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# A β-SUBUNIT NORMALIZES THE ELECTROPHYSIOLOGICAL PROPERTIES OF A CLONED N-TYPE $Ca^{2+}$ CHANNEL $\alpha_1$-SUBUNIT

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**Summary**—The electrophysiological and pharmacological properties of a cloned rat brain N-type $Ca^{2+}$ channel were determined by transient expression in *Xenopus* oocytes. Expression of the class B $Ca^{2+}$ channel $\alpha_1$ subunit, rbB-I, resulted in a high voltage-threshold current that activated slowly and showed little inactivation over 800 msec. Characteristic of N-type currents, the rbB-I current was completely blocked by ω-conotoxin GVIA and was insensitive to nifedipine and Bay K8644. The modulatory effects on the rbB-I current by cloned rat brain $Ca^{2+}$ channel $\alpha_2$ and $\beta_{1b}$ subunits were also examined. Coexpression of rbB-I with the $\beta_{1b}$ subunit caused significant changes in the properties of the rbB-I current making it more similar to N-type currents in neurons. These included: (1) an increase in the whole-cell current, (2) an increased rate of activation, (3) a shift of the voltage-dependence of inactivation to hyperpolarized potentials and (4) a pronounced inactivation of the current over 800 msec. Coexpression with the rat brain $\alpha_2$ subunit had no significant effect on the rbB-I current alone but appeared to potentiate the rbB-I + $\beta_{1b}$ whole cell current. The results show that coexpression with the brain $\beta_{1b}$ subunit normalizes the rbB-I N-type current, and suggests the possibility that differences in subunit composition may contribute to the heterogeneous properties described for N-type channels in neurons.

*Key words*—ω-conotoxin, cDNA, *Xenopus* oocytes, N-type calcium channel.

The rapid entry of calcium ions ($Ca^{2+}$) through channel proteins in neurons of the central nervous system (CNS) stimulates many physiological events including, neurotransmitter release, enzyme modulation, and changes in electrical spiking behaviour (see reviews by Miller, 1987; Tsien, Lipscombe, Madison, Bley and Fox, 1988). Several classes of $Ca^{2+}$ channels have been distinguished based upon distinct functional and pharmacological properties (called T, L, N and P types; see reviews by Bean, 1989a; Hess, 1990). Many neurons and neuronally derived cell lines express multiple types of $Ca^{2+}$ channels (for example: Nowycky, Fox and Tsien, 1985; Fox, Nowycky and Tsien, 1987; Llinas, Sugimori, Lin and Cherksey, 1989; Plummer, Logothetis and Hess, 1989; Regan, Sah and Bean, 1991; Mintz, Venema, Swiderek, Lee, Bean and Adams, 1992), and determination of the electrophysiological and modulatory characteristics of each $Ca^{2+}$ channel subtype is important to understanding the roles that the different $Ca^{2+}$ channels play in mediating various physiological processes.

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N-type $Ca^{2+}$ channels are found in many central and peripheral neurons and have been proposed to play a role in the release of neurotransmitter at certain synapses (Reynolds, Wagner, Snyder, Thayer, Olivera and Miller, 1986; Yeager, Yoshikami, Rivier, Cruz and Miljanüch, 1987; Miller, 1987; Hirning, Fox, McCleskey, Olivera, Thayer, Miller and Tsien, 1988; Stanley and Goping, 1991), in the migration of neurons (Komuro and Rakic, 1992) and in neuromodulatory processes (Dunlap and Fischbach, 1981; Wanke, Ferroni, Malgaroli, Ambrosini, Pozzan and Meldolesi, 1987; Lipscombe, Kongsamut and Tsien, 1989; Bean, 1989b; Bley and Tsien, 1990; Beech, Bernheim and Hille, 1992). While N-type $Ca^{2+}$ channels with diverse properties have been described, they can generally be distinguished by the combination of a number of criteria including, activation at potentials more positive than −30 mV (high voltage-threshold), inactivation during a prolonged depolarization, insensitivity to dihydropyridines (DHPs), and an irreversible block by the neuropeptide toxin ω-conotoxin GVIA (ω-CgTx; Olivera, Gray, Zeikus, McIntosh, Varga, de Santos and Cruz, 1985; Nowycky *et al.*, 1985; McCleskey, Fox, Feldman, Cruz, Olivera, Tsien and Yoshikami, 1987; Plummer, Logothetis and Hess, 1989; Regan *et al.*,

1991). Biochemical studies show that N-type $Ca^{2+}$ channels are multisubunit complexes (McEnery, Snowman, Sharp, Adams and Snyder, 1991; Sakamoto and Campbell, 1991; Ahlijanian, Striessnig and Catterall, 1991; Witcher, De Waard, Sakamoto, Franzini-Armstrong, Pragnell, Kahl and Campbell, 1993) including a pore-forming $\alpha_1$ subunit that is the target for $\omega$-CgTx binding, and $\alpha_2$ and $\beta$ subunits that are homologous to those found in skeletal muscle L-type $Ca^{2+}$ channels (Campbell, Leung and Sharp, 1988; Catterall, Seagar and Takahashi, 1988).

Molecular genetic characterizations have separated neuronal $Ca^{2+}$ channels into distinct classes based on differences in their $\alpha_1$ subunits (classes A, B, C, D and E; Tsien, Ellinor and Horne, 1991; Snutch and Reiner, 1992; Soong, Stea, Hodson, Dubel, Vincent and Snutch, 1993). A combination of exogenous expression and immunoprecipitation studies demonstrate that distinct neuronal $\alpha_1$ subunits determine many of the unique electrophysiological and pharmacological properties associated with physiologically defined $Ca^{2+}$ channels (Mori, Friedrich, Kim, Mikami, Nakai, Ruth, Bosse, Hofmann, Flockerzi, Furuichi, Mikoshiba, Imoto, Tanabe and Numa, 1991; Dubel, Starr, Hell, Ahlijanian, Enyeart, Catterall and Snutch, 1992a; Williams, Feldman, McCue, Brenner, Velicelebi, Ellis and Harpold, 1992a; Williams, Brust, Feldman, Patthi, Simerson, Maroufi, McCue, Velicelebi, Ellis and Harpold, 1992b; Soong *et al.*, 1993). Recent work has also shown that the electrophysiological characteristics of L-type $Ca^{2+}$ channel $\alpha_1$ subunits can be altered by coexpression with other subunits. In the case of cardiac and skeletal muscle L-type $\alpha_1$ subunits, expression of $\alpha_2$, $\beta$, $\gamma$ and $\delta$ subunits modulates a number of parameters including the level of expression, kinetic properties, and the voltage dependence of activation and inactivation (Lacerda, Kim, Ruth, Perez-Reyes, Flockerzi, Hofmann, Birnbaumer and Brown, 1991; Varadi, Lory, Schultz, Varadi and Schwartz, 1991; Wei, Perez-Reyes, Lacerda, Schuster, Brown and Birnbaumer, 1991; Singer, Biel, Lotan, Flockerzi, Hofmann and Dascal, 1991; Castellano, Wei, Birnbaumer and Perez-Reyes, 1993). In contrast, while whole-cell currents induced by brain class A, class B, and class D $\alpha_1$ subunits have been shown to be potentiated by coexpression of $\alpha_2$ and $\beta$ subunits (Mori *et al.*, 1991; Williams *et al.*, 1992a, b), there has been no report of any other modulatory effects by ancillary subunits on the electrophysiological properties of these neuronal $Ca^{2+}$ channels.

A previous study showed that a polyclonal antiserum generated against the rat brain class B $Ca^{2+}$ channel $\alpha_1$ subunit, rbB-I, selectively immunoprecipitated high-affinity $^{125}$I-labeled $\omega$-CgTx binding sites from rat brain membranes (Dubel *et al.*, 1992a). Immunocytochemistry reveals that rbB-I $Ca^{2+}$ channels are widely distributed in the rat CNS, and are predominantly localized along the surface of dendrites and at synaptic sites (Westenbroek, Hell, Warner, Dubel, Snutch and Catterall, 1992). In this study, we show that nuclear injection into *Xenopus* oocytes of an expression vector containing the rbB-I cDNA results in the functional expression of an N-type $Ca^{2+}$ channel. We also demonstrate that the electrophysiological properties of the rbB-I N-type $Ca^{2+}$ current are normalized by coexpression with a rat brain $Ca^{2+}$ channel $\beta$ subunit. The results provide direct evidence of a role for ancillary subunits in modulating the functional properties of a N-type $Ca^{2+}$ channel.

## METHODS

### *Construction of full-length rbB-I and subcloning of $\alpha_1$, $\alpha_2$ and $\beta_{1b}$ subunits*

Construction of expression plasmids encoding full-length rat brain $\alpha_2$ and $\beta_{1b}$ $Ca^{2+}$ channel subunits was accomplished by inserting the 3.3 kb Eco RI fragment of a rat brain $\beta_{1b}$ subunit cDNA (Pragnell, Sakamoto, Jay and Campbell, 1991), and the 4.2 kb Eco RI fragment of the rat brain $\alpha_2$ subunit cDNA (Dubel *et al.*, 1992b) into the mammalian expression vector pZEM229 (kindly provided by E. Mulvihill, Zymogenetics, Seattle) linearized with Eco RI. For subcloning of rbB-I, the pZEM229 vector was digested with Bam HI and the vector pZEM229R constructed by inserting a synthetic polylinker. A cDNA including the entire protein coding region of the rbB-I $Ca^{2+}$ channel $\alpha_1$ subunit (Dubel *et al.*, 1992a) was initially constructed in the phagemid Bluescript SK − (Stratagene, La Jolla, California). Briefly, a 3824 bp Sph I fragment of the rbB-79 cDNA was inserted into the cDNA rbB-1214 linearized with Sph I. From this construct, a 7.5 kb Kpn I fragment containing 90 bp of 5′ noncoding, the entire 7008 bp rbB-I coding region and 407 bp of 3′ noncoding sequence was excised and inserted into the Kpn I site of pZEM229R. Orientations of the subcloned $Ca^{2+}$ channel subunits were confirmed by restriction enzyme analysis and DNA sequencing.

### *Isolation and nuclear injection of Xenopus oocytes*

Ovaries were surgically removed from anaesthetized (0.17% 3-aminobenzoic acid ethyl ester; MS-222) mature female *Xenopus laevis* (Xenopus One, Ann Arbor, Michigan) and agitated for 3 hr in 2 mg/ml collagenase (type IA; Sigma) dissolved in a $Ca^{2+}$-free OR-2 solution containing (mM): NaCl, 82.5; KCl, 2; $MgCl_2$, 1; HEPES, 5 at a pH of 7.5. Oocytes were allowed to recover for 3–20 hr at 18°C in standard oocyte saline (SOS) containing (mM): NaCl, 100; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 5 at a pH of 7.5; supplemented with 2.5 mM sodium pyruvate and 10 $\mu$g/ml gentamycin sulphate (Sigma). Nuclear injections were performed on stage V and VI oocytes (Dumont, 1972) using a Drummond 10 $\mu$l microdispenser. In test cells, the position of the oocyte nucleus was determined by injection of 0.04% trypan blue dye followed by dissection of the stained

nucleus. Subsequent nuclear injections of DNA were performed by estimating the position of the nucleus under the pigmented animal pole. The pZEM229 vector was demonstrated to be suitable for nuclear expression by subcloning the Shaker $K^+$ channel (Iverson, Tanouye, Lester, Davidson and Rudy, 1988) into pZEM229 and successfully expressing robust Shaker A-type $K^+$ currents in microinjected *Xenopus* oocytes (data not shown). For $Ca^{2+}$ channel expression, approximately 1–2 ng of each expression plasmid (diluted in 140 mM KCl, 10 mM EGTA, 2 mM $MgCl_2$) was injected into the nucleus ($\approx$10–15 nl total volume) and surviving oocytes were maintained in supplemented SOS at 18°C for 2–5 days prior to electrophysiological recording. Control oocytes were either injected with the expression vector without any calcium channel subunits or were uninjected. Only oocytes expressing $Ba^{2+}$ currents of $\geqslant$20 nA were considered positive for the injected DNA and were included in the analyses.

### *Voltage-clamp protocols and data analysis*

Two-microelectrode voltage clamp experiments were performed on *Xenopus* oocytes using an Axoclamp-2A amplifier (Axon Instruments, Burlingame, California) connected to an IBM-compatible computer with PCLAMP version 5.5 software (Axon Instruments). Microelectrodes were filled with 3 M KCl and typically had resistances of 0.5–1.5 MΩ. $Ba^{2+}$ currents were isolated by recording in a media containing (mM): $BaCl_2$, 40; KCl, 2; tetraethylammonium chloride, 36; 4-amino-pyridine, 5; niflumic acid, 0.4; HEPES, 5 at pH 7.6. $\omega$-Conotoxin GVIA ($\omega$-CgTx, Research Biochemicals Inc., Natick, Massachusetts) was dissolved in SOS (see above) and applied to the oocytes at concentrations of 1–2 $\mu$M. Nifedipine (Sigma) and Bay K8644 (Research Biochemicals Inc.) stocks were dissolved in DMSO at a concentration of 10 mM and diluted in the above $Ba^{2+}$ recording solution to a final concentration of 10 $\mu$M.

Current recordings were analyzed using PCLAMP software and were plotted on a Hewlett-Packard Laserjet II printer after subtraction of capacitance and leakage currents and filtering at 1000 Hz. The voltage dependence of current activation was determined by normalizing the inward $Ba^{2+}$ currents elicited during steps from a holding potential of −100 mV to various test potentials [$I_{(step)}$]. Currents were normalized by dividing them by the maximum current recorded [$I_{(step)}/I_{(max)}$]. The voltage dependence of inactivation or steady-state inactivation was determined from normalized inward currents elicited during steps to +20 mV from various holding potentials (held for 20 sec). Both activation and inactivation data were fitted with smooth curves according to a modified version of the Boltzmann equation:

$$\frac{I_{(step)}}{I_{(max)}} = \frac{1}{\exp[(V_m - V_{1/2})/S_{1/2}] + 1}$$

where $I_{(step)}$ is the current recorded after each step divided by the maximum current $I_{(max)}$. $V_m$ is the step in the activation curves and the holding potential in the inactivation curves. $V_{1/2}$ is the potential where $I$ was half maximal. $S_{1/2}$ is the slope factor for the curve at $V_{1/2}$. The variables $S_{1/2}$ and $V_{1/2}$ were adjusted for best fit of the curve to the observed data and $V_{1/2(act)}$ or $V_{1/2(inact)}$ values were given in the text. The activation of the $Ba^{2+}$ currents for the various subunit combinations were compared by fitting them with single exponential curves using the Clampfit program in PCLAMP. The time constants ($\tau$) of these curves are compared in the text and in Table 1.

Significant differences between various parameters were determined using a Student's $t$-test with the significant level set at $P < 0.01$. All values given in the text and figures are mean ± SEM.

## RESULTS

### *Construction of a full-length rbB-I $\alpha_1$ subunit cDNA*

Five partial rat brain class B $\alpha_1$ subunit cDNAs were previously isolated and characterized by DNA sequencing (Dubel *et al.*, 1992a). The single open reading frame derived from the overlapping cDNAs encodes at 2336 amino acid protein with a predicted molecular mass of 262 kDa (designated rbB-I). For this study, a full-length rbB-I cDNA was initially constructed in the phagemid Bluescript SK-(Stratagene) using two cDNAs, rbB-1214 and rbB-79 (Fig. 1). Subsequently, a 7.5 kb Kpn I fragment of rbB-I was subcloned into the pZEM229 vertebrate expression vector that had been modified by insertion of a synthetic DNA polylinker (Fig. 1; see Methods). The pZEM229 vector is derived from pZEM228 (Clegg, Correll, Cadd and McKnight, 1987) and was chosen because the modified metallothionein promoter directs both high level constitutive and glucocorticoid-inducible transcription of downstream sequences. This vector has been previously used to efficiently express a cloned rat brain $Na^+$ channel (West, Scheuer, Maechler and Catterall, 1992).

### *rbB-I encodes a high-threshold, $\omega$-conotoxin-sensitive $Ca^{2+}$ channel*

The expression of rbB-I $Ca^{2+}$ channels was examined after microinjection of pZEM229 rbB-I DNA directly into the nuclei of stage V and VI *Xenopus* oocytes ($Ca^{2+}$ channel activity was recorded using 40 mM $Ba^{2+}$ as the charge carrier; see Methods). Two to five days after nuclear injection of rbB-I DNA, a small percentage (5–10%) of oocytes showed a significant inward $Ba^{2+}$ current induced by a voltage step of −100 mV to +10 mV [Fig. 2(A); 60.8 ± 7.5 nA; $n = 11$]. Current–voltage relations showed that the rbB-I $Ca^{2+}$ channels were high-threshold, first activating during voltage steps to −30 mV and peaking at +20 to +30 mV [Fig. 2(B)]. The rbB-I $Ba^{2+}$ current activated slowly ($\tau = 65.3 \pm 8.0$ msec; $n = 11$; see Table 1), reached peak values at 150–250 msec,

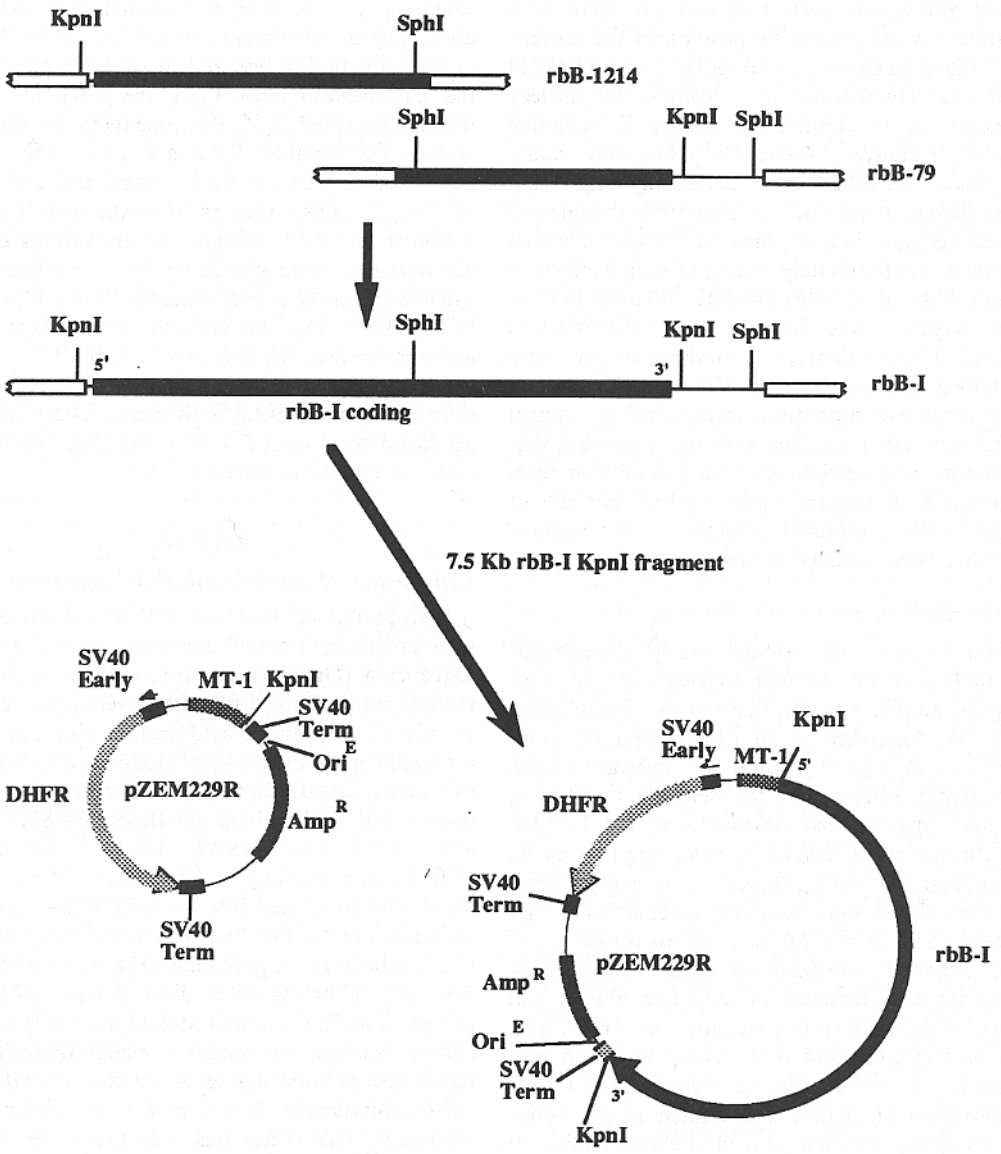


Fig. 1. Structure of rbB-I $\alpha_1$ subunit inserted into pZEM229R. The rbB-I sequence is represented by two overlapping cDNAs, rbB-1214 and rbB-79, inserted in the phagemid Bluescript SK − (top: protein coding regions are represented by filled boxes, 5′ and 3′ non-coding sequences by thin lines, and phagemid sequences by open boxes). The full-length rbB-I was constructed by digesting rbB-79 with Sph I enzyme and inserting into the Sph I site of rbB-1214. The rbB-I cDNA was subsequently digested with Kpn I and inserted into the expression vector pZEM229R. Transcription of rbB-I is driven by a modified mouse metallothionien 1 promoter and terminated by the SV40 termination signal. The vector also includes selectable markers for growth in bacteria (Amp R) and mammalian cells (DHFR). The rat brain $\alpha_2$ and $\beta_{1b}$ subunits were subcloned into a version of pZEM229R that has an Eco RI site in place of the Kpn I site.

and showed little inactivation (average ≈15–20%) over 800 msec [Fig. 2(A)]. The sensitivity of the rbB-I $Ba^{2+}$ current to various holding potentials was determined and the potential where one-half the current was inactivated [$V_{1/2(inact)}$], was calculated by fitting the data with a modified version of the Boltzmann equation (see Methods). The $V_{1/2(inact)}$ for these channels was −33.9 mV (±2.0 mV; $n = 10$; see Table 1) and almost 100% of the rbB-I $Ca^{2+}$ channels were available for opening at a holding potential of −60 mV, a value in the range of typical neuronal resting potentials.

Examination of the pharmacological properties of the rbB-I $Ba^{2+}$ current showed that it corresponded to that of an N-type $Ca^{2+}$ channel. Currents were initially recorded in high $Ba^{2+}$ saline and then the bath solution was switched to a low divalent saline (1.8 mM $Ca^{2+}$) containing 1–2 μM ω-CgTx (see

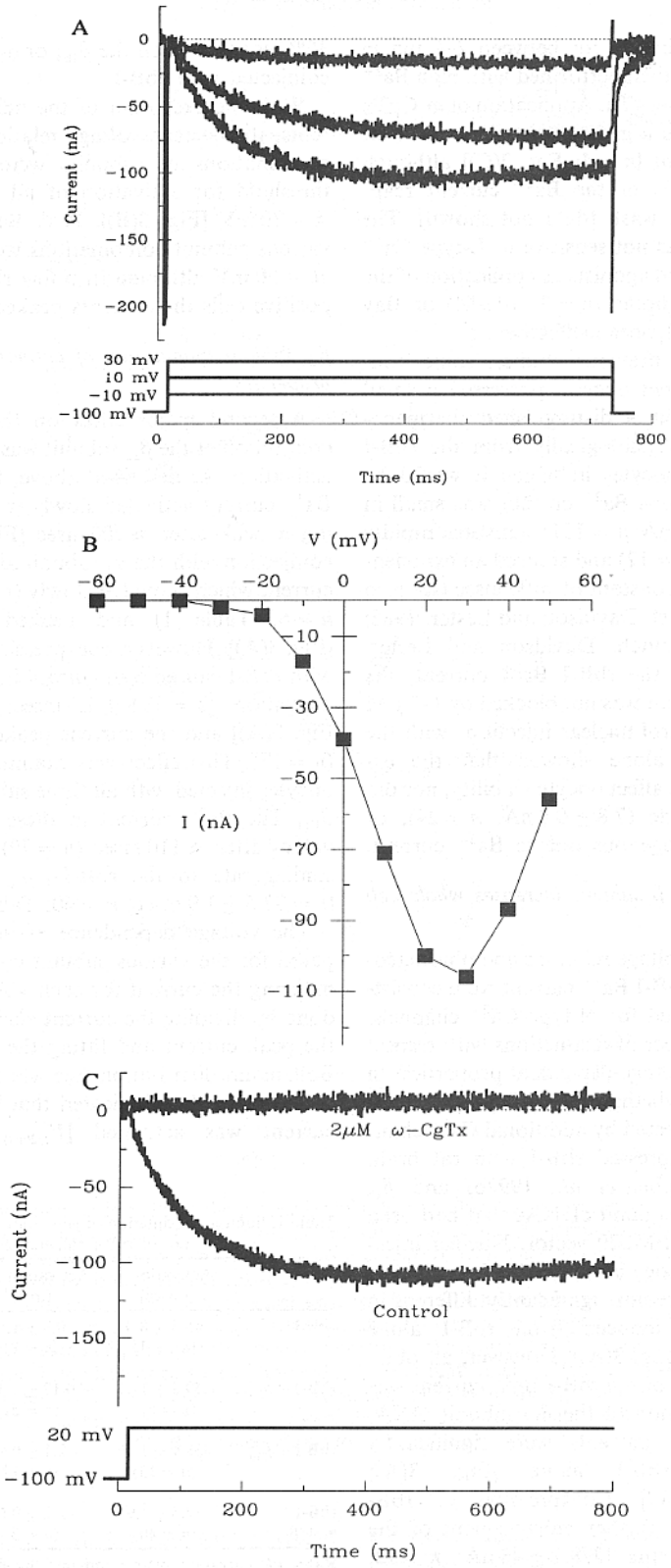


Fig. 2. The rbB-I $\alpha_1$ subunit encodes a high-threshold, $\omega$-CgTx-sensitive $Ca^{2+}$ channel. (A) Whole-cell voltage-clamp $Ba^{2+}$ currents from oocyte injected with rbB-I $\alpha_1$ $Ca^{2+}$ channel DNA. Note the slow activation of the current and minimal inactivation over several hundred milliseconds. (B) Current–voltage relation for rbB-I showing a high-threshold for activation ($\approx -30$ mV). The peak $Ba^{2+}$ current of $\approx 110$ nA was recorded during a step to $+30$ mV (holding potential; $-100$ mV). (C) Effect of $\omega$-CgTx on rbB-I whole-cell current. A short exposure (3 min) to 2 $\mu$M $\omega$-conotoxin GVIA ($\omega$-CgTx) in SOS (see Methods) caused a complete blockage of the rbB-I $Ba^{2+}$ current after washout of the toxin with normal $Ba^{2+}$ recording solution without $\omega$-CgTx.

Methods). After incubation for between 2–4 min in $\omega$-CgTx the bath was then performed with high $Ba^{2+}$ saline containing no $\omega$-CgTx. Application of $\omega$-CgTx consistently resulted in a greater than 95% block of the rbB-I $Ba^{2+}$ current [$n = 4$; Fig. 2(C)] although approximately 15–20% of the $Ba^{2+}$ current reappeared after a 10 min wash (data not shown). The rbB-I $Ba^{2+}$ current was not sensitive to L-type $Ca^{2+}$ channel antagonists and agonists as application of the dihydropyridines nifedipine ($n = 3$; 10 $\mu$M) or Bay K8644 ($n = 2$; 10 $\mu$M) were ineffective.

In agreement with previous studies, some non-injected control *Xenopus* oocytes possessed a small $Ba^{2+}$ current that could be distinguished pharmacologically and electrophysiologically from the rbB-I induced current. In oocytes in which it would be detected, the endogenous $Ba^{2+}$ current was small in magnitude ($10.0 \pm 0.6$ nA; $n = 137$), activated rapidly ($\tau = 1.6 \pm 0.3$ msec; $n = 12$) and showed an exponential decay with a time constant of $\approx$90 msec (see also Dascal, Snutch, Lubbert, Davidson and Lester, 1986; Leonard, Nargeot, Snutch, Davidson and Lester, 1987). In contrast to the rbB-I $Ba^{2+}$ current, the endogenous $Ba^{2+}$ current was not blocked by 1–2 $\mu$M $\omega$-CgTx ($n = 4$). Control nuclear injections with the pZEM229R plasmid alone showed that the expression vector did not affect oocyte viability, nor did it affect the magnitude ($7.8 \pm 0.8$ nA; $n = 24$), or waveform of the endogenous oocyte $Ba^{2+}$ current.

### *Coinjection of a brain β subunit increases whole-cell rbB-I current*

While the current–voltage relations and pharmacological profile of the rbB-I $Ba^{2+}$ current were consistent with those described for N-type $Ca^{2+}$ channels, there remained a number of distinctions with respect to some kinetic and voltage-dependent properties. In order to determine whether the rbB-I $\alpha_1$ subunit properties could be affected by additional $Ca^{2+}$ channel subunits, we coexpressed rbB-I with rat brain $Ca^{2+}$ channel $\alpha_2$ (Dubel *et al.*, 1992b) and $\beta_{1b}$ (Pragnell *et al.*, 1991) subunit cDNAs that had been subcloned into the pZEM229 vector. Nuclear injection of the $\alpha_2$ subunit together with rbB-I resulted in $Ba^{2+}$ currents that were not significantly different in magnitude to those induced from rbB-I alone [$42.5 \pm 9.5$ nA; $n = 8$; Fig. 3(A)]. However, an obvious increase in the size of the rbB-I $Ba^{2+}$ current was observed after coinjection of the $\beta_{1b}$ subunit DNA. The rbB-I + $\beta_{1b}$ $Ba^{2+}$ currents were significantly larger than the rbB-I alone [Fig. 3(A); $166.4 \pm 18.7$ nA; $n = 17$]. Coexpression of rbB-I + $\beta_{1b}$ + $\alpha_2$ resulted in further enhancement of the whole cell $Ba^{2+}$ currents [$278.2 \pm 43$ nA; $n = 72$; Fig. 3(A)] compared to those obtained with rbB-I + $\beta_{1b}$ [Fig. 3(A); currents were as large as 2.0 $\mu$A]. In addition, the frequency of positive cells (defined as having $Ba^{2+}$ currents >20 nA with the correct waveform) also increased from approximately 5–10% positive with rbB-I or rbB-I + $\alpha_2$ to between 25 and 35% positive when the $\beta_{1b}$, or $\alpha_2 + \beta_{1b}$ subunits were coinjected with rbB-I.

With the exception of the magnitude of the currents, the current–voltage relations for the different combinations of subunits were very similar. The threshold for activation of all rbB-I currents was $\approx -30$ mV [Fig. 3(B)]. Peak $Ba^{2+}$ currents for the various subunit combinations were usually recorded at +20 mV although in a few rbB-I and rbB-I + $\alpha_2$ positive cells the currents peaked at +30 mV.

### *$\beta_{1b}$ subunit modulation of activation and inactivation properties*

A second major effect on the rbB-I current by coinjection of the $\beta_{1b}$ subunit was an increased rate of activation. As described above, the whole-cell rbB-I $Ba^{2+}$ current activated slowly ($\tau = 65.3$ msec) reaching a peak after $\approx$200 msec [Fig. 3(A)]. Similarly, coinjection with the $\alpha_2$ subunit also produced a $Ba^{2+}$ current which activated slowly ($\tau = 59.2 \pm 11.1$ msec; $n = 6$; Table 1) and peaked around 200 msec [Fig. 3(A)]. However, coexpression of the $\beta_{1b}$ subunit with rbB-I caused a significant increase in the rate of activation [$\tau = 38.9 \pm 1.5$ msec; $n = 13$; Table 1; Fig. 3(A)] and the current peaked after $\approx$120 msec ($n = 17$). This effect was examined more closely in oocytes injected with all three subunits, rbB-I, $\alpha_2$ and $\beta_{1b}$. The $Ba^{2+}$ current in these cells reached peak values after $\approx$110 msec ($n = 70$) and activated at a similar rate to the rbB-I + $\beta_{1b}$ coinjected oocytes [$\tau = 32.5 \pm 1.9$ msec; $n = 40$; Table 1; Fig. 3(A)].

The voltage dependence of activation was compared for the various subunit combinations by normalizing the current for each voltage step. This was done by dividing the current elicited at each step by the peak current and fitting the data to a modified Boltzmann distribution (see Methods). Curves fitted to the rbB-I data indicated that half the rbB-I $Ba^{2+}$ current was activated [$V_{1/2(act)}$] at $0.8 \pm 1.4$ mV

Table 1. Subunit modulation of activation and inactivation properties of rbB-I calcium channels

| | Activation $\tau$ (msec) | Activation $V_{1/2}$ (mV) | Inactivation $V_{1/2}$ (mV) |
|---|---|---|---|
| rbB-I | $65.3 \pm 8.0$ ($n = 11$) | $0.8 \pm 1.4$ ($n = 8$) | $-33.9 \pm 2.0$ ($n = 10$) |
| rbB-I + $\alpha_2$ | $59.2 \pm 11.1$ ($n = 6$) | $-0.17 \pm 1.3$ ($n = 5$) | $-33.8 \pm 1.8$ ($n = 5$) |
| rbB-I + $\beta_{1b}$ | $38.9 \pm 1.5$* ($n = 13$) | $-2.1 \pm 0.9$ ($n = 12$) | $-59.6 \pm 2.1$* ($n = 3$) |
| rbB-I + $\alpha_2 + \beta_{1b}$ | $32.5 \pm 1.9$* ($n = 40$) | $-2.2 \pm 0.6$ ($n = 21$) | $-59.6 \pm 1.5$* ($n = 19$) |

Rates of activation were compared by fitting single exponential curves (using Clampfit program in PCLAMP) to the activating $Ba^{2+}$ currents and the tau ($\tau$) values were given in the table. The voltage dependence of activation and inactivation were determined for each subunit combination (see Methods) and $V_{1/2}$ values (voltage where half the current is activated or inactivated) were given in the table. All values were compared with rbB-I using a Student's *t*-test and significant differences ($P < 0.01$) were indicated by an asterisk (*).

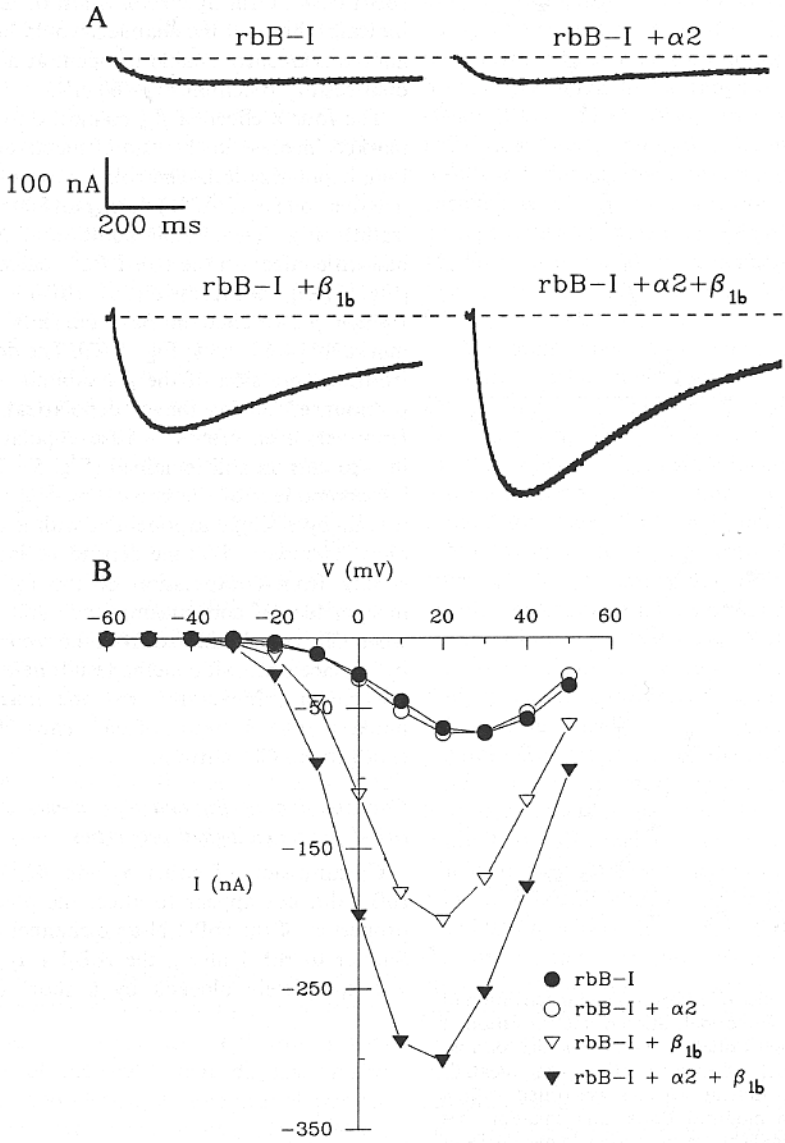


Fig. 3. Subunit composition affects the properties of rbB-I. (A) Representative traces of whole-cell $Ba^{2+}$ currents from oocytes coinjected with rbB-I $\alpha_1$ and rat brain $\alpha_2$ and $\beta_{1b}$ subunits. Note the significant increase in magnitude of the $Ba^{2+}$ current in oocytes coinjected with the rbB-I + $\beta_{1b}$, or rbB-I + $\beta_{1b}$ + $\alpha_2$ subunits compared to those with the rbB-I $\alpha_1$ alone, or with rbB-I + $\alpha_2$. The slow rate of activation of the rbB-I $Ba^{2+}$ current (top left; $\tau \approx 65$ msec) was significantly enhanced in oocytes coinjected with the $\beta_{1b}$ subunit (bottom traces; $\tau \approx 35$ msec). There was also pronounced inactivation (65–75%) of the $Ba^{2+}$ current over 800 msec in $\beta_{1b}$-coinjected oocytes (bottom traces) compared with relatively little inactivation (15–20%) in rbB-I (top left) or rbB-I + $\alpha_2$ (top right). (B) Current–voltage relations of $Ba^{2+}$ currents from coinjected oocytes. Both the threshold for activation and the peak of the $I/V$ curves were similar for all combinations of calcium channel subunits tested, although some rbB-I and rbB-I + $\alpha_2$ $Ba^{2+}$ currents (○, ●) peaked closer to +30 mV than +20 mV.

[Fig. 4(A); Table 1; $n = 8$]. Coexpression of rbB-I with the $\alpha_2$ and/or the $\beta_{1b}$ subunit did not significantly change $V_{1/2(act)}$ [Fig. 4(A); Table 1], indicating that the rbB-I $\alpha_1$ subunit is the major determinant of this electrophysiological characteristic and is not modified by the $\alpha_2$ and $\beta_{1b}$ subunits.

A third effect of coinjection of the brain $\beta_{1b}$ subunit with the rbB-I $\alpha_1$ subunit was observed as a shift in the steady state inactivation of the $Ba^{2+}$ current. The dependence of rbB-I $Ba^{2+}$ current inactivation upon voltage was examined by applying a 20 sec prepulse to various holding potentials prior to stepping to a test potential of +20 mV. Approximately 50% of the rbB-I $Ba^{2+}$ current was inhibited by a prepulse to −30 mV [$V_{1/2(inact)} = -33.9$ mV ± 2.0 mV; $n = 10$; Fig. 4(B), Table 1]. The steady state inactivation of

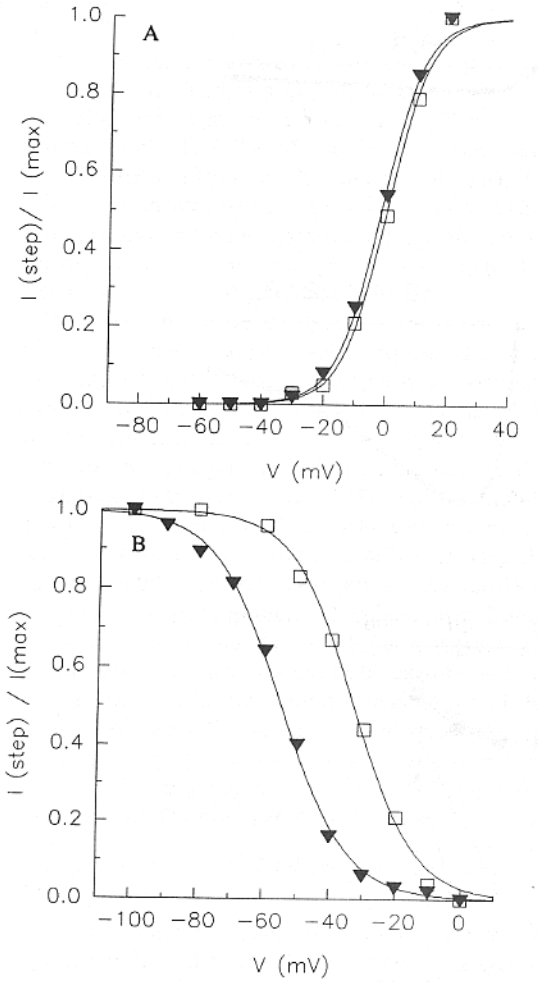


Fig. 4. Voltage dependence of activation and inactivation of rbB-I $Ba^{2+}$ currents. (A) Normalization of the activation of $Ba^{2+}$ currents was performed by dividing the current measured at steps from −100 mV to various test potentials by the peak current. Activation data was fitted with a smooth curve using a modified Boltzmann equation (see Methods). Note the similarity in the voltage dependence of activation between rbB-I (□) and rbB-I + $\alpha_2$ + $\beta_{1b}$ (▼). (B) The voltage dependence of inactivation or steady-state inactivation was determined by normalizing the current elicited upon a step to +20 mV from various holding potentials (V). A pronounced hyperpolarizing voltage shift (≈20 mV) was observed in oocytes coinjected with rbB-I and the brain $\beta_{1b}$ subunit (filled triangles (▼); rbB-I + $\beta_{1b}$ + $\alpha_2$) compared with rbB-I alone (□) or with rbB-I + $\alpha_2$ (not shown). A full comparison of the voltage dependence of activation and inactivation is given in Table 1.

rbB-I was shifted approximately 20 mV to more negative potentials by the presence of the brain $\beta_{1b}$ subunit [$V_{1/2(inact)} = -59.6 \pm 2.1$ mV; $n = 3$; Table 1] or the $\beta_{1b} + \alpha_2$ subunits [$V_{1/2(inact)} = -59.6 \pm 1.5$ mV; $n = 19$; Fig. 4(B); Table 1]. Coexpression of the brain $\alpha_2$ subunit alone did not have a significant effect on the rbB-I steady state inactivation [$V_{1/2(inact)} = -33.8 \pm 1.8$ mV; $n = 5$; Table 1]. The pronounced shift in the steady state inactivation of the rbB-I current during coexpression of the $\beta_{1b}$ subunit indicates that half the channels would be inactivated, and consequently unable to open, at a typical neuronal resting potential of −60 mV.

The fourth effect of $\beta_{1b}$ coinjection was seen as a marked increase in the rate of inactivation during a long depolarization. The rbB-I current showed little inactivation (≈15–20%) during an 800 msec depolarization [Fig. 3(A)]. While addition of the $\alpha_2$ subunit had little effect on the rbB-I $Ba^{2+}$ current waveform [Fig. 3(A)], coexpression of rbB-I + $\beta_{1b}$ or rbB-I + $\alpha_2$ + $\beta_{1b}$ resulted in $Ba^{2+}$ currents that decayed markedly [≈65–70%; Fig. 3(A)]. The decay resulting from coexpression of the $\beta_{1b}$ subunit was especially pronounced during longer depolarizations (Fig. 5). However, even after a 3.5 sec depolarization some inward current still remained (Fig. 5). The inactivating component of the rbB-I + $\alpha_2$ + $\beta_{1b}$ $Ba^{2+}$ current was fit by a single exponential with a time constant of ≈700 msec. The time-dependent inactivation resulting from coexpression of the $\beta_{1b}$ subunit was independent of current amplitude and still occurred when $Cl^-$ was eliminated from the recording solution by replacement with methanesulfonate ($n = 4$; data not shown), indicating that the inactivation was unlikely to be the result of $Ba^{2+}$ entry stimulating an endogenous $Cl^-$ current.

### *Coexpression of $\beta_{1b}$ and $\alpha_2$ subunits does not alter rbB-I pharmacological properties*

Coexpression of either $\alpha_2$ and $\beta_{1b}$ subunits with rbB-I did not appear to affect the pharmacological properties of the rbB-I N-type channel $Ba^{2+}$ current. Similar to rbB-I alone, the rbB-I + $\alpha_2$ $Ba^{2+}$ current was completely blocked by a short (2–4 min) ex-

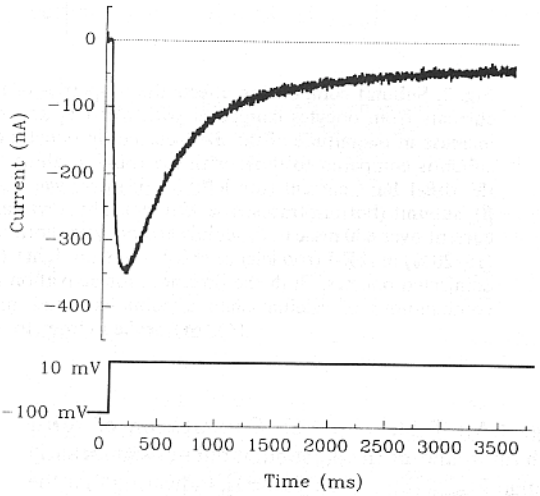


Fig. 5. Long test pulses show N-type rbB-I calcium channels do not completely inactivate. A long depolarization from −100 to +10 mV reveals that although a major component (≈75–85%) of the rbB-I + $\alpha_2$ + $\beta_{1b}$ $Ba^{2+}$ current inactivates within 1 sec another small component (≈15–25%) remains even after a prolonged depolarization of 3.5 sec.

posure to 1 $\mu$M $\omega$-CgTx ($n = 3$). This current was also insensitive to nifedipine (10 $\mu$M; $n = 3$) and Bay K8644 (10 $\mu$M; $n = 3$). Similarly, the rbB-I + $\beta_{1b}$ $Ba^{2+}$ current was also blocked by $\omega$-CgTx ($n = 8$) and unaffected by nifedipine at 10 $\mu$M ($n = 8$).

A more detailed pharmacological characterization was performed on oocytes coinjected with all three subunits. $Ba^{2+}$ currents recorded from rbB-I + $\alpha_2$ + $\beta_{1b}$ oocytes were again blocked completely (>95%) by a short exposure (2–4 min) to 1–2 $\mu$M $\omega$-CgTx [$n = 18$; Fig. 6(A)] but as with rbB-I alone some recovery ($\approx$20%) was observed after washing for 10–15 min. To confirm that the $\omega$-CgTx block was irreversible, several cells were exposed to $\omega$-CgTx for longer time periods as this toxin has a slow on-rate (Wagner, Snowman, Biswas, Olivera and Snyder, 1988). In all 6 cases where oocytes were treated with 1–2 $\mu$M $\omega$-CgTx for 10 min there was no recovery of the $Ba^{2+}$ current even after 15 min. The dihydropyridine antagonist, nifedipine (10 $\mu$M), had negligible effects on the $Ba^{2+}$ currents in rbB-I + $\alpha_2$ + $\beta_{1b}$ oocytes [$n = 18$; fig. 6(B)]. Similarly, the dihydropyridine agonist Bay K8644 (10 $\mu$M) had no effect on these channels [$n = 5$; Fig. 6(C)]. In control experiments, nifedipine and Bay K8644 both had pronounced effects on $Ba^{2+}$ currents in oocytes expressing the rat brain class C $\alpha_1$ subunit, rbC-II + $\alpha_2$ + $\beta_{1b}$ (Stea *et al.*). Addition of the non-specific $Ca^{2+}$ channel blocker $Cd^{2+}$ (10 $\mu$M) caused a virtually complete blockade (>90%) of the rbB-I + $\alpha_2$ + $\beta_{1b}$ $Ba^{2+}$ current ($n = 6$).

## DISCUSSION

### *The rat brain class B $\alpha_1$ subunit, rbB-I, encodes an N-type $Ca^{2+}$ channel and is modulated by a $\beta$ subunit*

High-threshold $Ca^{2+}$ channels represent a heterogenous class of molecules that display diverse pharmacological and electrophysiological characteristics (for reviews see Bean, 1989a; Hess, 1990). N-type $Ca^{2+}$ channels can be distinguished pharmacologically from other high-threshold $Ca^{2+}$ channels by their high affinity for $\omega$-CgTx (nanomolar range) and an irreversible block during exposure to micromolar concentrations of $\omega$-CgTx (Olivera *et al.*, 1985; Reynolds *et al.*, 1986; McCleskey *et al.*, 1987; Nowycky *et al.*, 1985; Wagner *et al.*, 1988; McEnery *et al.*, 1991; Plummer *et al.*, 1989; Regan *et al.*, 1991). The rbB-I $Ca^{2+}$ channel meets both of these criteria since: (1) antibodies against rbB-I selectively immunoprecipitate radiolabelled high-affinity rat brain $\omega$-CgTx binding sites (Dubel *et al.*, 1992a; Westenbroek *et al.*, 1992) and (2) $Ba^{2+}$ currents induced by expression of the rbB-I $\alpha_1$ subunit in *Xenopus* oocytes are completely blocked by 1–2 $\mu$M $\omega$-CgTx [Fig. 2(C)]. The insensitivity of the rbB-I $Ba^{2+}$ current to DHP agonists and antagonists is also consistent with that of an N-type current.

The electrophysiological properties of macroscopic N-type $Ca^{2+}$ currents is quite variable, but can generally be distinguished from other $Ca^{2+}$ currents by a decay in the current waveform during a prolonged depolarizing pulse and a pronounced inactivation at holding potentials in the range of $-60$ to $-40$ mV (Bean, 1989a; Hess, 1990). In several respects, the rbB-I $\alpha_1$ subunit expressed alone in *Xenopus* oocytes results in a current that appears distinct from N-type currents recorded from neurons. The rbB-I $Ba^{2+}$ current activates slowly ($\tau \approx 65$ msec), decays only 15–20% during an 800 msec depolarization, and inactivates at relatively positive holding potentials [$V_{1/2(inact)} = -33.9$ mV].

Two recent reports have confirmed that class B $\alpha_1$ subunits encode high-threshold, $\omega$-CgTx-sensitive $Ca^{2+}$ channels (Williams *et al.*, 1992b; Fujita, Mynlieff, Dirksen, Kim, Niidome, Nakai, Friedrich, Iwabe, Miyata, Furuichi, Furutama, Mikoshiba, Mori and Beam, 1993), although neither study addressed the issue of whether ancillary $Ca^{2+}$ channel subunits can modulate the electrophysiological properties of N-type currents. Utilizing the *Xenopus* oocyte expression system we examined the effects of coexpression of rat neuronal $\alpha_2$ and $\beta$ subunits on the rat N-type channel $\alpha_1$ subunit. A major finding of this study is that coexpression of a brain $Ca^{2+}$ channel $\beta_{1b}$ subunit dramatically alters a number of the electrophysiological properties of the rbB-I $\alpha_1$ subunit and results in currents more similar to those found for N-type channels in neurons. The effects of the $\beta_{1b}$ subunit on rbB-I include: (1) an enhancement of the whole current; (2) an increased rate of activation; (3) a hyperpolarizing shift in the voltage dependence of inactivation; and (4) an increased degree of decay of the current waveform during a prolonged depolarization. The observed modulatory effects by the $\beta_{1b}$ subunit is consistent with the finding that the purified brain $\omega$-CgTx receptor is a heteroligomeric complex that includes $\alpha_1$ and $\beta$ subunits (McEnery *et al.*, 1991; Sakamoto and Campbell, 1991; Witcher *et al.*, 1993) and provides strong evidence for a functional interaction between N-type channel $\alpha_1$ and $\beta$ subunits *in vivo*.

While the rbB-I $\alpha_1$ subunit is coexpressed in a rat neuronal cell line with the $\beta_{1b}$ subunit (T. P. Snutch and S. J. Dubel, unpublished results), further biochemical and immunological studies are required to confirm the exact subunit composition of rat brain N-type channels. Relevant to the present study, the N-type channel purified from rabbit brain has recently been shown to be composed of four subunits, $\alpha_1$, $\alpha_2$, $\beta$, and a novel 95 kDa component (Witcher *et al.*, 1993). The $\alpha_1$ subunit is immunologically identical to the rbB-I $\alpha_1$ used in this study and the $\alpha_2$ subunit is similar to that found in skeletal muscle L-type channels. The $\beta$ subunit associated with the rabbit N-type channel corresponds to the recently described $\beta_3$ subunit (Castellano *et al.*, 1993) and except for some amino acid differences at the amino and carboxy termini, is highly similar to the $\beta_{1b}$ subunit used in this study.

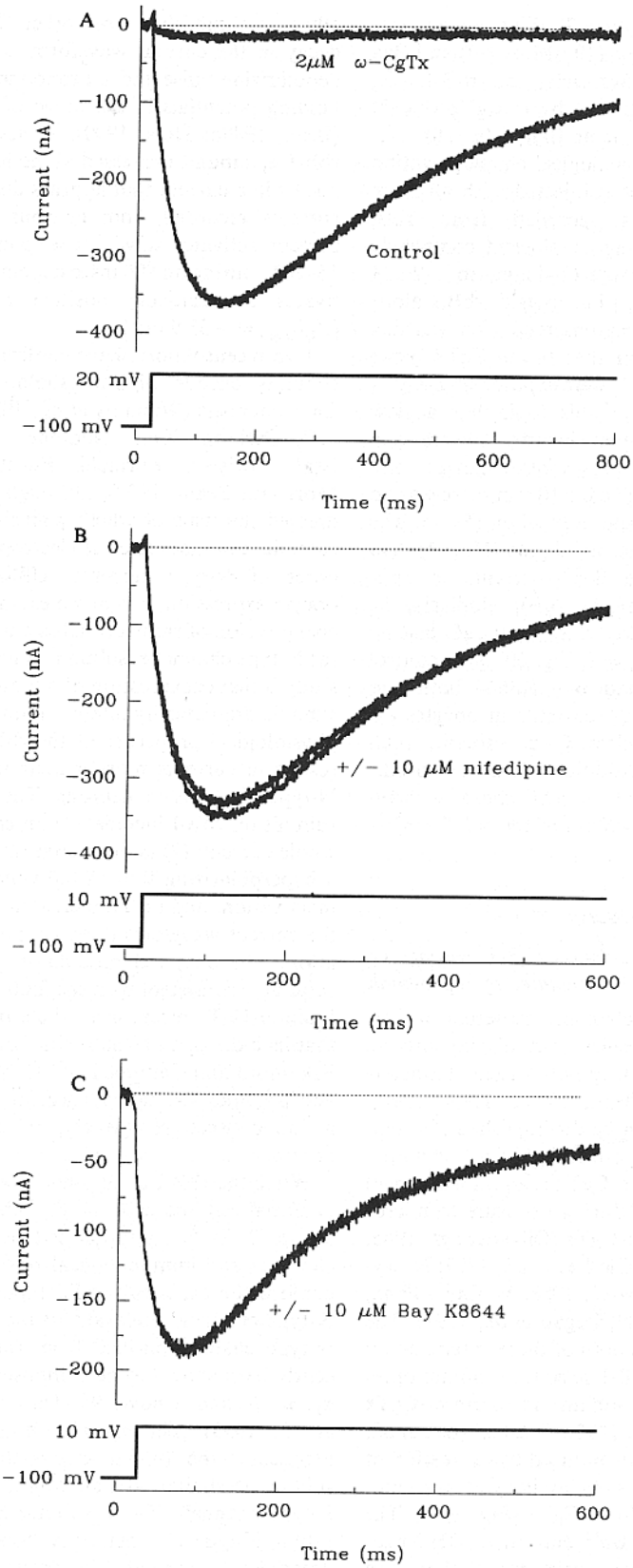


Fig. 6. Pharmacology of N-type rbB-I calcium channels. (A) rbB-I + $\alpha_2$ + $\beta_{1b}$ $Ba^{2+}$ currents are blocked (>95%) by 2 $\mu$M $\omega$-conotoxin GVIA ($\omega$-CgTx). (B) Exposure to the dihydropyridine antagonist nifedipine (10 $\mu$M) had negligible (<5%) effects on rbB-I + $\alpha_2$ + $\beta_{1b}$ $Ba^{2+}$ currents. (C) Similarly, the dihydropyridine agonist Bay K8644 (10 $\mu$M) had no effect on the $Ba^{2+}$ currents (rbB-I + $\alpha_2$ + $\beta_{1b}$) as traces are almost completely superimposed.

Some aspects of the modulation of rbB-I N-type channel properties by the rat brain $\beta_{1b}$ subunit are similar to those recently described for cloned cardiac and skeletal muscle L-type $Ca^{2+}$ channels. A prominent effect of a skeletal muscle $\beta$ subunit on stable cell lines expressing the skeletal muscle L-type $\alpha_1$ subunit is to both increase the rate of activation (up to $\approx$100-fold) and to increase inactivation during a prolonged depolarization (Lacerda *et al.*, 1991; Varadi *et al.*, 1991). Similarly, coexpression of cardiac L-type $\alpha_1$ and $\beta$ subunits in *Xenopus* oocytes results in an increased rate of activation over that of the $\alpha_1$ subunit alone (Wei *et al.*, 1991; Singer *et al.*, 1991; Perez-Reyes, Castellano, Kim, Bertrand, Baggstrom and Lacerda, Wei and Birnbaumer, 1992; Hullin, Singer-Lahat, Freichel, Biel, Dascal, Hofmann and Flockerzi, 1992). The $\beta_3$ cloned from rat brain (Castellano *et al.*, 1993) also accelerated the rates of activation and inactivation of cardiac L-type $\alpha_1$ currents in *Xenopus* oocytes.

Comparison of the rbB-I and rbB-I + $\beta_{1b}$ current activation curves shows that they are nearly identical and indicates that the $\beta_{1b}$ subunits has little affect on the voltage sensitivity of activation of rbB-I (Table 1). This result is in contrast to that found for interaction of cloned L-type $Ca^{2+}$ channel $\alpha_1$ and $\beta$ subunits. For example, a skeletal muscle $\beta$ subunit appears to affect the voltage-dependence of activation of the skeletal muscle $\alpha_1$ subunit at depolarized potentials (Lacerda *et al.*, 1991). Similarly coexpression of the cardiac $\beta_2$ subunit, or the brain $\beta_3$ subunit, were shown to affect the voltage sensitivity of activation of cardiac L-type $Ca^{2+}$ channel $\alpha_1$ subunits (Perez-Reyes *et al.*, 1992; Castellano *et al.*, 1993).

In contrast to the $\beta$ subunit modulatory effects, coinjection of the rat brain $\alpha_2$ subunit with rbB-I did not have any measurable effects compared to that of rbB-I alone. However, we did detect a moderate increase in the level of whole cell currents obtained when the $\alpha_2$ subunit was coinjected with rbB-I and the $\beta_{1b}$ subunit. The enhancement of whole cell currents induced by the $\alpha_2$ subunit is similar to that reported for other cloned neuronal and non-neuronal $Ca^{2+}$ channels (Mikami, Imoto, Tanabe, Niidome, Mori, Takeshima, Narumiya and Numa, 1989; Biel, Ruth, Bosse, Hullin, Stuhmer, Flockerzi and Hofmann, 1990; Mori *et al.*, 1991; Williams *et al.*, 1992a), although in some studies $\alpha_2$ subunit coexpression was also found to modulate the rates of activation and inactivation, and the voltage-dependence of inactivation (Singer *et al.*, 1991; Hullin *et al.*, 1992). While the wide variations in the $\alpha_1$ subunit electrophysiological properties modulated by $\alpha_2$ and $\beta_{1b}$ subunits reported is somewhat surprising, these results may reflect actual subunit interactions that occur in different types of $Ca^{2+}$ channels *in vivo*. However, it should also be noted that in some coexpression studies the resulting $Ca^{2+}$ channels were either brain/skeletal muscle or cardiac/skeletal muscle hybrids and thus some of the physiological properties of these hybrid channels may reflect differences in subunit interactions between tissue-specific isoforms.

While $\beta_{1b}$ subunit coexpression significantly increases the rate of activation of the rbB-I $Ba^{2+}$ current (Fig. 2; Table 1), it remains slower than that usually observed for N-type $Ca^{2+}$ channels in neurons (typically, $\tau \approx 1$–3 msec). There are number of possible explanations for the slow rate of activation of the rbB-I + $\beta_{1b}$ current. One possibility is that coexpression with additional $Ca^{2+}$ channel subunits might further modulate the gating properties of the rbB-I + $\beta_{1b}$ current. A second possibility is that distinct $Ca^{2+}$ channel $\beta$ subunits may differentially modulate the electrophysiological properties of N-type $Ca^{2+}$ channel $\alpha_1$ subunits. In support of this possibility, several different $\beta$ subunit isoforms have been shown to differentially modulate the rate of activation of the cardiac class C $\alpha_1$ subunit (Perez-Reyes *et al.*, 1992; Hullin *et al.*, 1992; Castellano *et al.*, 1993) and expression of multiple $\beta$ subunit isoforms has been demonstrated in the mammalian nervous system (Hullin *et al.*, 1992; Perez-Reyes *et al.*, 1992; Pragnell *et al.*, 1992; Williams *et al.*, 1992a; Castellano *et al.*, 1993). A third possibility is that the slower activation is an inherent property of the cloned rbB-I $\alpha_1$ subunit, reflecting either the existence of multiple N-type channel $\alpha_1$ subunits or the introduction of a mutation during the cDNA cloning of rbB-I. Both molecular cloning and biochemical evidence suggests that N-type channel $\alpha_1$ subunit isoforms exist in mammalian brain (Williams *et al.*, 1992b; Westenbroek *et al.*, 1992). Alternatively, amino acid substitutions resulting from nucleotide changes introduced into rbB-I during cloning could affect the gating properties of rbB-I or its interaction with the $\beta$ subunits.

### *Expression of N-type $Ca^{2+}$ channels in Xenopus oocytes*

The exogenous expression of voltage-gated $Ca^{2+}$ channels in *Xenopus* oocytes after microinjection of both natural and synthetic RNAs has proven to be a valuable tool for studying the structural and functional properties of these proteins (Dascal *et al.*, 1986; Leonard *et al.*, 1987; Mikami *et al.*, 1989; Biel *et al.*, 1990; Mori *et al.*, 1991; Singer *et al.*, 1991; Wei *et al.*, 1991; Williams *et al.*, 1992a). While uninjected oocytes possess an endogenous high-threshold $Ca^{2+}$ current (measured with 40 mM Ba as the charge carrier), the small magnitude (usually less than 10 nA), distinct waveform ($\tau$ inact $\approx$100 msec) and unique pharmacology (insensitive to DHPs and $\omega$-CgTx) of this current does not compromise examination of the much larger, and electrophysiologically and pharmacologically distinct $Ca^{2+}$ currents induced by exogenous RNAs (Dascal *et al.*, 1986; Leonard *et al.*, 1987). However, we note that upon coinjection with either the $\beta_{1b}$, or the $\alpha_2 + \beta_{1b}$ subunits we observed a small enhancement of the magnitude of the endogenous current ($\approx$30 nA; $n = 60$; data not

shown). This current was not sensitive to ω-CgTx (1 μM; $n = 7$). The waveform, and activation and inactivation characteristics of the enhanced current were similar to that found in uninjected oocytes, and suggests that β subunits are capable of associating with an endogenous oocyte $Ca^{2+}$ channel $\alpha_1$ subunit. While the molecular identity of the oocyte $\alpha_1$ subunit remains to be identified, its apparent ability to interact with the rat brain proteins suggests an evolutionary conservation of associations between $Ca^{2+}$ channel subunits (also see Singer *et al.*, 1991; Williams *et al.*, 1992a).

While oocytes are capable of reconstituting $Ca^{2+}$ channels with many of the electrophysiological and pharmacological properties of channels found in the tissue from which RNA is derived, a peculiar aspect of the oocyte system is the apparent selective expression of certain $Ca^{2+}$ channel subtypes. For example, microinjection of rat heart RNA preferentially induces the expression of L-type $Ca^{2+}$ channels (Dascal *et al.*, 1986; Snutch, Leonard, Nargeot, Lubbert, Davidson and Lester, 1987), while rat brain RNA directs the synthesis of a prominent high-threshold $Ca^{2+}$ channel that is insensitive to DHPs and ω-CgTx (Leonard *et al.*, 1987), but is blocked by a fraction from funnel-web spider venom (Lin, Rudy and Llinas, 1990). Furthermore, the apparent inability of oocytes to generate robust rat brain N-type $Ca^{2+}$ currents from microinjected RNA appears to be a selective phenomenon since oocytes can generate ω-CgTx-sensitive currents from both Torpedo electric lobe and human brain RNAs (Umbach and Gundersen, 1987; Gundersen, Umbach and Swartz, 1988). Consistent with our inability to detect N-type $Ca^{2+}$ channels from rat brain RNA, attempts to express $Ca^{2+}$ channels in oocytes injected with synthetic RNA from the full-length rbB-I cDNA have been unsuccessful (Dubel *et al.*, 1992a; unpublished results). The additional coinjection of synthetic RNAs for the rat brain $\beta_{1b}$ and $\alpha_2$ subunits used in this study also did not result in N-type $Ca^{2+}$ channels being expressed (data not shown). While detailed studies have not been performed, the selective expression of $Ca^{2+}$ channel subtypes may be related to the fact that the efficient translation of exogenous RNAs in oocytes appears to be influenced by 5′ and 3′ non-coding sequences. Indeed, the removal of 5′ noncoding regions containing additional ATG initiation codons (Kobilka, MacGregor, Daniel, Kobilka, Caron and Lefkowitz, 1987; White, Chen, Kleinfield, Kallah and Barchi, 1991) and the addition of 5′ and 3′ noncoding sequences found in *Xenopus* RNA (Krieg and Melton, 1984) have been shown to substantially increase the translatability of exogenous RNAs. The present study, together with the expression of other cloned rat brain $Ca^{2+}$ channel subtypes (Tomlinson, Stea, Bourinet, Charnet, Nargeot and Snutch, 1993; Soong *et al.*, 1993), indicate that nuclear expression of cDNAs driven by a strong vertebrate transcriptional promoter can overcome the inhibitory aspects associated with the expression of exogenous RNAs encoding some $Ca^{2+}$ channels.

*Conclusion*

The demonstrated ability of *Xenopus* oocytes to generate robust ω-CgTx-sensitive $Ba^{2+}$ currents after nuclear injection of the rbB-I cDNA will provide a convenient assay for the molecular and physiological study of N-type channels. Additionally, by coexpression of rbB-I and cloned neurotransmitter receptors, the oocyte assay will allow the mechanistic dissection of the modulatory effects on N-type $Ca^{2+}$ channels mediated by various neurotransmitters (for examples see: Wanke, Ferroni, Malgarolio, Ambrosini, Pozzan and Meldolesi, 1987; Lipscombe *et al.*, 1989; Bean, 1992b; Bley and Tsien, 1990; Beech *et al.*, 1992). Our results also suggest that in addition to the unique physiological properties encoded by individual subtypes of $Ca^{2+}$ channel $\alpha_1$ subunits (for reviews see Tsien *et al.*, 1991; Snutch and Reiner, 1992), coexpression of distinct β subunits may provide a general mechanism for generating further $Ca^{2+}$ channel functional heterogeneity in the nervous system.

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