14) from *Conus geographicus*. ωCgTx appears to block calcium channels at many vertebrate nerve endings (15–17). Single-channel recordings indicate that ωCgTx blocks both N and L channels (18, 19). Should it transpire that calcium channel proteins are not members of a relatively homologous family, our findings, along with those reported before (11–13), might be used in the context of an alternative strategy (20) to clone the genes for selected calcium channels.

METHODS

The techniques for isolating poly(A)$^+$ RNA and for injecting and culturing oocytes have been described previously (21, 22). In this study, however, the pooled electric lobe tissue (1.3 g, wet weight) of seven *Torpedo californica* (body length 22–30 cm) was the source of the mRNA. (The electric lobe contains the cell bodies of the neurons that innervate the electric organs of these fish.) Uninjected oocytes are referred to as control oocytes, while oocytes injected with the *Torpedo* mRNA are referred to as injected oocytes. Preliminary experiments indicated that it was necessary to culture the injected oocytes for 6–7 days before a satisfactory level of calcium channel activity appeared in these cells.

A two-electrode voltage clamp (23) was used to measure the transmembrane currents of oocytes that had been treated with collagenase (Sigma type I or IA at 1 mg·ml^{-1} for 1 hr at 20°C) to facilitate manual removal of the follicle cells. To maximize the current through oocyte Ca^{2+} channels, and to minimize the likelihood of activating the endogenous chloride current (11, 23), oocytes were bathed in the solution described previously (11). This recording solution (Ba-Ringer) contained 40 mM $Ba(OH)_2$, 50 mM NaOH, 2 mM KOH, and 5 mM Hepes, and methanesulfonic acid was used to adjust the pH to 7.4. Normally, 1 mM tetraethylammonium bromide was added to block the potassium currents that appear in the injected oocytes (21). When either Sr^{2+} (40 mM) or Ca^{2+} (40 mM) was substituted for Ba^{2+} in this Ba-Ringer, an endogenous chloride current (23), characterized by a large inward tail current lasting about 250 msec, was seen. No such tail currents were detected in the Ba-Ringer. All experiments were conducted at 20–22°C.

The study of barium currents of mRNA-injected oocytes was complicated by the presence in oocytes of endogenous voltage-gated calcium channels (11, 23). In general, the barium current of control oocytes was small during the summer months. The peak barium current recorded from 39 oocytes of two frogs was (mean ± SD) 5 ± 1.4 nA. However, control oocytes from some frogs (particularly during autumn and winter) did show a higher endogenous barium current (up to 30 nA). A similar variation among oocytes of this native barium current can be seen from other reports (11, 12). Since we were unable by pharmacological means to eliminate

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: ωCgTx, ω-conotoxin.

selectively the intrinsic barium current of these cells, when possible, we selected for mRNA injection batches of oocytes that had low endogenous barium currents. This expedient helped to reduce to a minimum the contribution of the native barium current to the total inward current of injected oocytes.

The ωCgTx (fraction GVIA) was purchased from Peninsula Laboratories (Belmont, CA). We thank A. Scriabine of Miles Laboratories (New Haven, CT) for the dihydropyridines used in this work.

RESULTS

In Ba-Ringer, uninjected control oocytes exhibit a cadmium-sensitive voltage-dependent inward current with peak amplitudes that range from 1 to 30 nA (Fig. 1). As reported earlier (11), this current is carried by barium ions (omission of barium eliminates the current, while replacement of sodium by choline or the use of tetrodotoxin (1 μM) does not affect the current), and it will be referred to as a barium current. Within 6–9 days of injecting oocytes with mRNA from *Torpedo* electric lobe, injected oocytes (with resting potentials more negative than −45 mV) manifest barium currents with amplitudes of 40–90 nA (Fig. 1). This current is eliminated when sodium replaces barium or when cadmium (2 mM) is present. Removal of sodium or addition of tetrodotoxin (1 μM) does not affect this barium current. The total barium current of the injected cells is less prone to inactivation than the barium current of control oocytes; moreover, there is a shift in the voltage at which maximum current is elicited in injected versus control oocytes (Figs. 1*D* and 2*B*). These results were our first indications of a dissimilarity of the barium current of injected and control oocytes.

ωCgTx. Subsequent experiments revealed that the peptide neurotoxin ωCgTx inhibited a portion of the barium current of injected oocytes but not of controls. When control oocytes were exposed to ωCgTx (1–20 μM for 0.5–2 hr), we detected no difference in the macroscopic properties of the barium current of these cells (data not shown). Thus, the endogenous calcium channel of *Xenopus* oocytes is resistant to ωCgTx at concentrations that block calcium channels in other cells (15–19). In contrast, a component of the barium current of injected oocytes is inhibited by ωCgTx (Fig. 2). When 1–10 μM ωCgTx is used, the barium current of injected oocytes declines by as much as 95% of the original amplitude (e.g., Fig. 2). This reduction of barium current is half maximal

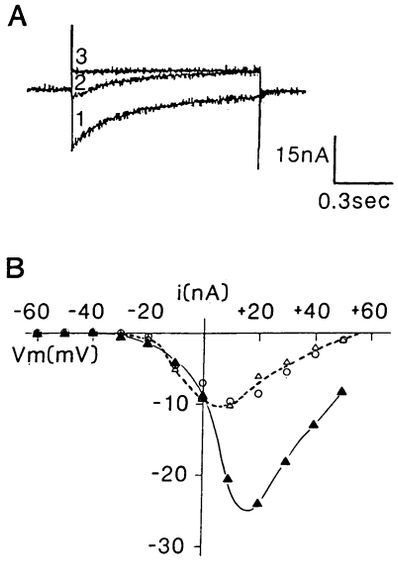

FIG. 2. Action of ωCgTx on barium currents. (*A*) Barium current of an oocyte (8 days after mRNA injection) evoked by a +70-mV step from V_{Hold} = −60 mV: trace 1, in Ba-Ringer; trace 2, after 30 min in Ba-Ringer + ωCgTx (10 μM); trace 3, in Ba-Ringer plus 2 mM $CdCl_2$. (*B*) Current–voltage relations for residual-current-corrected (i.e., Cd subtracted, see Fig. 1 legend) barium currents. △, ωCgTx-insensitive i_{Ba} (currents that remain after 30 min in Ba-Ringer plus 10 μM ωCgTx) of an injected oocyte; ▲, ωCgTx-sensitive i_{Ba} from the same oocyte. Values for ωCgTx-sensitive currents were obtained by subtracting the ωCgTx-insensitive current (△) from the total i_{Ba} measured in Ba-Ringer. ○, i_{Ba} measured in a control oocyte bathed in Ba-Ringer (note: exposure of this oocyte to ωCgTx for 60 min had no effect on the amplitude or shape of this current).

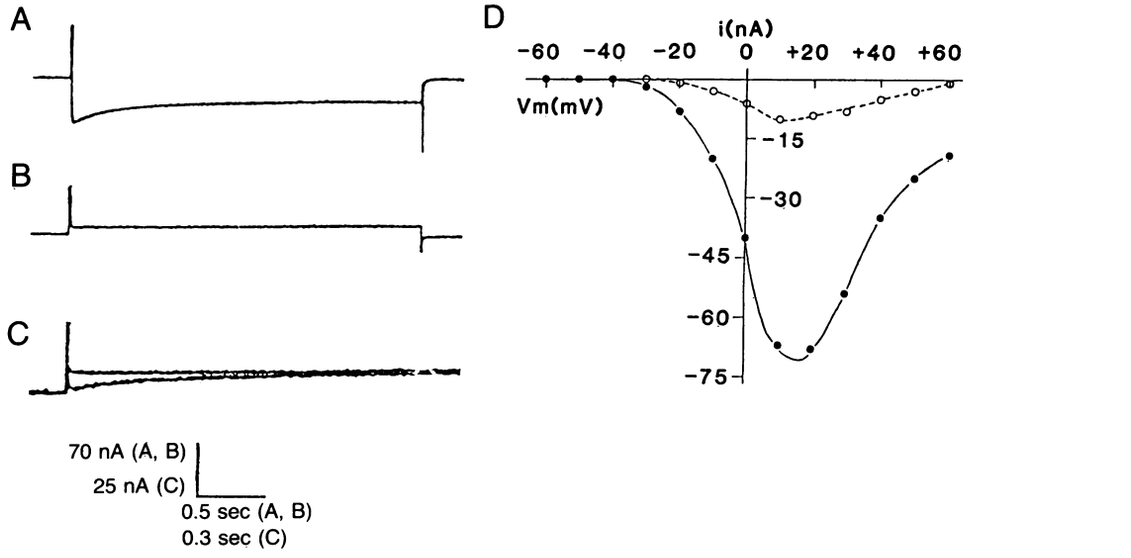

FIG. 1. Barium currents of injected and control oocytes. (*A*) Current recorded with a +70-mV step from V_{Hold} = −60 mV for an oocyte in Ba-Ringer 9 days after mRNA injection. (*B*) Same oocyte from *A* in Ba-Ringer + 2 mM $Cd(OH)_2$. (*C*) Superimposed current records during the same test protocol (V_{Hold} = −60 mV; test pulse to +10 mV) in a control oocyte bathed in Ba-Ringer (lower trace) and Ba-Ringer + 2 mM $Cd(OH)_2$ (upper trace). (*D*) Current–voltage relations for peak i_{Ba} of an injected oocyte (●) and a control oocyte (○). V_m = membrane potential. i_{Ba} was measured as the peak inward current (500-msec voltage step from V_{Hold} = −60 mV in Ba-Ringer) less the residual current (elicited with the same voltage step) in Ba-Ringer + 2 mM $Cd(OH)_2$. In all experiments the control and injected oocytes are taken from the same frog and cultured under identical conditions.

within 10 min at 22°C, and no recovery is seen even after repeated washing (for 1 hr) with toxin-free solution. Concentrations of ωCgTx above 10 μM (e.g., 20–30 μM) produce no further diminution of the barium current of injected oocytes. Data presented below suggest that this residual, ωCgTx-insensitive, barium current reflects barium permeation of the endogenous (ωCgTx-insensitive) calcium channels of the oocyte.

The current–voltage relation of an injected oocyte in Ba-Ringer before and 1 hr after ωCgTx (10 μM) is presented in Fig. 2*B*. After ωCgTx, a shift is seen in the voltage at which maximal current develops. The plot of toxin-insensitive barium current bears considerable resemblance to the *I–V* relation for barium current of uninjected control oocytes (Fig. 2*B*). Moreover, the concentration of cadmium ions needed to eliminate this ωCgTx-insensitive current matches the level necessary to inhibit the endogenous barium current of oocytes (Fig. 3). From these results we surmise that the total barium current of injected oocytes is a composite of the ωCgTx-sensitive current that develops after mRNA injection and the intrinsic toxin-resistant current of these cells.

Cadmium Ions. The IC_{50} for cadmium inhibition of the barium current of control oocytes is 11 ± 2 μM (n = 13; see Fig. 3). In contrast, the IC_{50} for cadmium inhibition of that component of the total barium current of injected oocytes that is blocked by ωCgTx is 0.1 ± 0.05 μM (n = 6). A representative illustration of this effect is given in Fig. 3. Thus, at 1 μM, one can see (Fig. 3) that cadmium ions virtually abolish the ωCgTx-sensitive barium current of oocytes without affecting the residual (i.e., endogenous) barium current of these cells.

Steady-State Inactivation. Measurements of steady-state inactivation can also be used to distinguish between the barium currents of control and injected oocytes. For control oocytes, 50% inactivation is observed with a prepulse to −49 mV (Fig. 4). A comparable decline of the total barium current of injected oocytes is not seen until −29 mV. When injected oocytes are first incubated with ωCgTx (10 μM), the inactivation of the residual current follows closely that of control oocytes (Fig. 4). If one then determines the inactivation of the ωCgTx-sensitive component of the total barium current, 50% inactivation is seen at −18 mV. A similar value is obtained when 1 μM cadmium is used instead of ωCgTx (data not shown). Neither cadmium ions nor ωCgTx had any effect on steady-state inactivation in control oocytes.

Additional Inactivation Parameters. Separate two-pulse experiments accentuate the apparent differences in inactivation (see Fig. 1) of the barium current of control and injected oocytes. Systematic variation of the amplitude or duration of a prepulse shows consistent differences in the extent to which barium currents are attenuated, particularly between the ωCgTx-sensitive current component of injected oocytes and the control currents (Fig. 5). These results indicate that the toxin-sensitive barium current of injected oocytes is less prone to inactivation by a conditioning prepulse and inactivates more slowly with a fixed prepulse duration than the endogenous current of the oocyte.

Dihydropyridines. We searched for other compounds that might selectively block (or activate) the barium current of injected or control oocytes. Among the drugs tested were several dihydropyridines. Neither nisoldipine nor nitrendipine (each at 1 μM) had any discernible effect on the amplitude of the barium current of control or injected oocytes. As a positive control, we tested whether nisoldipine would inhibit the barium current of oocytes injected with mRNA from neonatal rat heart. At 1 μM, nisoldipine reduced the barium current of these oocytes by 55%. This confirms an earlier report (11).

Actinomycin D. Finally, we sought evidence that the larger barium currents of mRNA-injected oocytes resulted directly from the translation of injected mRNA to yield calcium channel proteins. When oocytes were cultured continuously with actinomycin D at a concentration (50 $\mu g \cdot ml^{-1}$) that blocks 98–99% of RNA synthesis (24), there was no reduction in the amplitude of the ωCgTx-sensitive barium currents of these cells relative to injected oocytes cultured without this drug. This result implies that the endogenous synthesis of RNA by the oocyte plays little role in the expression of the barium current of injected oocytes.

DISCUSSION

The data presented here are consistent with the interpretation that *Xenopus* oocytes express an additional Ca channel after injection with mRNA from the electric lobe of *Torpedo*. Central to this interpretation is the assumption that we can discriminate between the endogenous calcium channels of the oocyte and those channels that appear after mRNA injection of these cells. Several observations support such a distinction.

The intrinsic barium current of oocytes was unaffected by ωCgTx at concentrations (1–20 μM) that reduce the amplitude of the barium current of injected oocytes to the level seen in control cells (Fig. 2). Therefore, in subsequent experiments we distinguished between the endogenous, ωCgTx-insensitive, barium current of oocytes and the toxin-sensitive component of the total barium current that could be seen after injection of oocytes with *Torpedo* electric lobe mRNA. Thus, the IC_{50} for cadmium-ion inhibition of the ωCgTx-sensitive barium current was approximately two orders of magnitude lower than the IC_{50} for the blockade of the native barium current (Fig. 3). These pharmacological

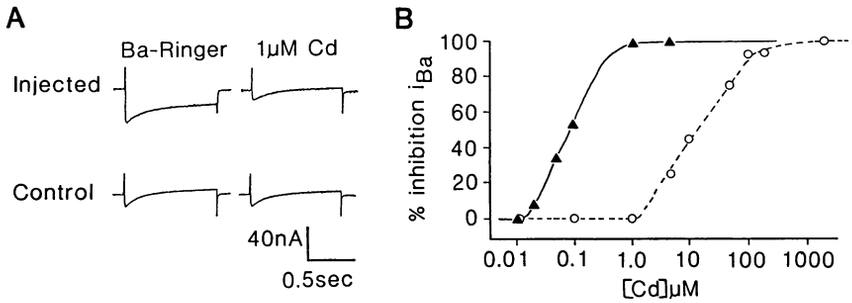

FIG. 3. Cadmium inhibition of barium currents. (*A*) Currents [with and without $Cd(OH)_2$] obtained with a +70-mV step from V_H = −60 mV for a control oocyte and an injected oocyte (9 days after injection). For both cells the current that remains after 1 μM Cd is unaffected by ωCgTx (not shown). (*B*) Representative dose–response curve for cadmium inhibition of peak inward barium currents (protocol as in *A*) of single control (○) and injected (▲) oocytes. For injected oocytes the data presented are for the cadmium sensitivity of that component of total barium current that is blocked by ωCgTx (10 μM). Prior exposure of control oocytes to ωCgTx did not affect this dose–response curve. Lines are the best fit by eye. Results were unchanged when $CdCl_2$ was used in place of $Cd(OH)_2$.

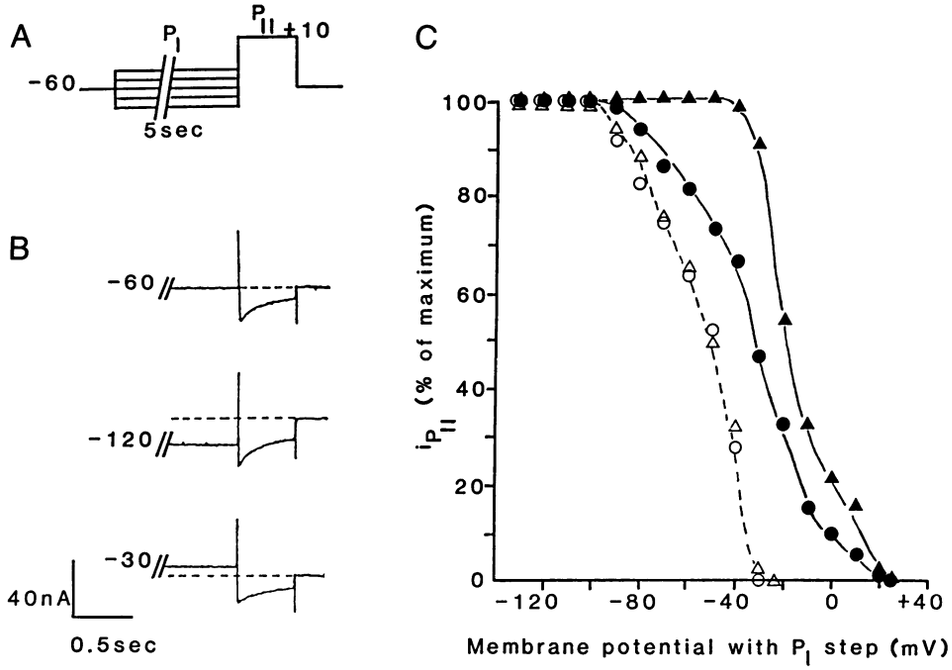

FIG. 4. Steady-state inactivation of barium currents in injected and control oocytes. (*A*) Experimental protocol: 5-sec conditioning pulses (P_I) of various amplitudes were given from a holding potential of −60 mV and immediately followed by a 500-msec voltage step (P_{II}) to +10 mV. (*B*) Currents recorded from an injected oocyte (8 days after injection) evoked by the test pulse P_{II} after P_I. The membrane potential (mV) during P_{II} is given to the left of each trace. (*C*) Plot of steady-state inactivation. Peak inward current during the test pulse is normalized to the maximal current observed and given as a function of membrane potential during the conditioning step. ●, Injected oocyte in Ba-Ringer; △, the same injected oocyte after 30-min exposure to 10 μM ωCgTx; ▲, ωCgTx-sensitive component of same injected oocyte obtained by subtracting currents in △ from ●; ○, control oocyte in Ba-Ringer (the steady-state inactivation of the control oocyte was unaltered after 1 hr in ωCgTx). Lines are best fit by eye.

results indicate that mRNA-injected oocytes express a form of calcium channel not found in uninjected controls. Additional support for this conclusion comes from the disparate inactivation characteristics of the ωCgTx-sensitive and ωCgTx-insensitive barium currents. As seen in Figs. 1 and 5, the barium current of control oocytes is considerably more prone to inactivation than either the total or ωCgTx-sensitive component of the barium current of injected oocytes.

The ωCgTx-sensitive calcium channel of injected oocytes has properties that are qualitatively and quantitatively similar to those (17) reported for the calcium channel that controls transmitter release from electric organ synaptosomes [Yeager and co-workers (17) studied synaptosomal calcium channels by monitoring the evoked release of ATP from synaptosomes]. Both channels are resistant to blockade by dihydropyridine calcium channel antagonists. ωCgTx blocks ATP output and oocyte barium current in a similar dose range. The cadmium sensitivity of the calcium channel of electric organ synaptosomes was not well quantitated (17); however, Reynolds and co-workers (16) reported an IC_{50} of 1 μM for cadmium inhibition of calcium flux in rat brain synaptosomes. The physiological significance of the submicromolar sensitivity to cadmium of the calcium channel of injected oocytes remains to be clarified. Finally, in our recording solution (which reduces the possibility of calcium-mediated inactivation), the barium current of injected oocytes exhibits relatively little inactivation compared with control oocytes. Rapid inactivation is absent at other nerve-ending calcium channels (25–27). These similarities support the hypothesis that the mRNA from *Torpedo* electric lobe induces the expression of a "nerve-ending-like" calcium channel in oocytes. This postulate can be tested by obtaining single-channel records of both types of calcium channel.

The ωCgTx-sensitive barium currents we detected are different from those previously identified in mRNA-injected oocytes (11–13). mRNA from rat heart or brain has been shown to induce the expression in oocytes of dihydropyridine-sensitive and dihydropyridine-insensitive currents (11–13). ωCgTx appears not to affect either of these currents (12). Moreover, the IC_{50} for cadmium blockade of the barium current of oocytes injected with rat brain mRNA is 6 μM (12). This level of cadmium is considerably higher than that needed to produce a similar reduction of the barium current of oocytes injected with *Torpedo* electric lobe mRNA (see Fig. 3). These data indicate that *Xenopus* oocytes can express a variety of pharmacologically distinctive calcium channels.

The most parsimonious explanation for the appearance of novel calcium channels in our mRNA-primed oocytes is that the channels were assembled as a consequence of the translation of the appropriate *Torpedo* mRNA(s). However, we cannot exclude more circuitous possibilities (e.g., modification of endogenous channels or activation of cryptic channels). Because novel calcium channels appear in injected oocytes treated with actinomycin D, it is unlikely that mRNA injection acts via transcription of oocyte DNA. Thus, the *Xenopus* genome appears to play no direct role in the expression of these calcium channels. Moreover, it has only been with *Torpedo* electric lobe mRNA that we have seen barium currents that are sensitive to inhibition by micromolar concentrations of ωCgTx and cadmium. Poly(A)$^+$ RNA from rat or human brain, rat heart, *Torpedo* electric organ, and two clonal cell lines (PC-12 from rat and DDT_1 from hamster) has not yielded similar currents (unpublished observations). The high density of relatively uniform synapses (in the electric organ) formed by the electromotor neurons may have contributed to this success.

Assuming a single-channel current of 1 pA for calcium channels in solutions containing high barium (2), we estimate that our "best" oocytes incorporated 8×10^4 calcium channels into their plasma membrane. Ignoring the abundant

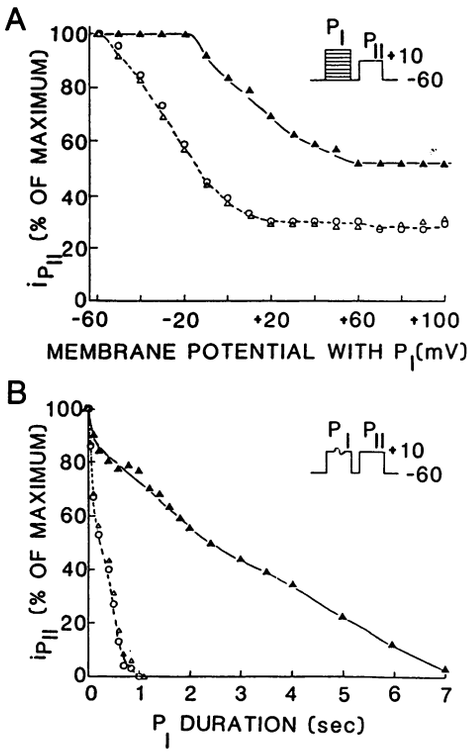

FIG. 5. Two-pulse studies of the barium current of control and injected oocytes. (*A*) Dependence of peak inward current on prepulse voltage. (*B*) Dependence on prepulse duration. (*Insets*) Experimental protocols. Test-pulse duration, 500 msec; interval, 200 msec. ▲, ωCgTx-sensitive component of barium current of injected oocyte. △, ωCgTx-insensitive component of barium current of injected oocyte. ○, Control oocyte.

microvilli at the surface of the oocyte, this corresponds to a channel density of less than 1 per 30 μm^2 of membrane. This channel density is at least an order of magnitude lower than that observed in other cells that have been studied by patch clamping (2). Obtaining mRNA preparations that are enriched (using physical fractionation or gene cloning) in transcripts coding for calcium channels should allow us to identify with greater certainty the nature of the calcium channel we have "transplanted" to the oocyte.

This paper is dedicated to the memory of Dr. Roger Eckert. We thank Drs. D. Armstrong and G. Augustine for helpful comments, and G. Miljanich for providing access to work in press. This work was supported by a contract from the Army Research Office (DAAG 29-85-K-0113). Funds for this work were also supplied by a grant-in-aid from the American Heart Association. J.A.U. was supported by the Laubisch Fund of the University of California at Los Angeles and by National Institutes of Health Grant NS 23851. C.B.G. is a Sloan Foundation Fellow in Neuroscience and is supported by National Institutes of Health Grant NS 00827.

1. Hagiwara, S. & Byerly, L. (1981) *Annu. Rev. Neurosci.* **4,** 69–125.
2. Tsien, R. W. (1983) *Annu. Rev. Physiol.* **45,** 341–358.
3. Reuter, H. (1983) *Nature (London)* **301,** 569–574.
4. Carbone, E. & Lux, H. D. (1984) *Nature (London)* **310,** 501–502.
5. Llinas, R. & Yarom, Y. (1981) *J. Physiol. (London)* **315,** 549–567.
6. Hume, J. R. & Giles, W. (1983) *J. Gen. Physiol.* **81,** 153–194.
7. Nilius, B., Hess, P., Lansman, J. B. & Tsien, R. W. (1985) *Nature (London)* **316,** 443–446.
8. Nowycky, M. C., Fox, A. P. & Tsien, R. W. (1985) *Nature (London)* **316,** 440–443.
9. Hagiwara, S., Ozawa, S. & Sand, O. (1975) *J. Gen. Physiol.* **65,** 617–644.
10. Chesnoy-Marchais, D. (1985) *J. Physiol. (London)* **367,** 457–488.
11. Dascal, N., Snutch, T. P., Lübbert, H., Davidson, N. & Lester, H. A. (1986) *Science* **231,** 1147–1150.
12. Leonard, J. P., Nargeot, J., Snutch, T. P., Davidson, N. & Lester, H. A. (1987) *J. Neurosci.* **7,** 875–881.
13. Miledi, R., Parker, I. & Sumikawa, K. (1986) *J. Physiol. (London)* **374,** 38P.
14. Olivera, B. M., McIntosh, J. M., Cruz, L. J., Luque, F. A. & Gray, W. R. (1984) *Biochemistry* **23,** 5087–5090.
15. Kerr, L. M. & Yoshikami, D. (1984) *Nature (London)* **308,** 282–284.
16. Reynolds, I. J., Wagner, J. A., Snyder, S. H., Thayer, S. A., Olivera, B. M. & Miller, R. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8804–8807.
17. Yeager, R. E., Yoshikami, O., Rivier, J., Cruz, L. J. & Miljanich, G. P. (1987) *J. Neurosci.*, in press.
18. Feldman, D. & Yoshikami, D. (1985) *Soc. Neurosci. Abstr.* **11,** 517.
19. McCleskey, E. W., Fox, A. P., Feldman, D. & Tsien, R. W. (1986) *J. Exp. Biol.* **124,** 177–190.
20. Noma, Y., Sideras, P., Naito, T., Bergstedt-Lindquist, S., Azuma, C., Severinson, E., Tanabe, T., Kinashi, T., Matsuda, F., Yaoita, Y. & Honjo, T. (1986) *Nature (London)* **319,** 640–646.
21. Gundersen, C. B., Miledi, R. & Parker, I. (1984) *J. Physiol. (London)* **353,** 231–248.
22. Gundersen, C. B., Jenden, D. J. & Miledi, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 608–611.
23. Miledi, R. (1982) *Proc. R. Soc. London Ser. B* **215,** 491–497.
24. Adamson, E. D. & Woodland, H. R. (1977) *Dev. Biol.* **57,** 136–149.
25. Llinas, R., Steinberg, I. Z. & Walton, K. (1981) *Biophys. J.* **33,** 289–322.
26. Nachshen, D. A. (1985) *J. Physiol. (London)* **361,** 251–268.
27. Suszkiw, J. B., O'Leary, M. E., Murawsky, M. M. & Wang, T. (1986) *J. Neurosci.* **6,** 1349–1357.