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Ca Channels Induced in *Xenopus* Oocytes by Rat Brain mRNA

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RNA was isolated from brains of 16-d-old rats and poly(A) samples were injected into stage V and VI oocytes. After allowing 2–5 d for expression, most oocytes were exposed to medium in which the K had been replaced by Cs for 24 hr prior to recording. Ba currents were usually measured in Cl-free Ba-methanesulfonate saline.

I_{Ba} in noninjected oocytes was often undetectable, but ranged up to 50 nA (22 ± 4 nA, $n = 21$). In contrast, injected oocytes showed a peak I_{Ba} of 339 ± 42 nA ($n = 33$). The threshold for activation of I_{Ba} was −40 mV, with peak currents at +10 to +20 mV. After a peak, currents decayed to a nearly steady level along a single-exponential time course ($\tau = 650 \pm 50$ msec at +20 mV). The maintained current was 67 ± 6% ($n = 9$) of the early peak amplitude. A prepulse duration of 5 sec was needed to examine the inactivation of barium currents in injected oocytes. The inward I_{Ba} could be observed in $BaCl_2$ solutions at potentials positive to E_{Cl} and also in Na-free salines, indicating that neither Cl^- nor Na^+ was carrying the inward current.

Although I_{Ba} displayed voltage-independent blockade by Cd (50% inhibition at 6 μM), the peptide Ca channel antagonist, ω-CgTX (1 μM), and the organic Ca channel-blocking agents (verapamil, compound W-7, and nifedipine) were uniformly ineffective. No effects were observed with the dihydropyridine antagonist nifedipine (even at 10 μM, or when cells were held at −40 mV) or agonist Bay K-8644. However, I_{Ba} was enhanced via activation of protein kinase C with 4-β-phorbol dibutyrate (PBT_2). In contrast, use of forskolin to activate protein kinase A did not alter I_{Ba}. However, experiments in the presence of Cd revealed that forskolin decreased I_K. Ca channels produced by rat brain mRNA were thus in contrast to the nifedipine-sensitive, Bay K-8644- and forskolin-enhanced Ca channels observed after injection of rat heart mRNA (Dascal et al., 1986).

Voltage-dependent calcium channels occur in most neurons and in several other cell types. Clearly, these channels form a diverse group (Carbone and Lux, 1984a, b; Armstrong and Matteson, 1985; Nowycky et al., 1985). Questions that have yet to be answered concern the detailed variation in Ca channel characteristics between cell types, the evolutionary relations among them, and the relationship between their structure and function. As one approach to answering these questions, we have begun to apply the technique of expressing genes for Ca channels in foreign cells. Barnard, Miledi, and their colleagues have demonstrated the expression of various ion channels by mRNA injection in *Xenopus* oocytes (Gundersen et al., 1983; Houamed et al., 1984); we have shown that voltage-dependent Ca channels can be studied in this way as well (Dascal et al., 1986).

With few exceptions, exogenous mRNA is translated in *Xenopus* oocytes so as to produce proteins that retain the properties of the donor tissue. Posttranslational modifications, assembly, secretion, sorting, and other processes proceed appropriately (for a review, see Soreq, 1985). For membrane channels, these similarities extend to pharmacology, kinetics, single-channel conductance, and several other characteristics (Dascal, 1987). One may therefore hope to proceed with a characterization of Ca channel classes based on electrophysiological measurements after injection of RNA from various tissues into *Xenopus* oocytes. The oocyte system may ultimately allow access to presynaptic Ca channels that are difficult to study in the intact tissue.

The present experiments examine the Ca channels induced by the injection of mRNA isolated from total rat brain into *Xenopus* oocytes. We find that robust Ca channel currents (actually carried by Ba for the usual technical reasons) are induced by rat brain mRNA. Their characteristics differ from those, previously described, induced by injection of mRNA from rat heart. We find major differences that reinforce present concepts about the diversity of Ca channels.

Materials and Methods

RNA was isolated from fresh brains of 16-d-old rats by a lithium chloride–urea procedure (Dierks et al., 1981) or by a modification of the guanidine hydrochloride method of Chirgwin et al. (1979). The poly(A) mRNA was isolated from total RNA by chromatography on oligo (dT) cellulose type III (Collaborative Research) by the standard binding and elution protocol of Maniatis et al. (1982). A greater yield of high-molecular-weight RNA was obtained by pretreating the column with a poly(A)$^-$ fraction from a previous run at roughly 1 mg/ml in binding buffer, followed by an elution buffer wash. The columns were stored in 0.02% sodium azide and never rewashed with NaOH.

Adult female *X. laevis* were anesthetized in 0.17% MS-222 (tricaine methanesulfonate) prior to surgery. Following surgical removal from the frog, oocytes were cleaned of overlying follicle cells by agitation for 3 hr in 2 mg/ml collagenase (Sigma; Type 1A) in Ca-free saline of the following composition (in millimolar concentrations): NaCl, 82.5; KCl, 2.0; $MgCl_2$, 1.0; HEPES, 5.0 (titrated to pH 7.4). After washing, stage V and VI oocytes were injected, using a method similar to that of Contreras et al. (1981), with 70 ng samples of poly(A) RNA from rat brains. Noninjected oocytes served as controls. Oocytes were incubated for 3–6 d at room temperature (20°C) in saline of the following composition: NaCl, 96.0 mM; KCl, 2.0 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1.0 mM; HEPES, 5.0 mM; pyruvate, 2.5 mM; theophylline, 0.5 mM; penicillin, 100 U/ml; streptomycin, 100 μg/ml (titrated to pH 7.4). In order to

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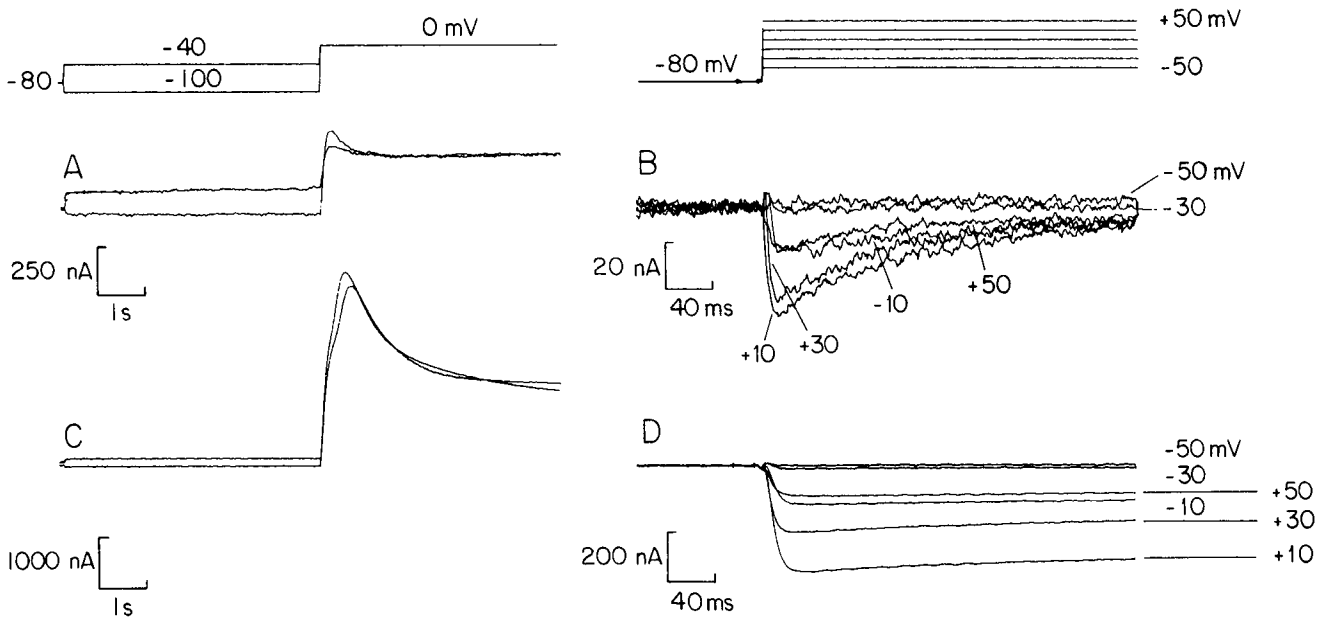

Figure 1. A comparison ICl_{Ca} (*left*) and I_{Ba} (*right*) from a noninjected (*A, B*) and an mRNA-injected (*C, D*) oocyte. ICl_{Ca} elicited by depolarization to 0 mV from a holding potential of −100 or −40 mV in normal saline was larger for mRNA-injected oocytes (*C*), and was less inactivated by holding at −40 mV, than ICl_{Ca} from noninjected oocytes (*A*). I_{Ba} in BaMS saline was isolated by subtraction of Cd-insensitive currents (see Fig. 2). I_{Ba} was considerably larger in mRNA-injected oocytes (*D*) than in noninjected cells (*B*). Note the relatively rapid decay of I_{Ba} in noninjected oocytes. Also note differences in calibration bars for each panel.

reduce K conductance to facilitate measurement of I_{Ba}, some oocytes were exposed to medium in which the K had been replaced by Cs for at least 24 hr before voltage-clamping.

Oocytes were voltage-clamped using a standard 2-intracellular microelectrode circuit. Microelectrodes were filled with 3 M KCl and had resistances from 0.5 to 2.0 MΩ. Resting potential and Ca-dependent Cl current were measured in saline of the same ionic composition as the standard incubation medium, but in the absence of pyruvate, theophylline, and antibiotics. Ba currents were measured in $BaCl_2$ or, more usually, Cl-free Ba-methanesulfonate (BaMS) saline of the following composition (millimolar): $Ba(OH)_2$, 40; NaOH, 50; KOH, 2; HEPES, 5 (titrated to pH 7.4 with methanesulfonic acid). Na-free BaMS saline used in some experiments contained *N*-methyl-D-glucamine as an Na replacement. All Ba currents were measured in the presence of 1 μM TTX. All drugs used were obtained from Sigma, with the exception of omega conotoxin GVIA (ω-CgTX; gift of Dr. B. M. Olivera), oleoylacetylglycerol (OAG; Calbiochem) and Bay K-8644 (Bayer).

Results

Noninjected control oocytes in normal saline showed a slow, transient, outward current when depolarized to 0 mV from a conditioning voltage of −40 or −100 mV (Fig. 1*A*). This current was due to activation of a Ca-dependent Cl conductance (gCl_{Ca}) by the influx of Ca through voltage-dependent Ca channels in the plasma membrane (Miledi, 1982; Barish, 1983). This Cl current was relatively small in noninjected oocytes ($\bar{X}$ = 88 ± 22 nA; n = 22) and was inactivated by a depolarized holding potential; it was only 20 ± 6 nA when elicited from a holding potential of −40 mV. However, the inward Ca current underlying the gCl_{Ca} was difficult to detect directly because it was masked by the outward Cl current. As described earlier (Dascal et al., 1986), we used 40 mM BaMS saline to reveal the small inward current (Fig. 1*B*) because Ba often permeates Ca channels better than Ca does, and also fails to activate gCl_{Ca} in oocytes (Barish, 1983). The peak Ba current varied from <10 nA (7 cases) to as much as 50 nA ($\bar{X}$ = 22 ± 4 nA; n = 21). The current was transient, with an exponential decay-time constant of roughly 100 msec.

In contrast to noninjected cells, oocytes injected with poly(A) RNA from rat brains showed large ICl_{Ca} currents both from conditioning voltages of −40 mV (1285 ± 117 nA; n = 26) and from −100 mV (1596 ± 140 nA; n = 26) in normal saline (Fig. 1*C*). Ba currents recorded in Cl-free BaMS were also much larger (339 ± 42 nA; n = 33)—roughly 15 times the control amplitude (Fig. 1*D*). All injected oocytes that had Ca-dependent Cl conductances >1000 nA in normal saline revealed peak Ba currents >150 nA when the bath was changed to BaMS saline. Peak I_{Ba} elicited after a 5 sec conditioning prepulse at −40 mV was 76 ± 2% of the maximal current (n = 6). Thus, both brain RNA-directed ICl_{Ca} and I_{Ba} showed less inactivation than the corresponding endogenous currents.

To obtain a more quantitative estimate of the Ba current through Ca channels, the inward Ba current in Cl-free saline was isolated from contaminating outward K currents by subtracting currents recorded in the presence of Cd from prior records of total current. In order to further reduce I_K, most oocytes were exposed to incubation medium in which the K had been replaced by Cs for 24 hr prior to electrophysiology. This procedure reduced I_K, measured as the outward current at +50 mV in the presence of Cd, by about 70%. Injected oocytes pretreated with Cs had resting potentials that were, on average, 13 mV more negative than untreated oocytes when recorded in normal (i.e., K-containing) saline. This hyperpolarization began within a few seconds of changing the bath from K-free to normal saline and stabilized in 1 min. The hyperpolarization could be reversed in a few minutes with 1 μM strophanthidin, a Na-K-pump blocker. While Cs pretreatment did not completely eliminate I_K, it did help isolate I_{Ba}.

Figure 2 shows currents recorded before (*A*) and after (*B*) the addition of 100 μM Cd to the bath for a Cs-pretreated cell. The large inward I_{Ba} seen in Figure 2*A* is blocked, and the outward K current is revealed (Fig. 2*B*). Figure 2*C* shows the subtraction

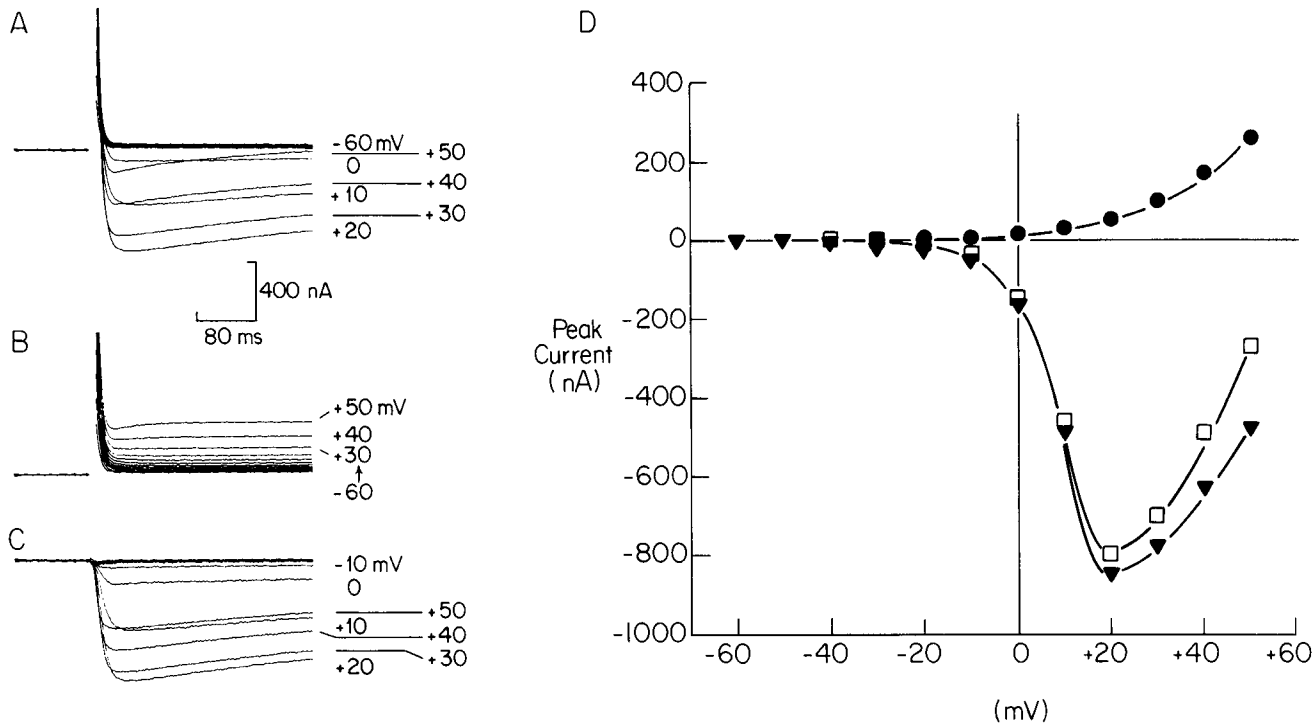

Figure 2. Subtraction of Cd-insensitive currents. *A,* Total currents elicted by depolarizing voltage steps, from a holding potential of −80 mV to voltages from −60 to +50 mV. *B,* The same voltage protocol in the presence of 100 μM Cd reveals Cd-insensitive outward currents carried by potassium. Subtraction of traces in *B* from total current in *A* yields the purely Cd-sensitive I_{Ba} shown in *C.* Apparent rapid inactivation of current at +40 and + 50 mV was due to activation of I_K. Current–voltage relationships for each family of traces (*A–C*) are plotted in *D. Open squares,* total current (leak subtracted); *filled squares,* Cd-insensitive outward current (leak subtracted); *triangles,* Cd-sensitive I_{Ba} (data of panel *C*).

of the Cd-insensitive currents of Figure 2*B* from total current traces in Figure 2*A*. This subtraction seems to reveal a nearly pure I_{Ba}, and suggests that the apparently more rapid inactivation of total currents elicited by depolarization to +40 and +50 mV is due to activation of I_K. The current–voltage relationships for each of the families of traces in Figure 2, *A–C* are shown in Figure 2*D*. The I–V curves for total current and for total current minus Cd-insensitive currents are similar, but diverge at $V_m >$ +20 mV, where I_K is activated. I_K is only 5% of the total current at +20 mV, because peak I_{Ba} (at +20 mV) was 850 nA in this cell. Unless otherwise noted, Ba current measurements refer to Cd subtracted traces. The threshold for activation of I_{Ba} was −40 mV, with peak currents elicited at +10 to +20 mV in different cells.

Figure 2 also shows that I_{Ba} undergoes relatively little inactivation during a 300 msec depolarization. Figure 3*A* shows currents produced by much longer test pulses, from −60 to +50 mV. In experiments like that of Figure 3, more than ~80% of the inactivation time course can be described by a single exponential with a time constant of 655 ± 53 msec (n = 9 cells). There is also a component that decays very slowly during a 3 sec depolarization. This current remaining after 3 sec is 67 ± 6% (n = 9) of the early peak amplitude. When a 5 sec conditioning prepulse to −20 mV was given before depolarization to +10 mV (Fig. 3*B*), peak current was inhibited more than 50%. All oocytes subjected to this pulse protocol showed less than 50% inactivation of I_{Ba} at voltages more negative than −20 mV.

Cl-free BaMS saline, rather than $BaCl_2$ saline, was used for most experiments because, in addition to the I_K normally seen in BaMS saline, I_{Ba} in $BaCl_2$ saline was often followed by a different outward current. This current may have been dependent on the Ba influx because it was absent in 100 μM Cd. Perhaps a large I_{Ba} can release Ca from intracellular stores and thereby induce ICl_{Ca} in $BaCl_2$ but not in BaMS saline. In any case, an inward current in Cl-free BaMS saline could be due to an efflux of intracellular Cl^-. This possibility can be rejected because similar large inward Ba currents can be recorded in $BaCl_2$ saline (Fig. 4), where Cl current would have been outward at potentials more positive than E_{Cl} (−25 mV).

In addition to Ba, Na could conceivably carry inward current in BaMS saline. Although all experiments were performed in the presence of 1 μM TTX, which completely blocks the voltage-dependent Na current produced by injection of rat brain mRNA (Gundersen et al., 1983a), other Na currents can be produced in oocytes under certain circumstances. For instance, prolonged depolarization induces a maintained Na current in noninjected oocytes (Baud et al., 1982). However, Na does not carry a noticeable inward current under our conditions, since use of Na-free (*N*-methyl-D-glucamine-replaced) and Cl-free Ba saline produced very typical inward currents (n = 5 cells).

The conclusion that the inward current in BaMS was I_{Ba} through Ca channels is further supported by the high sensitivity to blockade by Cd (Fig. 5). Cd at 10 μM blocked >50% of the net inward current; blockade was essentially complete at 500 μM. Use of Cd at concentrations >100 μM sometimes caused a dramatic increase in leak current. Another inorganic Ca channel inhibitor, Ni, was effective only at higher concentrations. An analysis similar to that of Figure 5 showed an apparent dissociation constant for Ni of 500 μM. The organic Ca-channel blocker verapamil (10 μM) was ineffective in blocking I_{Ba} (n = 3 cells). An

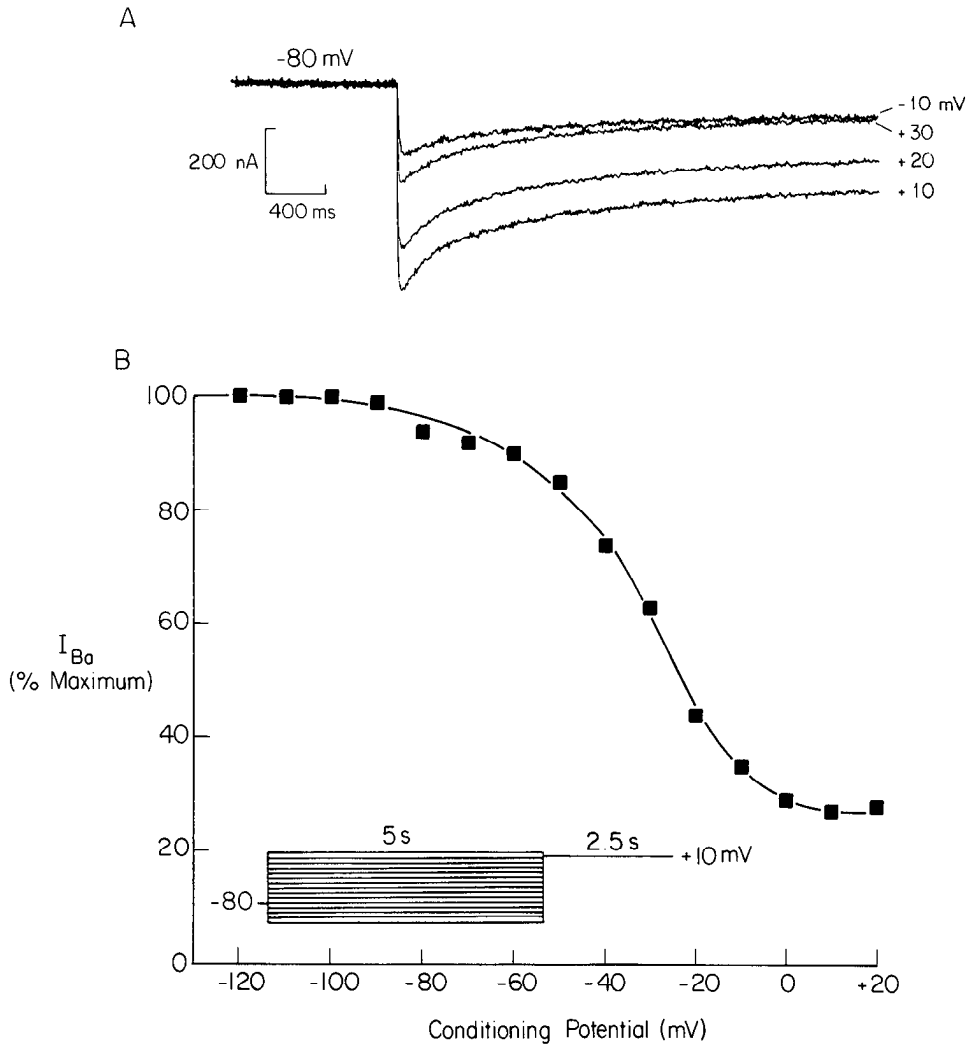

Figure 3. Inactivation of I_{Ba}. *A*, Three second depolarizing steps from −80 mV to indicated voltages revealed slow inactivation of I_{Ba} to a nearly steady plateau. *B*, Five second conditioning prepulses before a standard test pulse (to +10 mV) showed >50% inactivation of peak I_{Ba} for prepulses >−20 mV. Inactivation was incomplete even for prepulses to +20 mV. Cd-insensitive currents were subtracted out for both *A* and *B*.

agent that blocks *Paramecium* calcium channels, *N*-(6 aminohexyl)-5-chloro-1-naphthalenesulfonamide (compound W-7 at 100 μM), was also ineffective (n = 4 cells). Nifedipine at 10 μM (n = 8 cells) had no effect, even when cells were held at depolarized voltages (−40 mV) to allow Ca channels to enter a higher-affinity inactivated state (Bean, 1984; Sanguinetti and Kass, 1984). The dihydropyridine Ca agonist Bay K-8644 also failed to affect I_{Ba} (n = 3 cells), although I_{Ba} induced by mRNA from rat heart was enhanced by Bay K-8644 (n = 2 cells). The peptide calcium channel inhibitor from the marine snail *Conus geographus*, ω-CgTX, was also ineffective. At 1 μM, μ-CgTX failed to alter I_{Ba} in BaMS saline or ICl_{Ca} in normal saline even after 30 min.

Ba currents were strikingly enhanced by PBT_2 (Fig. 6), presumably via activation of protein kinase C. Five to 10 min after adding PBT_2 to the bath (Fig. 6*A*), I_{Ba} was enhanced 42 ± 5% (n = 11). The effect was independent of dose over the range of 100 nM to 1 μM. Control experiments were performed using the parent stereoisomer 4-α-phorbol. Slight decreases in peak Ba current (8 ± 3%; n = 4) were found after application of 1 μM 4-α-phorbol PBT_2. The increase in inward current due to PBT_2 followed the I–V curve for Ba current (Fig. 6*B*), suggesting that enhanced Ca-channel activity was responsible. Phorbol esters produced no major changes in the waveform or voltage sensitivity of I_{Ba}. The effect of PBT_2 was smaller in Na-free saline (10 ± 6%; n = 3). Although I_K is relatively small at voltages that elicit peak I_{Ba}, control experiments were carried out in which phorbol esters were applied after the addition of 100 μM Cd to the bath, to further rule out potential effects of phorbol esters on I_K. These control experiments revealed no effects of phorbol esters on I_K (n = 4 cells). Another C-kinase activator, OAG, also enhanced I_{Ba} in injected oocytes. The effect of 100 μM OAG was more rapid in onset (<1 min) but was smaller (26 ± 3%; n = 3).

In contrast to the effects of C-kinase activation by phorbol ester, 50 μM forskolin, used to activate protein kinase A, did not alter I_{Ba} even when cells were preincubated for 1 hr in 10 mM theophylline to inhibit phosphodiesterases. However, forskolin did have an inhibitory effect on I_K. In the presence of Cd, forskolin decreased I_K 34 ± 7%, (n = 7). Attempts to modulate I_{Ba} by acetylcholine and isoproterenol yielded no change in I_{Ba}.

Discussion

A major finding of this study is the distinction, both in kinetic and pharmacological terms, between the Ba currents induced by heart mRNA (Dascal et al., 1986) and those induced by brain RNA (the present study). Heart RNA directs the synthesis of channels producing 2 components. The sustained component resembles the dihydropyridine-sensitive, A-kinase-modulated channels that underlie the slow inward current (the "L" channels of Tsien and collaborators; see Nilius et al., 1985). The transient

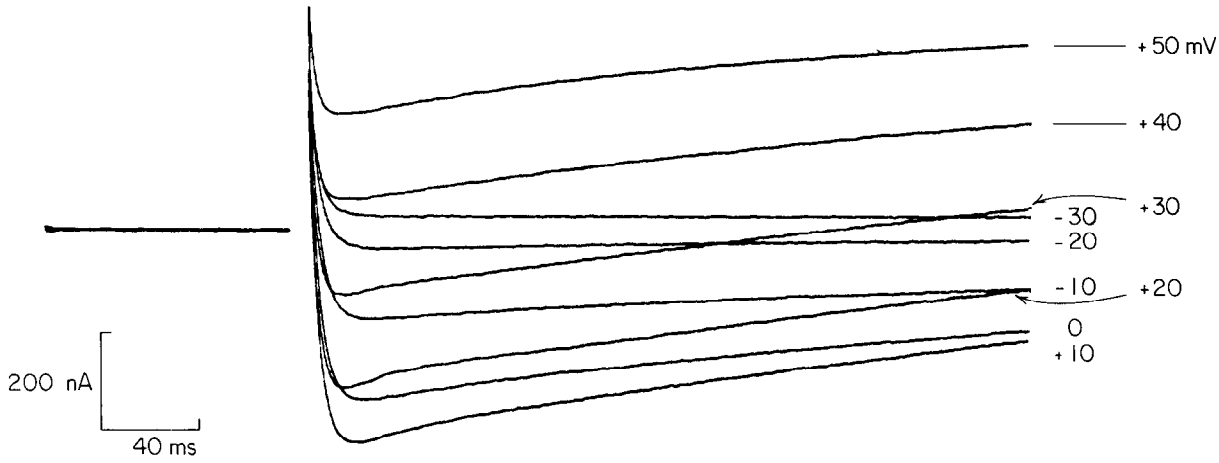

Figure 4. I_{Ba} in $BaCl_2$ saline. I_{Ba} was elicited by depolarizing voltage steps from a holding potential of −80 mV to indicated voltages. Inward currents at voltages positive to −20 mV could not be carried by Cl as $E_{Cl} = -25$ mV. Cd-insensitive currents were not subtracted in this example.

component is not well characterized, but may resemble the "T" channels (Bean, 1985; Nilius et al., 1985).

Although our preliminary studies suggested that a rapidly decaying (150–200 ms) transient component of Ba current occurs in *Xenopus* oocytes after injection of rat brain mRNA (Dascal et al., 1986), we now believe that the apparent rapid transient component resulted artifactually when the large K currents induced by brain RNA distorted the time course of the Ba current. In the present study, we obtained larger signals and better suppression of K currents; we detected only a single type of current which is, however, still partially transient (time constant of ~650 ms). We searched for additional components by examining the waveforms and current–voltage relationships as influenced by holding potential, by Cd blockade, by dihydropyridines, and by kinase activation. No additional components were detected, although single-channel data would be more decisive. For purposes of comparison, we will summarize the properties of this current (with Ba as the charge carrier): relatively slow inactivation, peak current at +15 mV, half-maximal blockade by Cd at <10 μM, insensitivity to the dihydropyridines, insensitivity to ω-CgTX, insensitivity to forskolin, and enhancement by phorbol esters.

Comparison with vertebrate CNS Ca channels. Other studies have examined dihydropyridine sensitivity in a variety of vertebrate CNS neurons. Ca action potentials and I_{Ca} from *Xenopus* spinal cord, guinea pig olfactory cortex, and rat locus coeruleus neurons are resistant to the dihydropyridine "calcium antagonists" (Bixby and Spitzer, 1984; Williams et al., 1984; Kuan et al., 1985). Indirect examination of Ca-channel activity by measurement of neurotransmitter release also suggests the presence of dihydropyridine "calcium antagonist"-resistant Ca channels in cultured nigrostriatal neurons (Shalaby et al., 1984). In one case, however (hippocampal neurons), a maintained I_{Ca} is sensitive to nimodopine (Brown et al., 1985).

In our study, it is possible that if a small amount of mRNA encoding dihydropyridine-sensitive Ca channels were present, we would not detect these channels above the background of the larger dihydropyridine-insensitive current. It will be of interest to examine currents induced by mRNA from specific brain regions and from rats of various ages. It is also possible that the channels expressed in oocytes lacked a subunit or the correct postranslational modifications necessary for dihydropyridine sensitivity. This latter possibility seems unlikely, given that heart mRNA injections induced dihydropyridine-sensitive Ca channels in oocytes.

All neurons presumably express the voltage-dependent Ca currents involved in transmitter release. Could such channels be responsible for our I_{Ba}? Presynaptic Ca channels have not been studied with voltage-clamp techniques in vertebrate tissues. Work on ^{45}Ca fluxes in synaptosomes, however, suggests that depolarization-activated Ca channels inactivate within 1 sec, and that this inactivation has a substantial voltage-dependent component (Nachshen, 1985; Turner and Goldin, 1985a, b; Suszkiw et al., 1986). One would still expect to observe voltage-dependent inactivation with Ba as the charge carrier, and the time constant is similar to our observations. There are conflicting reports on Ca-channel blockade by dihydropyridines in synaptosomes (Nachshen and Blaustein, 1979; Turner and Gol-

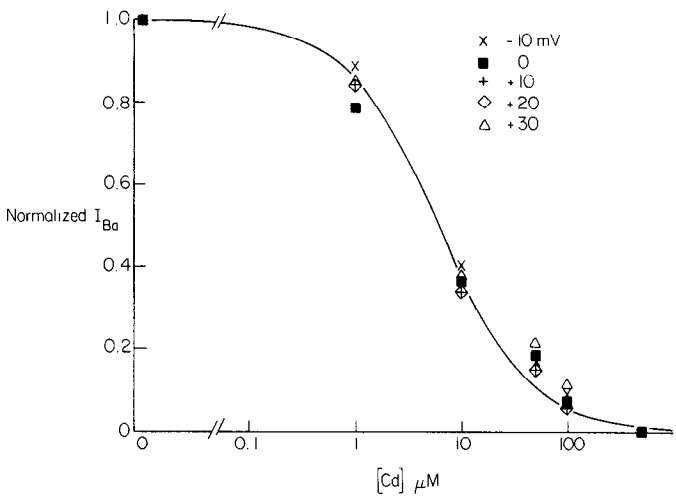

Figure 5. Cd dose–response curve. The degree of inhibition by Cd of peak I_{Ba} was similar at the various voltages examined (−10 to +30 mV). The response in the presence of 500 μM Cd was defined as complete blockade and used to subtract Cd-insensitive currents from total currents at all other concentrations of Cd. The *smooth curve* represents theoretical single-site competitive inhibition, with a dissociation constant for Cd of 6 μM.

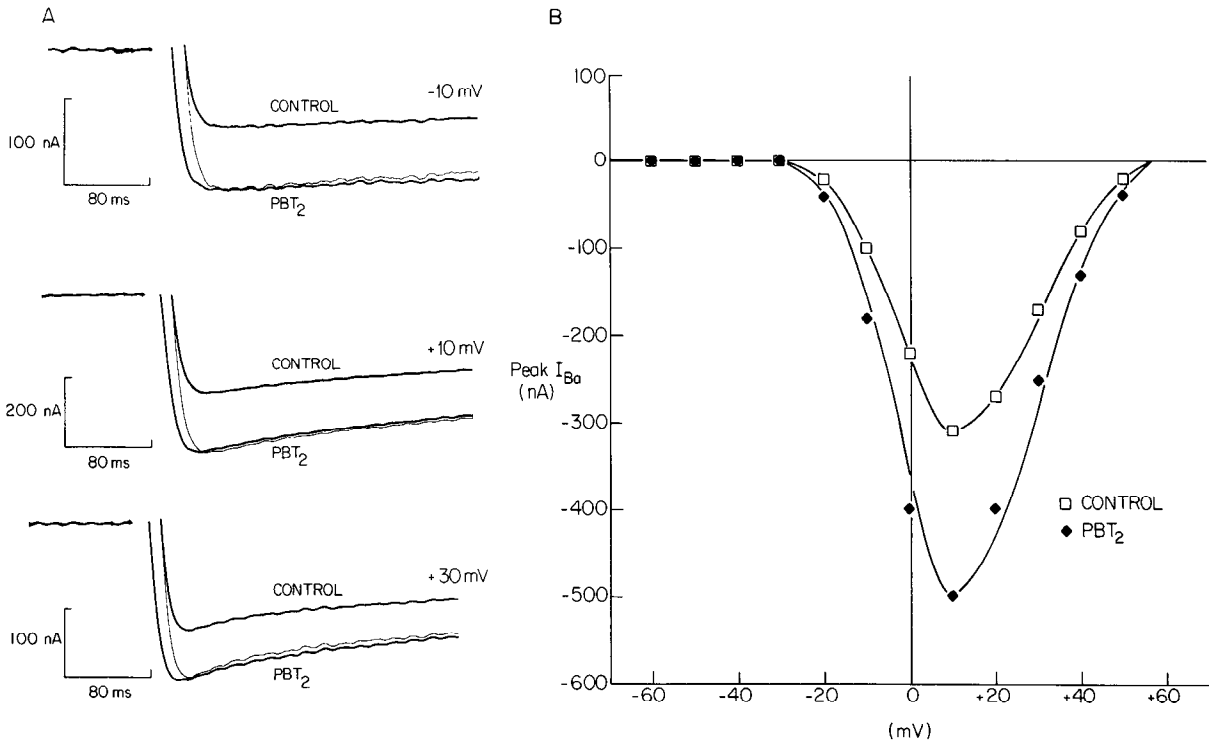

Figure 6. Enhancement of I_{Ba} by C-kinase activator PBT_2. *A,* Depolarization from a holding potential of −80 mV to indicated voltages elicited I_{Ba}, which was enhanced ~60% after 15 min in 100 nM PBT_2. *Thin trace* is control current scaled up by factors between 1.5 and 1.8 to show that the kinetics of I_{Ba} are unchanged after PBT_2. *B,* Current–voltage relationship before and after 100 nM PBT_2 for the same cell as in *A. Smooth curve* was fitted to *open squares* (control values) by eye. This curve was then scaled up 1.6× and superimposed on the post-PBT_2 values (*filled diamonds*). The close fit of the scaled control curve and the post-PBT_2 values suggests that the PBT_2 effect does not depend on voltage over the range tested. Cd-insensitive currents were subtracted.

din, 1985a, b; Suszkiw et al., 1986). Dihydropyridine-resistant ^{45}Ca uptake into a rat brain synaptosome preparation is partially inhibited by ω-CgTX (Reynolds et al., 1986). The failure of ω-CgTX to block Ca channels in our system thus remains to be explained. Overall, the available data are consistent with the hypothesis that the channels observed in the present study include the presynaptic voltage-dependent Ca channels involved in transmitter release.

Modulation. Although Ba currents directed by heart mRNA are increased by β-agonists and by forskolin (both presumably acting via cAMP-activated protein kinase), the present experiments reveal no such effect on Ba currents directed by brain mRNA. Forskolin nonetheless seems to be activating a kinase, and that kinase does seem able to influence membrane proteins: we find that forskolin diminishes the K currents directed by brain mRNA, as well as affecting the heart mRNA-encoded Ca channels, as indicated above.

Phorbol esters, on the other hand, enhanced the brain mRNA-encoded Ca channels. The action was presumably mediated via C-kinase, since the enhancement was also observed with the C-kinase activator OAG, but not with the inactive phorbol ester analog, 4-α-phorbol. Some mammalian Ca currents are *decreased* by C-kinase activation (Baraban et al., 1985; Werz and MacDonald, 1985; Rane and Dunlap, 1986), but increases have been observed with molluscan neurosecretory and nerve cells (De Riemer et al., 1985; Paupardin-Tritsch et al., 1985) and traced to the appearance of a new population of Ca-channel openings with a larger conductance (Strong et al., 1986). Is the increase we observed due to phosphorylation of the channels? It is impossible to answer this question decisively at present, but the enhancement was much smaller in Na-free Ringer's solution. One must therefore consider C-kinase activation of Na–proton and Na–Ca exchange systems (Swann and Whitaker, 1985); increased intracellular pH (Kurachi, 1982) or pCa (Tillotson, 1979) could increase currents through Ca channels. We believe that future experiments with cell-free patches will give more decisive answers to questions about the mechanism of Ca-channel modulation in oocytes injected with brain mRNA.

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