# βγ subunits of GTP-binding proteins inhibit muscarinic receptor stimulation of phospholipase C

### (*Xenopus* oocyte/inositol trisphosphate/chloride current)

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**ABSTRACT** This study examines the mechanism of guanine nucleotide-binding protein (G protein) coupling of receptors to phospholipase C. The *Xenopus* oocyte has a muscarinic receptor-activated $Cl^-$ current that is mediated by inositol 1,4,5-trisphosphate. Modulation of the muscarinic receptor-evoked $Cl^-$ current was examined under voltage clamp in oocytes injected with resolved G-protein subunits. The presence of an $\alpha$ subunit of G proteins in oocytes was shown by pertussis toxin-labeling of a 41-kDa band in oocyte membranes. The presence of the $\beta$ subunit of G proteins was demonstrated by immunoblotting experiments with an antiserum (U-49) that is specific for the $\beta$ subunit. Pertussis toxin treatment of oocytes resulted in the uncoupling of muscarinic receptors from activation of the $Cl^-$ current. Cells microinjected with 1.5 ng of human erythrocyte $\beta\gamma$-subunit complex or 1.0 ng of bovine brain $\beta\gamma$-subunit complex showed approximately a 95% reduction in the evoked $Cl^-$ current. Cells injected with equal volumes of protein storage vehicle showed no change in response. Cells injected with boiled $\beta\gamma$ subunits, bovine serum albumin, or resolved $\alpha$ subunits also showed no reduction in response. Cells injected with various concentrations of $\beta\gamma$ subunits showed a concentration dependence with half-maximal inhibition of the muscarinic activated $Cl^-$ current at about 10 nM. Cells injected with 1.0 ng of bovine brain $\beta\gamma$ subunits could not respond to bath-applied agonist but could generate the $Cl^-$ current on intracellular injection of inositol 1,4,5-trisphosphate. These observations suggest that there is a G protein responsible for muscarinic receptor-mediated signal transduction through phospholipase C and that it is an $\alpha\beta\gamma$ heterotrimer. It appears that the mode of action of the G protein in the phospholipase C system may be similar to that of the hormone-activated adenylyl cyclase.

Receptor-mediated stimulation of phosphatidylinositol 4,5-bisphosphate breakdown to inositol 1,4,5-trisphosphate (Ins$P_3$) is thought to be mediated through guanine nucleotide-binding proteins (G proteins) (1–3). Evidence that supports this line of reasoning includes: (*i*) the GTP requirement of coupling of receptors to phospholipase C, (*ii*) the stimulatory effects of nonhydrolyzable analogs of GTP on Ins$P_3$ production, and (*iii*) the guanine nucleotide sensitivity of agonist interactions with receptors known to stimulate Ins$P_3$ production. However the lack of reports on successful reconstitution of purified G proteins with the various purified phospholipase C and the lack of a reproducible reconstitution of receptor coupling to Ins$P_3$ production by purified G proteins are not consistent with the hypotheses of G-protein involvement. Hence, we reasoned that studies examining the role of

G-protein subunits in signal transduction through phospholipase C would be informative.

The *Xenopus* oocyte has muscarinic acetylcholine receptors (4–6) that stimulate Ins$P_3$ production (7) and activate $Cl^-$ channels (5–9). Intracellular injection of Ins$P_3$ mimics the muscarinic activation of the $Cl^-$ conductance (8), and both the muscarinic and Ins$P_3$-mediated effects are dependent upon the increase of intracellular $Ca^{2+}$ (8). Our present understanding of the pathway is: muscarinic receptor occupancy increases cellular Ins$P_3$ levels, and this in turn increases cellular $Ca^{2+}$ concentration, which results in opening of the $Cl^-$ channels. It has been shown that the magnitude of the $Cl^-$ current is proportional to the intracellular concentration of Ins$P_3$ (8). Thus, measurement of the $Cl^-$ current can be used to monitor Ins$P_3$ production and phospholipase C activity.

Studies on both the hormone-stimulated adenylyl cyclase and the light-sensitive cGMP phosphodiesterase have shown that the $\alpha$ subunits of G proteins, which have the guanine nucleotide-binding site, interact with the effector system and modulate its activity. The $\beta\gamma$-subunit complex, which consists of a 35- or 36-kDa $\beta$ subunit and the 5- to 8-kDa $\gamma$ subunit, inhibits signal transduction by associating with the $\alpha$ subunit (1). We reasoned that if the coupling mechanisms of the inositol phospholipid breakdown system are similar to that in the adenylyl cyclase system, then injection of excess $\beta\gamma$ subunits should inhibit muscarinic stimulation of Ins$P_3$ production and hence the chloride current. In this report we demonstrate that intracellular injection of $\beta\gamma$ subunits of G proteins into *Xenopus* oocytes results in attenuation of the muscarinic stimulation of the $Cl^-$ current and that this blockade is prior to Ins$P_3$ production.

## MATERIALS AND METHODS

**Materials.** Mature female *Xenopus laevis* were obtained from NASCO (Fort Atkinson, WI). [$^{32}$P]NAD$^+$ was synthesized by the procedure of Cassel and Pfeuffer (10). Antiserum U-49 was the gift of S. Mumby and A. Gilman (University of Texas Southwestern Medical Center, Dallas). Ins$P_3$ was purchased from Boehringer Mannheim, and pertussis toxin (PTX) was purchased from List Biological Laboratories (Campbell, CA). Fresh bovine brains were obtained from a local slaughterhouse. Sources of all other materials have been described (11).

**Oocyte Preparation.** Oocytes were surgically removed from mature *Xenopus laevis* under tricaine anesthesia. Oocytes were separated either manually with microforceps

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Abbreviations: Ins$P_3$, D-*myo*-inositol 1,4,5-trisphosphate; PTX, pertussis toxin; G protein, guanine nucleotide-binding protein; $G_i$, guanine nucleotide-inhibitory factor; $G_s$, guanine nucleotide-stimulatory factor; $G_o$, guanine nucleotide-binding regulatory factor.
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or by treatment with collagenase [agitation for 2 hr in CaCl$_2$-free medium containing 82.5 mM NaCl, 2.0 mM KCl, 1.0 mM MgCl$_2$, 5.0 mM Hepes, 2.5 mM sodium pyruvate, 100 units of penicillin per ml, 100 $\mu$g of streptomycin per ml, and 2 mg of collagenase (Sigma type 1A) per ml]. Cells were maintained at 20°C for 3–4 days in frog Ringer's solution (ND96) (96.0 mM NaCl/2.0 mM KCl/1.8 mM CaCl$_2$/5.0 mM Hepes) supplemented with 2.5 mM sodium pyruvate, 100 units of penicillin per ml, and 100 $\mu$g of streptomycin per ml. All solutions were at pH 7.5 ± 0.2.

**Intracellular Injections.** Resolved G-protein subunits, bovine serum albumin (Armour, Kankakee, IL), or buffer were injected into oocytes by using a Drummond microinjector prior to voltage clamping. All cells were injected in the animal pole. Ins$P_3$ was injected into cells with a Picospritzer pressure injection system while recording from the cell under voltage clamp as described (8).

**Electrophysiological Measurements.** Oocytes were assayed 1–4 days postoperatively by using the two-electrode voltage-clamp technique. Individual oocytes were placed in a 0.5-ml bath constantly perfused with ND96 at room temperature. The oocyte was voltage-clamped between −50 and −70 mV with 3 M KCl microelectrodes (0.5–2.0 M$\Omega$). Acetylcholine was applied by superfusion. T$_{out}$, a Cl$^-$ conductance that is activated by a voltage-gated Ca$^{2+}$ channel (11), was evoked by a microcomputer-controlled voltage step protocol. Cells were injected with the Drummond microinjector in groups of at least five and allowed to recover for 5–20 min. The cells were then assayed at a rate of about 1 every 5 min by the following protocol. The cell was voltage clamped for 1 min, the resting potential and input resistance were recorded, and T$_{out}$ was evoked and recorded. After a 3-min pause, acetylcholine was applied. Data are reported as the mean peak amplitude of induced Cl$^-$ current ± SEM.
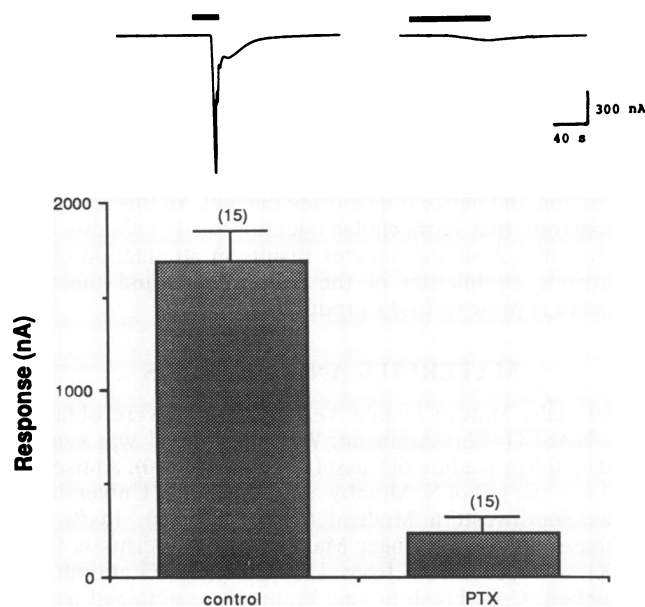
**G-Protein Subunits.** G proteins were purified from human erythrocytes or bovine brain as described (12). $\beta\gamma$ subunits were obtained during the second DEAE-Sephacel purification. The $\beta\gamma$ subunits were concentrated to 50 $\mu$g/ml and stored at −70°C. $\alpha$ subunits of brain G proteins consisting mainly of guanine nucleotide-inhibitory protein (G$_i$) and guanine nucleotide-binding regulatory protein (G$_o$) (15–20%) were obtained by heptylamine-Sepharose chromatography of G protein that had been treated with 10 mM NaF/ 10 $\mu$M AlCl$_3$/25 mM MgCl$_2$ for 1 hr at room temperature (22–24°C). The $\alpha$-subunit peak on the heptylamine-Sepharose was identified by Coomassie blue staining of sodium dodecyl sulfate/polyacrylamide gels. The samples containing the protein were pooled and loaded on to a DEAE-Toyopearl column (Pierce), washed free of NaF, MgCl$_2$, and AlCl$_3$, and eluted with 200 mM NaCl. The $\alpha$ subunits were concentrated to a final concentration of 100 $\mu$g/ml. The proteins were stored in 10 mM sodium Hepes/1 mM EDTA/20 mM 2-mercaptoethanol/30% ethylene glycol/0.1% purified Lubrol-Px at pH 8.0.

**Analysis of G-Protein Subunits of *Xenopus* Oocytes.** Oocyte membranes were prepared by the procedure of Kobilka *et al.* (13). PTX labeling and immunoblotting analysis of the $\beta$ subunit of the *Xenopus* oocyte were performed as described (14). Sodium dodecyl sulfate/polyacrylamide gel electrophoresis was performed by the procedure of Laemmli (15). Proteins were measured by the fluorescamine method (12).
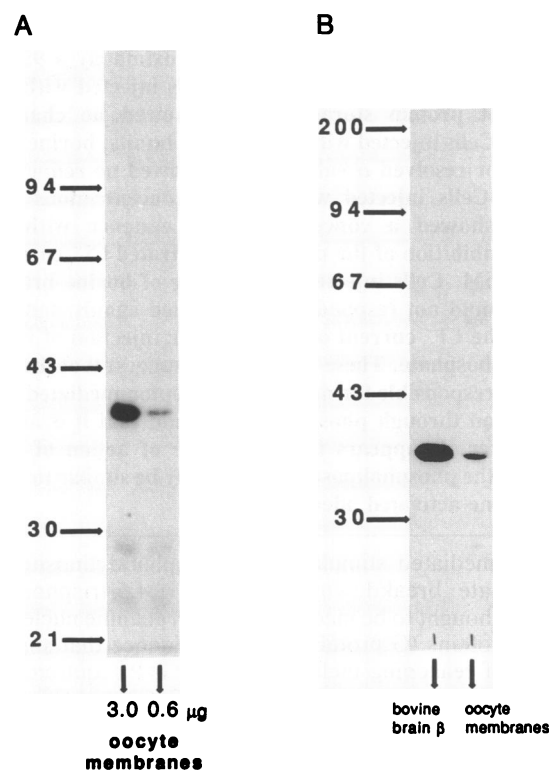


FIG. 1. (*Lower*) Effect of PTX treatment on the muscarinic receptor-evoked Cl$^-$ current in *Xenopus* oocytes. Fifteen cells were treated with toxin (2 $\mu$g/ml) for 26 hr and then examined under voltage clamp for responsiveness to acetylcholine (1 $\mu$M). The bar graph shows the mean ± SEM of the amplitude of the acetylcholine-stimulated Cl$^-$ current in nA. (*Upper*) Characteristic waveform of the acetylcholine-evoked Cl$^-$ current in a control cell (*Upper Left*) compared to a representative response in a toxin-treated cell (*Upper Right*). Currents were recorded with a two-electrode voltage clamp at a holding potential of −70 mV. The bar above the current trace indicates the duration of acetylcholine application.



FIG. 2. (*A*) PTX-catalyzed ADP-ribosylation of *Xenopus* oocyte membranes. (*B*) Immunoblotting analysis of oocyte membranes with antiserum U-49 specific for the $\beta_{36}$ subunit of G proteins. Indicated concentrations of oocyte membranes were incubated with activated PTX and [$^{32}$P]NAD$^+$ (10$^7$ cpm; 1 $\mu$M) in the presence of other additives as described (14). Immunoblotting with U-49 antiserum (1: 10,000 dilution) was performed as described (14); 250 ng of purified bovine brain $\beta\gamma$ dimer and 50 $\mu$g of oocyte membranes were used. For detailed methodologies, see ref. 14. The PTX labeling autoradiogram is on Kodak XAR-5 film exposed for 48 hr. The immunoblotting autoradiogram is on DuPont Cronex film exposed for 48 hr.

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## RESULTS

Acetylcholine (1 $\mu$M) elicited an inward Cl$^-$ current in almost all oocytes (Fig. 1 *Upper*) that was similar to the well-known Ins$P_3$-mediated muscarinic cholinergic response of the oocyte (4, 5, 7–9). Most oocytes were harvested from a frog known to yield cells that have a collagenase-insensitive muscarinic response. This was done to avoid the complications of using follicle-enclosed oocytes and to facilitate the injection of proteins and Ins$P_3$ into the cells (8). Some experiments were performed in follicle-enclosed cells. Each frog gave oocytes with a distinct mean amplitude. The range of responses across all cells was 500–3000 nA.

One of the *Bordatella pertussis* toxins is known to ADP-ribosylate certain G proteins, thereby uncoupling receptors from G proteins and thus from their effector systems (1). By treating one group of cells with PTX and comparing it to a nontreated control group, it was shown that the acetylcholine-stimulated Cl$^-$ current is sensitive to PTX treatment (Fig. 1 *Lower*). The toxin-treated group had a mean response of 237 ± 82 nA ($n$ = 15), whereas the control group mean response was 1683 ± 163 nA ($n$ = 15). This result suggested that the acetylcholine response in the native oocyte was probably mediated by a PTX-sensitive G protein.

We then analyzed the oocyte membranes for the presence of G-protein subunits. Incubation of oocyte membranes with [$^{32}$P]NAD$^+$ and activated PTX resulted in the labeling of a 41-kDa protein (Fig. 2A) as reported (16). The size of this protein is indicative of the $\alpha$ subunit of the G$_i$ family of proteins (1). U-49 is an antiserum that was raised against a synthetic peptide encoding a sequence from the $\beta_{36}$ subunit of G proteins (17). Immunoblotting experiments using the U-49 antiserum showed that the oocyte membrane contains the $\beta_{36}$ subunit (Fig. 2B).

These data indicate that the native *Xenopus* oocyte contains both the $\alpha$ and $\beta$ subunits of G proteins and that the muscarinic receptor is coupled to phospholipase C through a PTX-sensitive substrate. The amount of Ins$P_3$ produced is measurable as the amplitude of receptor-activated Cl$^-$ current (7, 8). Hence, we used this system for analyzing the role of G-protein subunits in modulating signal transduction through phospholipase C.

First we tested the effect of $\beta\gamma$ subunits on the acetylcholine-elicited Cl$^-$ current. One group of cells was injected intracellularly with 1.5 ng of the human erythrocyte $\beta\gamma$ dimer per cell in a 40-nl volume. (The oocyte has an approximate volume of 1.0 $\mu$l.) These cells were then analyzed under voltage clamp for responsiveness to acetylcholine. Each cell's resting membrane potential and input resistance were also recorded. These results were compared to a control noninjected group of cells. Fig. 3A shows that the erythrocyte $\beta\gamma$ dimer reduces the acetylcholine-elicited Cl$^-$ current by
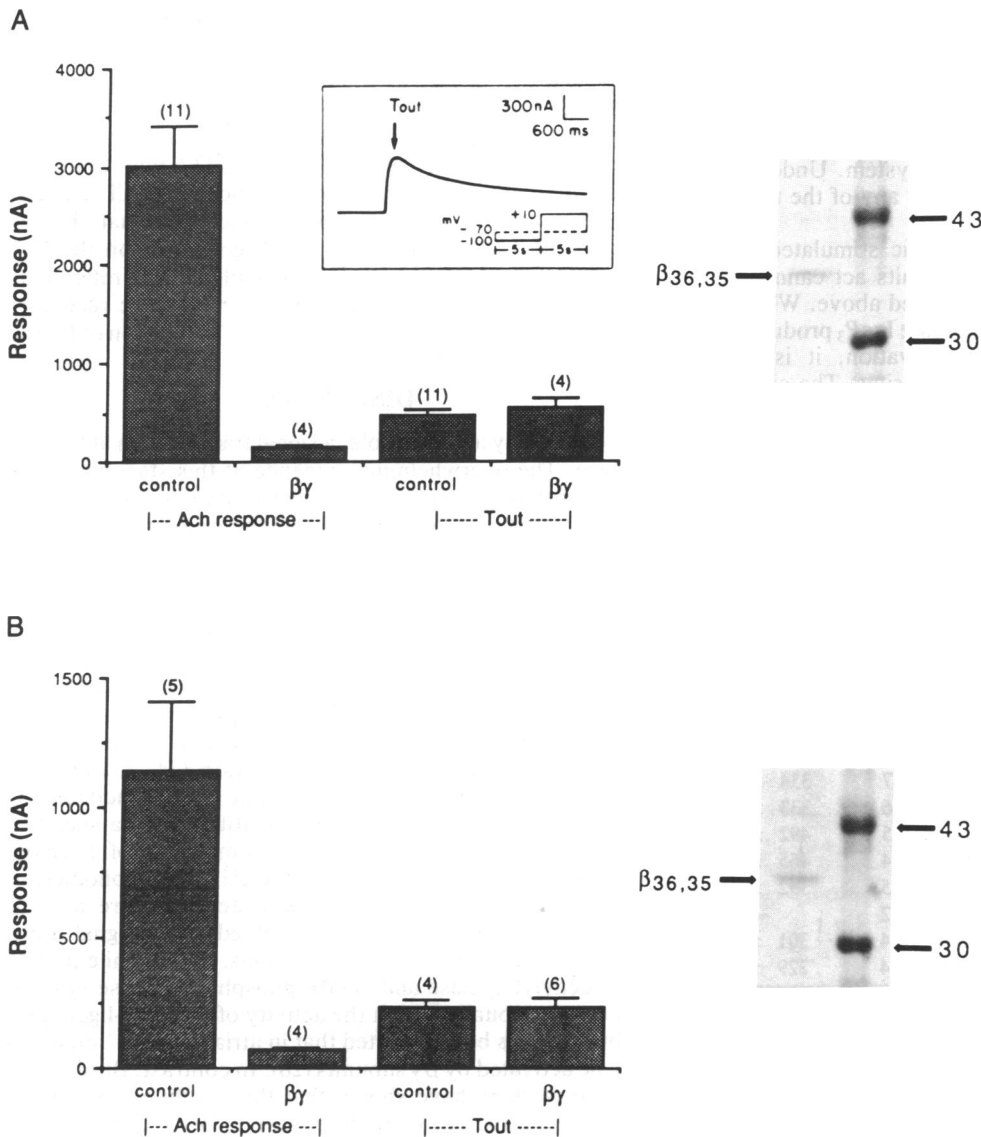


FIG. 3. Effect of intracellular injection of human erythrocyte $\beta\gamma$ dimer at 1.5 ng per cell (*A*) and bovine brain $\beta\gamma$ dimer at 1.0 ng per cell (*B*) on the muscarinic receptor-evoked Cl$^-$ current in *Xenopus* oocytes. (*A Left*) Each column of the bar graph represents the mean ± SEM amplitude of the activated current for a group of cells (the number of cells is indicated above each column). The two columns at the left compare noninjected control cells to cells injected with 1.5 ng (in a fixed volume of 40 nl) of human erythrocyte $\beta\gamma$ subunits. The two columns on the right compare the voltage-activated T$_{out}$ currents of the same cells. (*Inset*) Representative T$_{out}$ current trace and the voltage-step protocol used to elicit the T$_{out}$. A cell is voltage-clamped at a holding potential of −70 mV. When it is prepulsed to −100 mV for 5 s and then "stepped" rapidly to +10 mV, the T$_{out}$ Cl$^-$ current will result. $\beta\gamma$ subunits inhibit the muscarinic response by about 95% but do not affect the T$_{out}$ Cl$^-$ current in the same cell. (*A Right*) Coomassie blue staining profile of the $\beta\gamma$ subunits used in the experiment; 0.5 $\mu$g of $\beta\gamma$ subunits was loaded onto the gel and electrophoresed, fixed, stained, and destained. (*Lower*) Experiments are the same as in *Upper* except that bovine brain $\beta\gamma$ subunits were used at 1.0 ng of dimer per cell. ACh, acetylcholine.

about 95%. The injected cells did not show a difference in resting potential ($47.3 \pm 1.5$ mV) and input resistance ($0.43 \pm 0.1$ M$\Omega$) as compared to noninjected controls (resting potential = $49.5 \pm 1.5$ mV and input resistance = $0.73 \pm 0.2$ M$\Omega$). This experiment was repeated with 1.0 ng of bovine brain $\beta\gamma$ dimer per cell in a 40-nl volume (Fig. 3B). The brain $\beta\gamma$ dimer caused an $\approx$94% reduction in the receptor-activated Cl$^-$ current. These injected cells also did not show a change in resting potential and input resistance.

The native oocyte has a voltage-dependent Cl$^-$ conductance known as $T_{out}$ (11). This current is elicited by prepulsing the cell from the resting potential to $-100$ mV for 5 s and then jumping the potential to $+10$ mV for 5 s. Presently there is no evidence that $T_{out}$ is mediated by a G protein. Therefore, we used $T_{out}$ as a same-cell control for the specificity of the injected $\beta\gamma$ subunits. Fig. 3 shows that the injected $\beta\gamma$ had no effect on $T_{out}$. This suggests that the injected proteins specifically affected only the receptor-mediated Cl$^-$ current.

The specificity of the material injected into the cells was examined (Table 1). Injection of the $\beta\gamma$ storage vehicle did not have an effect on the acetylcholine response or $T_{out}$. Injection of bovine serum albumin (1.0 ng per cell), boiled $\beta\gamma$ subunits (1.0 ng per cell), or resolved $\alpha$ subunits (1.0 ng per cell), each in 40 nl of buffer, did not inhibit the muscarinic stimulated Cl$^-$ current. We also tested the effect of various concentrations of $\beta\gamma$ subunits injected into oocytes in a fixed volume (Fig. 4). It was found that the $\beta\gamma$ subunits half-maximally inhibited the muscarinic stimulated Cl$^-$ current at about 0.4 ng per cell. At a cell volume of 1.0 $\mu$l, this is approximately equal to 10 nM. This is consistent with the findings of Cerione *et al.* (18), who showed $\beta\gamma$ inhibition of guanine nucleotide-stimulatory factor (G$_s$) coupling of $\beta$-adrenergic receptors to adenylyl cyclase to be in the 10–100 nM range in a completely reconstituted system. Under the same conditions, $T_{out}$ was not affected by any of the tested concentrations of $\beta\gamma$ subunits.

Since we are measuring the muscarinic stimulated Cl$^-$ current, the locus at which the $\beta\gamma$ subunits act cannot be ascertained from the experiments described above. While it is likely that the $\beta\gamma$ subunits act to attenuate Ins$P_3$ production stimulated by muscarinic receptor activation, it is also possible that the $\beta\gamma$ subunits act at other sites. The experiment in Fig. 5 was designed to determine the locus of the $\beta\gamma$-subunit effects. In this experiment we tested the response of control cells and cells injected with 1 ng of $\beta\gamma$ subunits to intracellular injection of Ins$P_3$. Bath application of acetyl-

Table 1. Effects of intracellular injection of various proteins and vehicle on the acetylcholine-stimulated Cl$^-$ current and $T_{out}$

| Exp. | Material injected | n | Chloride current, nA | |
|------|-------------------|---|----------------------|---|
| | | | Acetylcholine (1 $\mu$M) | $T_{out}$ |
| A | None | 3 | $1216 \pm 148$ | — |
| | Vehicle | 3 | $1151 \pm 174$ | — |
| | $\beta\gamma$ subunits | 3 | $166 \pm 52$ | — |
| B | Vehicle | 7 | $1436 \pm 287$ | $334 \pm 31$ |
| | $\beta\gamma$ subunits | 7 | $153 \pm 16$ | $333 \pm 26$ |
| C | None | 5 | $1035 \pm 175$ | $492 \pm 59$ |
| | BSA | 5 | $1095 \pm 134$ | $465 \pm 34$ |
| | $\beta\gamma$ (boiled) | 3 | $925 \pm 175$ | $392 \pm 26$ |
| | $\beta\gamma$ subunits | 4 | $68 \pm 12$ | — |
| D | None | 11 | $1559 \pm 174$ | $301 \pm 16$ |
| | $\alpha$ subunits | 6 | $2675 \pm 384$ | $229 \pm 23$ |

Cells were injected with $\beta\gamma$ subunits (1.0 ng per cell), boiled $\beta\gamma$ subunits (1.0 ng per cell), $\alpha$ subunits (1.0 ng per cell), or bovine serum albumin (BSA) (1.0 ng per cell) in the vehicle or with vehicle alone. Vehicle is the protein storage buffer (10 mM sodium Hepes/1 mM EDTA/20 mM 2-mercaptoethanol/30% ethylene glycol/0.1% purified Lubrol-Px, pH 8.0). All injections were in a 40-nl volume.
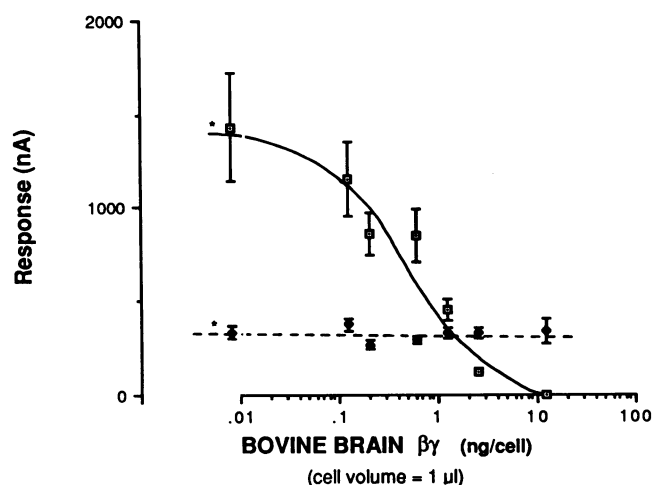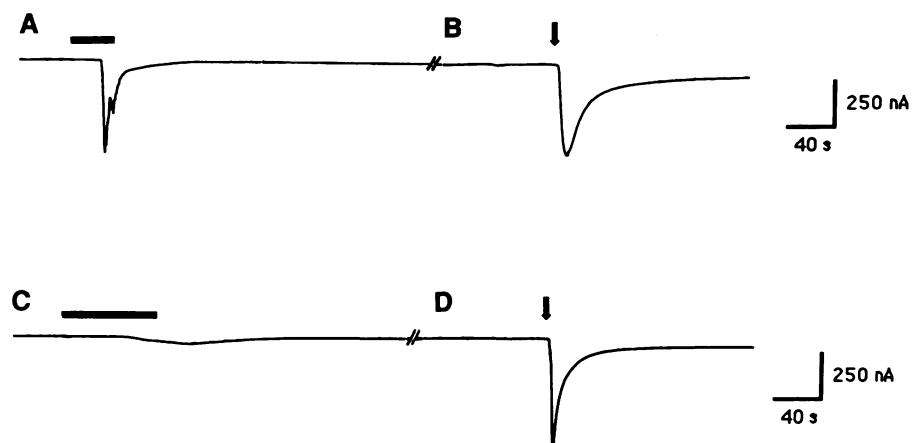


FIG. 4. Effect of various concentrations of bovine brain $\beta\gamma$ subunits on the muscarinic receptor-evoked Cl$^-$ current and on the voltage-activated $T_{out}$. □, Mean $\pm$ SEM of the peak acetylcholine-evoked Cl$^-$ current in at least five cells; ◆, mean $\pm$ SEM of the peak $T_{out}$ current in the same cells. Cells were injected with various amounts of bovine brain $\beta\gamma$ dimer in a fixed volume (40 nl). The stars denote the group of cells injected with only vehicle. The oocyte has an average volume of 1 $\mu$l. The half-maximal inhibition was at $\approx$0.4 ng per cell.

choline to a control oocyte elicited the inward Cl$^-$ current (Fig. 5, trace A). Injection of Ins$P_3$ (2.6 pmol) into the same cell 20 min later caused a similar Cl$^-$ current (Fig. 5, trace B). A cell that had been injected with 1 ng of $\beta\gamma$ subunits did not respond appreciably to bath application of acetylcholine (Fig. 5, trace C). However, injection of Ins$P_3$ (2.6 pmol) into the same cell 15 min later resulted in activation of the Cl$^-$ current (Fig. 5, trace D). This experiment indicates that the $\beta\gamma$ subunits do not appear to have a direct effect on the Cl$^-$ channels or the release of Ca$^{2+}$ from endoplasmic reticulum; rather, $\beta\gamma$ subunits interfere with the muscarinic receptor-activated Cl$^-$ current at a site prior to the generation of Ins$P_3$.

## DISCUSSION

G proteins play a pivotal role in signal transduction at the cell surface. The overwhelming evidence at this stage indicates that G protein-coupled receptors never directly interact with their effector systems; rather, they communicate with appropriate G proteins, which in turn modulate the activity of the effector system (19). Currently, at least six G proteins whose $\alpha$ subunits are substrates of PTX are known. These are $G_t$, $G_o$ and $G_{i\text{-}1,2,3}$, and $G_{43}$. Several cellular signal-transduction systems are also affected by PTX. In addition to inhibition of adenylyl cyclase, these include stimulation of Ins$P_3$ production (20), stimulation of phospholipase A$_2$ activity (21), stimulation of hyperpolarizing K$^+$ channels (22), and both inhibition (23) and stimulation (24, 25) of Ca$^{2+}$ channels. However, at the present time several individual G proteins have not been uniquely identified with a specified effector function as has $G_s$ with stimulation of adenylyl cyclase and $G_t$ with activation of the cGMP phosphodiesterase. Furthermore, recent data indicate that there may be differences in the mechanisms involved in the signal transduction process in the various systems. Thus, while in both the adenylyl cyclase and cGMP-phosphodiesterase systems excess $\beta\gamma$ subunits inhibit the activity of the GTP-liganded $\alpha$ subunit, it has been reported that in atrial cells, K$^+$ channels can be activated by $\beta\gamma$ subunits (26). In contrast, Birnbaumer and co-workers have shown that the $\alpha$ subunit of purified human erythrocyte $G_i$ protein(s) can open K$^+$ channels (27).

FIG. 5.   Effect of intracellular injections of $\beta\gamma$ subunits on application of acetylcholine and intracellular injection of Ins$P_3$. Traces: A, response of a control cell to 1 $\mu$M acetylcholine (the bar denotes length of application). B, response of the same cell 20 min later to intracellular injection of 2.6 pmol of Ins$P_3$ in 2.6 nl (the arrow indicates the point of injection); C, response of a $\beta\gamma$ dimer-injected cell (1.0 ng of bovine brain $\beta\gamma$ dimer) to 1 $\mu$M acetylcholine; D, response of the same cell 15 min later to intracellular injection of 2.6 pmol of Ins$P_3$. Intracellularly injected Ins$P_3$ is able to activate the Cl$^-$ current. Cells were voltage-clamped at a holding potential of $-70$ mV. This procedure was performed on eight $\beta\gamma$ dimer-injected cells.

The $\beta\gamma$ subunits have also been reported to stimulate phospholipase $A_2$ activity in retinal rod cells (28).

In the light of the diversity of data that exists, it is reasonable to examine the role of individual subunits in each signal transduction system. This was of particular interest in the phospholipase C system, where in spite of widespread reports on the effects of guanine nucleotides, no reproducible effects of G proteins have been reported. From our initial experiments, it became obvious that injection of $\beta\gamma$ subunits vastly attenuated muscarinic stimulation of the Cl$^-$ current. The data presented here show that this effect is specifically observed only when $\beta\gamma$ subunits are injected, not when unrelated proteins such as bovine serum albumin or $\alpha$ subunits are injected. Injection of the $\alpha$ subunits of brain G proteins generally gave small but significant stimulation of the muscarinic response. The basis for this stimulation is currently being explored in our laboratories. In contrast, $\beta\gamma$ subunits inhibited signal transduction extensively. Since this inhibition is relieved by the intracellular injection of Ins$P_3$, it is reasonable to conclude that $\beta\gamma$ subunits have their effects by disrupting communication between the agonist-occupied receptor and phospholipase C. However, our data do not allow us to unequivocally rule out direct effects of $\beta\gamma$ subunits on phospholipase C or the muscarinic receptor, though this would appear unlikely. Since the $\beta\gamma$ subunits of G proteins interact only with the $\alpha$ subunit of signal-transducing G proteins but not with other GTP-binding proteins such as the *ras* gene product $p_{21}$ (29), it appears most likely that a G protein that is an $\alpha\beta\gamma$ heterotrimer interfaces between the muscarinic receptor and phospholipase C. Further, it appears likely in the phospholipase C system as well that an $\alpha$ subunit activates the effector function.

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