Ca^{2+} current enhancement by $\alpha 2/\delta$ and β subunits in *Xenopus* oocytes: contribution of changes in channel gating and $\alpha 1$ protein level

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1. A combined biochemical and electrophysiological approach was used to determine the mechanism by which the auxiliary subunits of Ca^{2+} channel enhance the macroscopic Ca^{2+} currents. *Xenopus* oocytes were injected with RNA of the main pore-forming subunit (cardiac: $\alpha 1C$), and various combinations of RNAs of the auxiliary subunits ($\alpha 2/\delta$ and $\beta 2A$).

2. The single channel open probability (P_o; measured at 0 mV) was increased ~3-, ~8- and ~35-fold by $\alpha 2/\delta$, $\beta 2A$ and $\alpha 2/\delta + \beta 2A$, respectively. The whole-cell Ca^{2+} channel current was increased ~8- to 10-fold by either $\alpha 2/\delta$ or $\beta 2A$, and synergistically >100-fold by $\alpha 2/\delta + \beta 2A$. The amount of ^{35}S-labelled $\alpha 1$ protein in the plasma membrane was not changed by coexpression of $\beta 2A$, but was tripled by coexpression of $\alpha 2/\delta$ (either with or without β).

3. We conclude that the increase in macroscopic current by $\alpha 2/\delta$ is equally due to changes in amount of $\alpha 1$ in the plasma membrane and an increase in P_o, whereas all of the effect of $\beta 2A$ is due to an increase in P_o. The synergy between $\alpha 2/\delta$ and β in increasing the macroscopic current is due mainly to synergistic changes in channel gating.

Voltage-dependent Ca^{2+} channels consist of a main pore-forming subunit ($\alpha 1$) and auxiliary subunits which may vary among tissues and depend on the subtype of $\alpha 1$. Yet two subunits ($\alpha 2/\delta$ and one of the isoforms of β) are always present and seem to be required for proper channel function and modulation (for reviews see Hofmann, Biel & Flockerzi, 1994; Isom, De Jongh & Catterall, 1994). A well-studied model is the cardiac L-type Ca^{2+} channel. Expression of the cardiac L-type $\alpha 1$ subunit alone ($\alpha 1C$) in mammalian cells and *Xenopus* oocytes is sufficient to produce functional dihydropyridine (DHP)-sensitive Ca^{2+} channels, but the macroscopic (whole-cell) currents are small, and strongly enhanced by coexpression of either $\alpha 2/\delta$ or β (Mikami *et al.* 1989; Lacerda *et al.* 1991; Mori *et al.* 1991; Singer, Biel, Lotan, Flockerzi, Hofmann & Dascal, 1991; Varadi, Lory, Schultz, Varadi & Schwartz, 1991). Coexpression with $\alpha 1$ of both $\alpha 2/\delta$ and β subunits increases the macroscopic currents to magnitudes far exceeding the sum of those seen in either $\alpha 1 + \alpha 2/\delta$ or $\alpha 1 + \beta$ compositions, suggesting a synergistic interaction between $\alpha 2/\delta$, β and $\alpha 1$ (Mori *et al.* 1991; Singer *et al.* 1991).

Both $\alpha 2/\delta$ and β alter some of the macroscopic parameters of the Ca^{2+} channel current (kinetics, voltage dependency of activation and inactivation: see Hofmann *et al.* 1994). Coexpression of β increases the proportion of long openings and the total open probability (P_o) (Wakamori, Mikala, Schwartz & Yatani, 1993; Neely, Olcese, Baldelli, Wei, Birnbaumer & Stefani, 1995), but it is not known whether these factors are sufficient to account for all of the observed increase in the whole-cell current. The effect of $\alpha 2/\delta$ or the simultaneous coexpression of $\alpha 2/\delta$ and β on single channel properties has not been studied.

It is also unclear whether the coexpression of subunits changes the level of $\alpha 1$ protein in the plasma membrane. The effects of $\alpha 2/\delta$, or a simultaneous expression of $\alpha 2/\delta$ and β have not been studied at all, and controversies still abound even with respect to the much better studied β subunit. Coexpression of β with cardiac or skeletal muscle $\alpha 1$ increases the amount of DHP-binding sites in transfected mammalian cells (Lacerda *et al.* 1991; Varadi *et al.* 1991; Nishimura, Takeshima, Hofmann, Flockerzi &

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This manuscript was accepted as a Short Paper for rapid publication.

Imoto, 1993). However, no effect of coexpression of β on the amount of $\alpha 1$ protein has been found by an immunoblot method (Nishimura *et al.* 1993). Importantly, these findings pertain to the amount of $\alpha 1$ protein in a whole-cell membrane fraction and may not adequately reflect the amount of functional protein in the plasma membrane. Neely, Olcese, Wei, Birnbaumer & Stefani (1993) addressed this problem by studying gating and macroscopic ionic currents in oocytes expressing $\alpha 1$ or $\alpha 1 + \beta$, and found that the addition of β does not change the gating current (which is proportional to the amount of voltage sensor, i.e. $\alpha 1$ subunit, in the plasma membrane) but increases the macroscopic current, suggesting an improved coupling between activation gating and the opening of the channel without a change in the amount of $\alpha 1$ in the membrane.

To assess quantitatively the contribution of effects of changes in channel gating *vs.* changes in protein level to the whole-cell Ca^{2+} current enhancement by $\alpha 2/\delta$ and β, we combined direct measurements of the $\alpha 1$ protein in plasma membrane and internal fractions, and measurements of macroscopic and single-channel currents, in *Xenopus* oocytes expressing Ca^{2+} channel subunits.

METHODS

RNAs specific for the cardiac $\alpha 1C$ and $\beta 2A$ and the skeletal muscle $\alpha 2/\delta$ subunits were synthesized *in vitro* using Asp718-cleaved pCaH, NotI-cleaved pCaB2a and SalI-cleaved pCaA2 as templates (Singer *et al.* 1991; Hullin *et al.* 1992). Capped $\alpha 1$, $\alpha 2/\delta$ and $\beta 2A$ mRNAs were synthesized *in vitro* using Sp6 (for $\alpha 1$ and $\alpha 2/\delta$) and T7 (for $\beta 2A$) RNA polymerases as described in Dascal & Lotan (1992). All materials for molecular biology were obtained from Boehringer-Mannheim.

Xenopus laevis frogs were anaesthetized in a 0·15% (w/v) solution of tricaine methanesulphonate (MS222), portions of ovary were removed through a small incision on the abdomen, the incision was sutured, and the animal was returned to water (Dascal & Lotan, 1992). In each experiment, defolliculated oocytes (Dascal & Lotan, 1992) were injected with equal amounts (by weight) of the mRNAs of the various subunits in the desired combinations (5 ng of each subunit for biochemical experiments and whole-cell current records, and 1 ng for patch clamp). Injected oocytes were incubated at 22 °C in ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM Hepes; pH 7·5) supplemented with 1 mM $CaCl_2$, 2·5 mM sodium pyruvate and 50 μg ml^{-1} gentamicin (termed NDE solution).

Oocytes were injected with mRNAs and incubated in NDE solution, containing 0·5 mCi ml^{-1} [^{35}S]methionine–cysteine (Amersham) for 3–4 days at 22 °C. Plasma membranes together with the vitelline membranes (extracellular collagen-like matrix) were removed manually with fine forceps after a 5–15 min incubation in low osmolarity solution (5 mM NaCl, 1 mM phenylmethanesulphonyl fluoride (PMSF), 1 μM pepstatin, 1 mM 1,10-phenanthroline, 5 mM Hepes–NaOH; pH 7·5. The remainder of the cells, consisting of cytoplasm and intracellular organelles (cell interior, or internal fraction) was processed separately (for experimental details see Ivanina, Perets, Thornhill, Levin, Dascal & Lotan, 1994). Twenty plasma membranes and ten cell interiors were solubilized in 100 μl buffer (4% SDS (w/v), 10 mM EDTA, 50 mM Tris, 1 mM PMSF, 1 mM pepstatin, 1 mM 1,10-phenanthroline; pH 7·5) and heated to 100 °C for 2 min. Following the addition of 100 μl H_2O and 800 μl immunoprecipitation buffer (190 mM NaCl, 6 mM EDTA, 50 mM Tris (pH 7·5), 2·5% (v/v) Triton X-100), homogenates were centrifuged for 10 min at 1000 *g* at 4 °C. The supernatant was incubated for 16 h with the Card-C polyclonal antibody directed against fourteen (residues 2156–2169) of the last sixteen *C*-terminal amino acids of $\alpha 1$ (T. Puri & M. Hosey, unpublished data). The antibody–antigen complex was incubated for 1 h at 4 °C with protein A sepharose and then pelleted by centrifugation for 1 min at 8000 *g*. Immunoprecipitates were washed 3 times with immunowash buffer (150 mM NaCl, 6 mM EDTA, 50 mM Tris–HCl, 0·1% (v/v) Triton X-100, 0·02% (w/v) SDS; pH 7·5). Samples were boiled in SDS gel loading buffer and electrophoresed on 3–8% SDS–polyacrylamide gel together with standard molecular mass markers (45–205 kDa). Gels were dried and placed in a PhosphorImager (Molecular Dynamics, Kemsing, UK) cassette for 1–3 days. The protein bands of the image were estimated quantitatively using the software ImageQuant (Molecular Dynamics). All materials were obtained from Sigma.

Whole-cell currents were recorded using two-electrode voltage clamp as described by Singer *et al.* (1991), in a solution containing 40 mM $Ba(OH)_2$, 50 mM NaOH, 2 mM KOH and 5 mM Hepes, titrated to pH 7·5 with methanesulphonic acid. Single channel recordings were performed in the cell-attached mode using an Axopatch 200 amplifier (Axon Instruments). Pipettes contained 110 mM $BaCl_2$ and 10 mM Hepes–NaOH (pH 7·5). The oocytes were bathed in a solution containing 100 mM KCl, 1 mM $MgCl_2$ and 10 mM Hepes–KOH (pH 7·5). Currents were filtered at 2 kHz (4-pole Bessel), and sampled at 10 kHz. Voltage steps from −80 to 0 mV lasting 140 ms were delivered every 1 s. Leak and capacitative currents were subtracted from the traces using blank sweeps during the analysis session. Data acquisition and analysis were performed using pCLAMP software (Axon Instruments). Averaged data are presented as means ± S.E.M.

RESULTS

Two-electrode recordings from whole oocytes in 40 mM external Ba^{2+} showed, in agreement with earlier findings (Singer *et al.* 1991), that coexpression in the oocytes of $\alpha 1$ with either $\alpha 2/\delta$ or $\beta 2A$ strongly (7·5- to 11-fold at 0 mV) enhanced the whole-cell Ca^{2+} channel currents, compared with $\alpha 1$ alone (see Fig. 2*A*). Coexpression of both $\alpha 2/\delta$ and $\beta 2A$ resulted in a 115-fold increase. To assess the contribution of changes in channel gating to these effects, we have performed recordings of single channel activity of the channel in different subunit combinations and estimated the probability of a single channel to open (P_o) in the absence of any Ca^{2+} channel agonists. To allow a direct comparison between macroscopic and single channel data, in all cases the currents were measured at 0 mV (holding potential was −80 mV). The analysis was done in patches estimated to have one or two channels only. Since P_o was extremely low (see below and Fig. 1*A*), in all cases, after recording a series of 300–700 sweeps, the DHP agonist (−)-Bay K 8644 (1 μM) was added to the bath (this caused augmentation of P_o to 1–6%; see Fig. 1*B*), and the actual number of channels in a patch was estimated from the number of overlapping openings in a series of >300 sweeps.

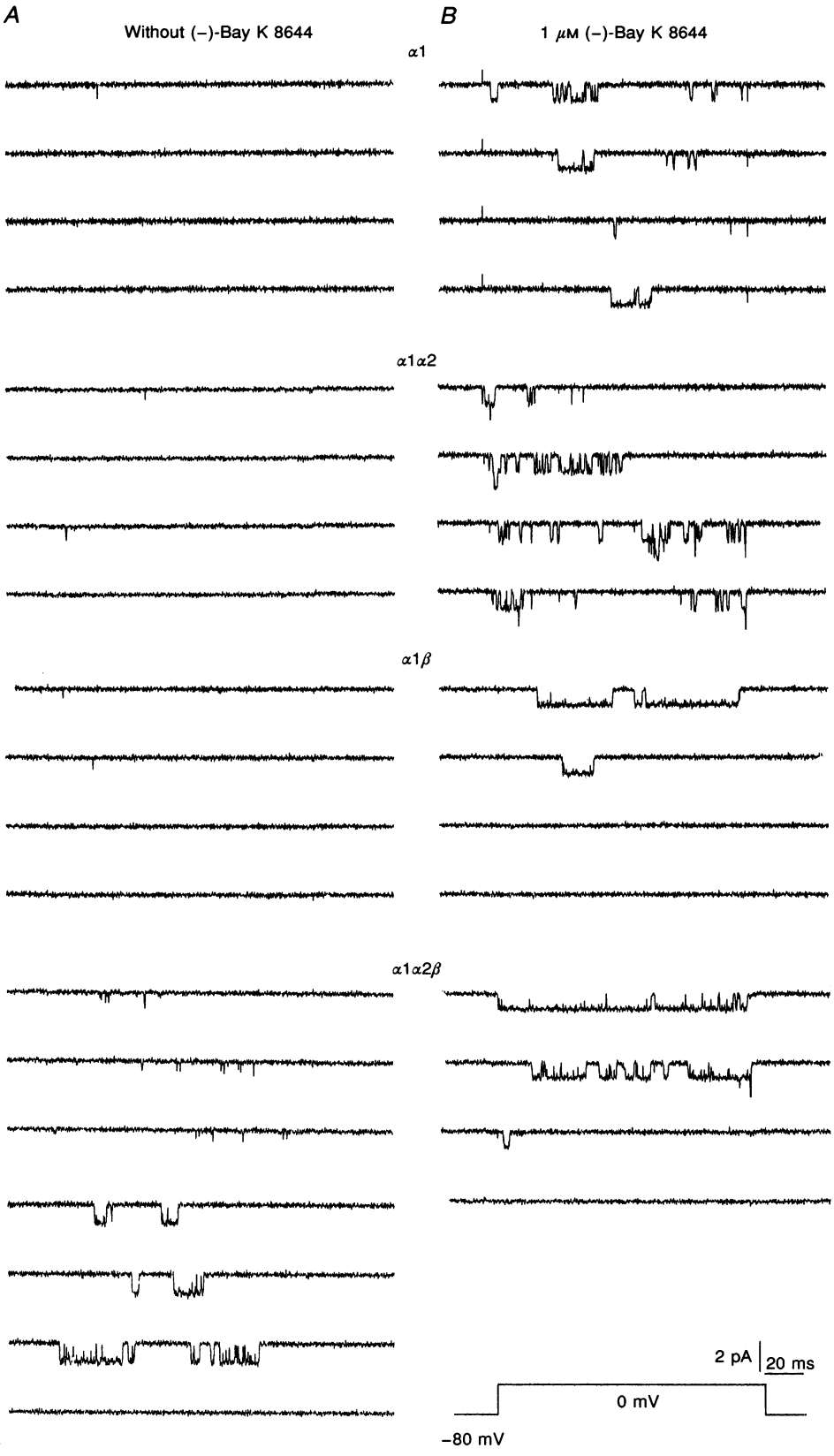

Figure 1

Representative records of activity of channels of different subunit composition (consecutive traces) at 0 mV, in the absence (*A*) and presence (*B*) of 1 μM (−)-Bay K 8644 in the bath.

P_o of a single channel (in the absence of (−)-Bay K 8644) was then calculated based on the number of channels estimated using the above procedure.

Figure 1 shows typical records of unitary Ba^{2+} currents at 0 mV via single channels of different subunit composition, in the absence (Fig. 1*A*) and presence (Fig. 1*B*) of 1 μM (−)-Bay K 8644. The channels composed of α1 alone showed extremely rare short openings; 97% of traces were empty. The pattern of rare brief openings, termed mode 1 (Hess, Lansman & Tsien, 1984), predominated in all subunit combinations. Mode 2, characterized by long openings (2–9 ms) was rare in the activity of the channel composed of α1 alone and α1 + α2/δ or α1 + β2A, accounting for 1·5–2·5% of the observed openings. In the case of the 'full' channel composition (α1 + α2/δ + β), long openings were observed more frequently and, in one of four patches, bursts of openings appeared comprising 13% of the active sweeps (Fig. 1*A*).

Figure 2*B* shows the mean P_o values at 0 mV in channels of different subunit composition in the absence of (−)-Bay K 8644. Channels composed of α1 alone showed the lowest P_o and short openings with a mean open time (t_o) in mode 1 of 0·3 ± 0·01 ms (Fig. 2*B*, inset). (The values of t_o should be regarded only as an indicator, since the 2 kHz analog filtering might distort the shortest openings.) Coexpression of α2/δ caused a 3·4-fold increase in P_o without changing the t_o in mode 1. Coexpression of β2A increased the P_o 8-fold, while t_o in mode 1 was prolonged by about 80%. The effect of β2A on t_o of mode 1 was independent of α2/δ (Fig. 2*B*, inset). Coexpression of both α2/δ and β with α1 resulted in a dramatic increase in P_o, about 35-fold in comparison with α1 alone. This is a synergistic (more than additive) effect. Since t_o in mode 1 in the full subunit composition was only 52% higher than in channels composed of α1 alone, the increase in P_o was mainly due to a shift of channel activity to the active gating modes. Preliminary analysis showed that α2/δ increased the number of active sweeps; coexpression of β2A both decreased the fraction of null sweeps and increased the fraction of modes 1 and 2 (data not shown; see Neely *et al.* 1995). Figure 2*C* shows that none of the

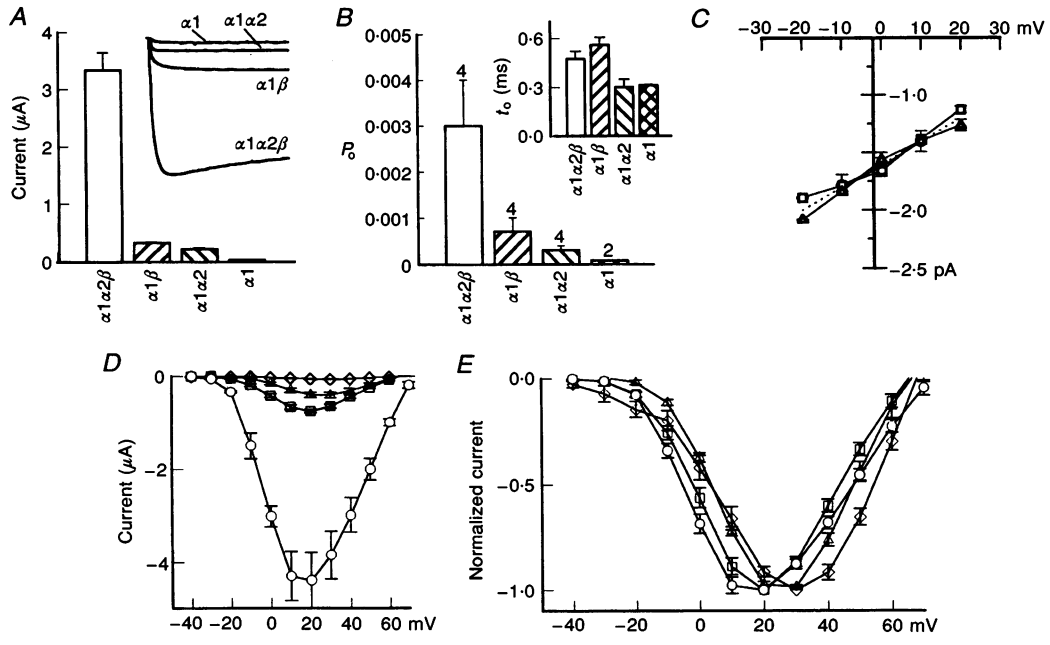

Figure 2. Analysis of whole-cell and single channel Ba^{2+} currents via the Ca^{2+} channels of different subunit compositions

A, mean amplitudes ± S.E.M. of whole-cell Ba^{2+} currents from 12–20 cells measured in this series of experiments. The inset shows representative macroscopic currents in 40 mM external Ba^{2+} measured with voltage steps from −80 to 0 mV. *B*, P_o at 0 mV in the absence of (−)-Bay K 8644. The inset shows the mean open time in mode 1 (t_o) in the same patches as P_o. The numbers above the bars indicate the number of patches. *C*, single channel current–voltage relations (○, α1 + α2/δ + β2A; □, α1 + β2A; △, α1 + α2/δ). The single channel amplitudes were measured for long openings (> 2 ms) in the presence of (−)-Bay K 8644. The amplitudes of currents via single channels composed of α1 alone were measured only at 0 and 10 mV and were identical to those in all other subunit combinations (not shown). *D* and *E*, representative whole-cell current–voltage relations from 3–5 oocytes of the same donor for each subunit combination (○, α1 + α2/δ + β2A; □, α1 + β2A; △, α1 + α2/δ; ◇, α1): *D*, row data; *E*, same data normalized to the maximal amplitude to facilitate the comparison between the different subunit combinations.

subunits altered the single channel conductance, which in our recording conditions was 19 pS.

As reported previously (e.g. Lacerda *et al.* 1991; Singer *et al.* 1991), β2A, but not $\alpha 2/\delta$, shifted the macroscopic current–voltage relationship to negative potentials by about 10 mV (Fig. 2*D* and *E*). However, the stronger increase in P_o at 0 mV caused by β2A compared with $\alpha 2/\delta$ was probably not due to this shift, because comparison of P_o of channels composed of $\alpha 1 + \alpha 2/\delta$ at +10 mV with P_o of channels composed of $\alpha 1 + \alpha 2/\delta + \beta$2A at 0 mV, gave similar results (data not shown).

In order to assess the effect of the auxiliary subunits on expression of the $\alpha 1$ subunit, we compared the amount of synthesized $\alpha 1$ subunit alone and with coexpressed $\alpha 2/\delta$ and/or β2A subunits, in oocytes labelled *in vivo* by incubation in medium containing [^{35}S]methionine–cysteine. The channels were isolated separately from manually dissected plasma membranes and from the rest of the cell (cell interior) by detergent solubilization, followed by immunoprecipitation with the Card-C antibody and analysis by SDS–polyacrylamide gel electrophoresis. The amount of expressed $\alpha 1$ subunit was quantified by measuring the relative intensity of $\alpha 1$ bands on SDS gels (Ivanina *et al.* 1994).

Autoradiograms of typical gels are shown in Fig. 3. In all subunit combinations, the $\alpha 1$ subunit was detected both in the plasma membranes and in the internal fraction (presumably internal membranes) as a diffuse band migrating above 205 kDa, somewhat larger than reported for $\alpha 1$ purified from heart (Chang & Hosey, 1988); a smear labelling was also observed at higher apparent molecular weight (~260 kDa). No such bands were detected in native (not injected with RNA) oocytes. The calculated molecular weight of the $\alpha 1$ subunit is 243 kDa (Mikami *et al.* 1989). If a *C*-terminally truncated channel was also expressed in the oocytes, as has been reported for skeletal muscle $\alpha 1$ (De Jongh, Warner, Colvin & Catterall, 1991), and $\alpha 1$C expressed in CHO cells (Yoshida, Takahashi, Nishimura, Takeshima & Kokubun, 1992), it would not be detected in our experiments. Independent evidence that the 205 kDa band represents a full-length $\alpha 1$ polypeptide comes from comparison with its expression in insect SF9 cells using recombinant baculovirus (T. Puri & M. Hosey, unpublished data). On SDS gels, the baculovirus-expressed $\alpha 1$ subunit migrated and was of a similar size to that expressed in oocytes. The baculovirus-expressed protein has been shown to be a full-length $\alpha 1$ as it was recognized by: (1) the Card-C antibody, demonstrating that it had an intact *C*-terminus; (2) a monoclonal antibody recognizing an epitope tag that was engineered into the *N*-terminus, and (3) an antibody against an internal domain (T. Puri & M. Hosey, unpublished data).

To compare data obtained in different oocyte batches, the intensities of the 205 kDa bands in each subunit combination were normalized with respect to the band having the maximal intensity (usually the full subunit composition). The results of the experiments (4 oocyte batches for plasma membrane and 5 for cell interior) are summarized in Fig. 4. In the plasma membrane, coexpression of the $\alpha 2/\delta$ subunit increased the amount of $\alpha 1$ about 3-fold compared with $\alpha 1$ alone, whereas the β2A subunit did not change the level of $\alpha 1$. Coexpression of both $\alpha 2/\delta$ and β2A with $\alpha 1$ increased the amount of $\alpha 1$ in the

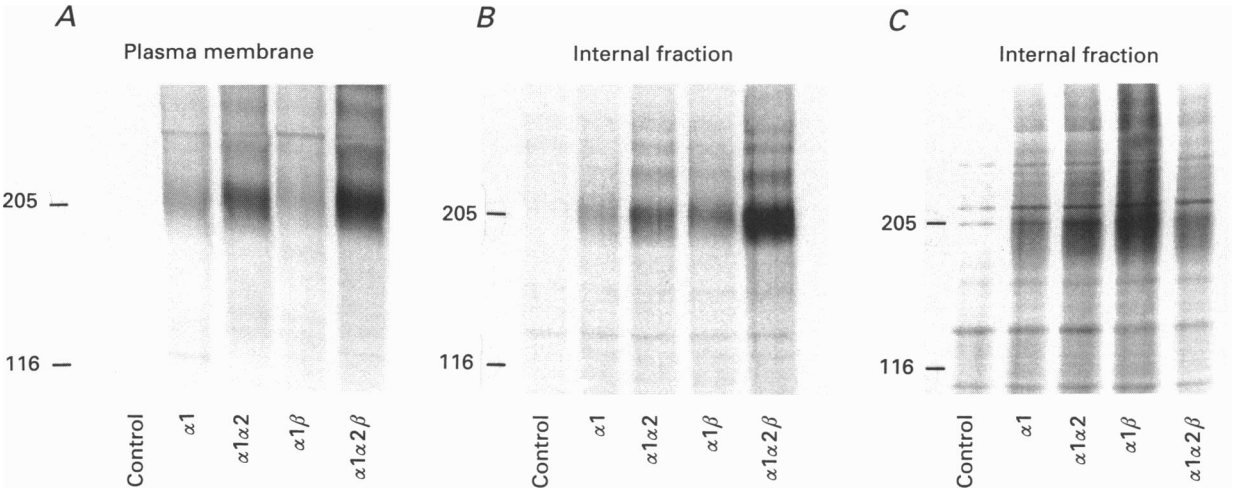

Figure 3

SDS–polyacrylamide gel electrophoresis analysis of the $\alpha 1$ protein immunoprecipitated from oocyte plasma membranes (*A*) and internal fractions (*B* and *C*; the two examples represent two separate experiments performed on different batches of oocytes). In each lane, immunoprecipitates from 20 (plasma membranes) or 10 (internal fraction) oocytes were loaded. The lane termed 'Control' represents immunoprecipitates from native oocytes which have not been injected with RNA.

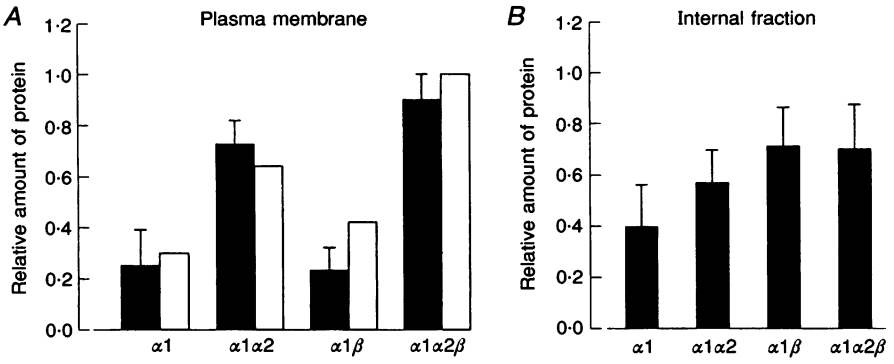

Figure 4

Summary of the effects of $\alpha 2$ and β subunits on the relative amount of $\alpha 1$ protein in the plasma membrane (*A*; 4 oocyte batches) and cell interior (*B*; 5 oocyte batches). Band intensities were measured using PhosphorImager and normalized as explained in the text and presented as means ± S.E.M. (filled bars). The open bars represent the relative amounts of functional channels calculated from the electrophysiological data.

plasma membrane 3·6-fold, as with $\alpha 2/\delta$ alone (Figs 3*A* and 4*A*). Statistical analysis (one-way ANOVA test followed by paired comparison of groups using the Bonferroni *t* test method) showed that the increase in the $\alpha 1$ level in plasma membrane caused by $\alpha 2/\delta$ was significant ($P = 0{\cdot}001$), whereas the 'addition' of $\beta 2$A either with or without $\alpha 2/\delta$ did not change the level of $\alpha 1$ ($P > 0{\cdot}05$).

In the internal fraction the variability among oocyte batches was more pronounced than in plasma membrane; therefore two examples of internal fraction radiograms are shown (Fig. 3*B* and *C*). The level of $\alpha 1$ in the internal fraction was approximately equal in all subunit combinations, except when $\alpha 1$ was expressed alone, when its amount seemed to be somewhat lower (Fig. 4*B*), but this was not statistically significant (one-way ANOVA; $P = 0{\cdot}473$).

The total amount of $\alpha 1$ expressed in an oocyte was estimated by summarizing the absolute intensities of the $\alpha 1$ subunit in plasma membrane and cell interior. By this analysis, the expression of the $\alpha 1$ subunit alone in the oocyte was about 2-fold lower than when coexpressed with one or both of the auxiliary subunits (data not shown). We have also compared the relative amounts of functional $\alpha 1$ subunits in the plasma membrane predicted from the electrophysiological analysis, with the amounts of $\alpha 1$ protein detected in the plasma membrane by the immunological analysis (Fig. 4*A*). Since

$$I = iNP_o,$$

where I is the whole-cell current, i is the single channel current and N is the number of functional channels, and since i does not depend on the presence of the auxiliary subunits (Fig. 2*C*), the values of N for different subunit combinations are related as I/P_o. The calculated relative values of N (open bars in Fig. 4*A*) are in good agreement (within a factor of 1·7 or better) with the observed relative amounts of the $\alpha 1$ subunit in the plasma membrane, implying that most of the $\alpha 1$ protein detected in the plasma membrane by the Card-C antibody is functional (or, if there is a pool of functional channels in plasma membrane, its proportion is not significantly changed by the auxiliary subunits).

DISCUSSION

Auxiliary subunits dramatically modulate Ca^{2+} channel properties; the underlying mechanisms are still poorly understood. The novel findings of this study concern the ubiquitous $\alpha 2/\delta$ subunit (which, unlike β, has been somewhat neglected and its role less well studied) and the mechanism of synergy between $\alpha 2/\delta$ and β. We report that $\alpha 2/\delta$ substantially elevates (triples) the amount of $\alpha 1$ protein in the plasma membrane of *Xenopus* oocytes. Candidate mechanisms are an enhanced translocation of $\alpha 1$ to the plasma membrane, or an improved stability of $\alpha 1$. It will be of interest to distinguish between these possibilities in future experiments. The increase in the amount of $\alpha 1$ in the plasma membrane probably explains part of the enhancement of whole-cell current caused by $\alpha 2/\delta$. An additional proportion of the enhancement is certainly due to the ~3-fold rise in P_o suggesting that $\alpha 2/\delta$ alters the gating of the channel. The combination of $\alpha 2/\delta$ effects on gating (× 3) and on the amount of $\alpha 1$ in the plasma membrane (× 3) would be expected to increase the whole-cell current by a factor of 9, which is in good agreement with the observed ~7·5-fold enhancement.

The effects of $\beta 2$A are different from those of $\alpha 2/\delta$. Our results show a modest (2-fold) increase in the total cellular

$\alpha 1$ by coexpression of $\beta 2A$; this is consistent with the lower estimates of the increase in DHP binding sites, caused by coexpression of the β-subunit with $\alpha 1$ in transfected mammalian cells (Lacerda *et al.* 1991; Varadi *et al.* 1991; Nishimura *et al.* 1993). However, this increase, at least in *Xenopus* oocytes, appears to be functionally irrelevant since, as our data show, $\beta 2A$ does not alter the amount of the functionally important Ca^{2+} channels (those located in the plasma membrane). In contrast, $\beta 2A$ strongly alters the gating of the channel, as reflected in an ~8-fold increase in P_o. This change alone may almost fully explain the observed increase in the macroscopic current (~11-fold); the remaining discrepancy is probably within the limits of inherent experimental inaccuracies of the measurements. The increase in P_o found here corroborates the results of Wakamori *et al.* (1993) and Neely *et al.* (1995), although quantitatively our estimate is higher than that reported by Wakamori *et al.* (1993; ~2-fold). Our conclusion that the enhancement of the whole-cell current by $\beta 2A$ is entirely due to changes in channel gating rather than to a change in its amount is in full agreement with that of Neely *et al.* (1993) achieved by an independent method (comparison of gating currents in channels composed of $\alpha 1$ or $\alpha 1 + \beta 2A$).

The effects of the β subunit may vary with type of expression system used. Thus, in a mammalian cell line (HEK 293), coexpression of $\beta 2A$ significantly increases the amount of $\alpha 1$ in the membrane fraction (Sun *et al.* 1994; A. Chien, X. L. Zhao & M. Hosey, unpublished results); an increase in total gating charge is observed upon coexpression of β with $\alpha 1$ in these cells (Kemp, Perez-Garcia & Marban, 1995). The oocyte may be missing the machinery necessary for the translocation of β to the membrane, or the translocation of $\alpha 1$ (without β) to the plasma membrane may be more efficient in the oocytes than in some mammalian cell lines. In contrast, changes in Ca^{2+} channel gating by the β-subunit are universal and occur in all expression systems used (Hofmann *et al.* 1994), although it is not known whether the changes in kinetic parameters are the same in oocytes and other cell types.

The synergistic effect of $\alpha 2/\delta$ and β on the whole-cell Ca^{2+} channel current cannot be due to an enrichment of $\alpha 1$ in the plasma membrane, since it is practically the same as with $\alpha 2/\delta$ alone. Therefore, the synergy is entirely due to changes in gating effected by the two subunits in concert, as reflected by the 35-fold increase in P_o. This increase, together with the 3·6-fold increase in the amount of the $\alpha 1$ protein in the plasma membrane, predicts a total ~125-fold increase in the macroscopic current amplitude, in good agreement with the observed ~115-fold enhancement.

To conclude, the presented results demonstrate that the increase in macroscopic current by $\alpha 2/\delta$ is due equally to changes in the amount of $\alpha 1$ in the plasma membrane and to an increase in P_o, whereas all of the effect of β is due to an increase in P_o. The synergistic (more than additive) enhancement of the macroscopic current by simultaneous coexpression of $\alpha 2/\delta$ and β is due to altered channel gating. Elucidation of the exact molecular mechanism by which each of the auxiliary subunits changes the gating of Ca^{2+} channels, and to the mechanism of $\alpha 2/\delta$ and β synergism, remains a challenge for the future.

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Acknowledgements

We are indebted to F. Hofmann and V. Flockerzi for supplying the cDNAs of the Ca^{2+} channel subunits. This study has been supported partially by grants from the Muscular Dystrophy Association and from the Israel Academy of Sciences and Humanities (N.D.), and by a National Institutes of Health grant (HL 23306) to M.H.

Received 20 July 1995; accepted 6 September 1995.