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Functional Coupling of the β_2-Adrenoceptor to a Pertussis Toxin-Sensitive G Protein in Cardiac Myocytes

RUI-PING XIAO, XIANGWU JI, and EDWARD G. LAKATTA

Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224

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SUMMARY

Recently we demonstrated that the effects of β_2-adrenoceptor (AR) stimulation to augment Ca^{2+} current (I_{Ca}), cytosolic Ca^{2+} (Ca_i) transients, and contractility in rat ventricular myocytes are largely dissociated from its effect to increase cellular cAMP levels. This result suggested that β_2ARs might be coupled to signaling pathways other than the $G_{s\alpha}$-mediated activation of adenylyl cyclase. Here we show that pertussis toxin (PTX) pretreatment specifically potentiates the responses of rat heart cells to β_2AR but not β_1AR stimulation. After PTX pretreatment, 1) the dose-response curve for the effects of the β_2AR agonist zinterol on contraction amplitude is shifted leftward and upward (EC_{50} changed from about 1.0 μM to 70 nM), 2) in indo-1-loaded cells, the maximal effects of zinterol (10^{-5} M) on Ca_i transient and contraction amplitudes are additionally increased 1.7- and 2.0-fold, respectively, over those in control cells, and 3) the increase in I_{Ca} amplitude induced by the same zinterol concentration is potentiated by 2.5-fold. Similar effects of PTX are observed when β_2ARs are stimulated by isoproterenol in the presence of a selective β_1AR blocker, CGP 20712A. All effects of β_2AR agonists in both PTX-treated and control cells are abolished by a selective β_2AR blocker, ICI 118,551. In contrast, neither the base-line I_{Ca}, Ca_i transient, and contraction in the absence of βAR stimulation nor the β_1AR-mediated augmentations of these parameters are significantly altered by PTX treatment. These results demonstrate, for the first time, that the G_s-coupled β_2AR can simultaneously activate a pathway that leads to functional inhibition in cardiac cells via a PTX-sensitive G protein. The activation of more than one G protein during β_2AR stimulation, leading to functionally opposite effects, may provide a mechanism to protect the heart from Ca^{2+} overload and arrhythmias during the response to stress.

The interaction of cell surface receptors with G proteins plays a critical role in cellular signal transduction. It has been well established that both β_1- and β_2AR subtypes increase the activity of adenylyl cyclase via an interaction with G_s, raising the cellular cAMP concentration and the level of cAMP-dependent protein phosphorylation (1–4). However, whereas β_1AR stimulation and β_2AR stimulation increase total cellular cAMP levels to similar extents in rat ventricular myocytes, the effects of β_2AR stimulation on I_{Ca}, Ca_i transients, and contraction are largely dissociated from its effect to increase cAMP content (5). In addition, activation of the two βAR subtypes elicits qualitatively different cell responses at the levels of ionic channels, myofilaments, and SR (6). Specifically, β_1AR but not β_2AR stimulation induces spontaneous Ca^{2+} release, markedly accelerates the kinetics of Ca_i transients and contraction, and markedly increases phospholamban phosphorylation (5). These findings suggest that, compared with β_1AR stimulation, the β_2AR-activated G_s pathway is significantly modified, counteracted, or paralleled by additional transduction mechanisms.

It has been recently shown that G_s-coupled receptors can interact with G_i/G_o as well. This is most clearly exemplified by the observation that glucagon activates cardiac L-type I_{Ca} not only by G_s-mediated activation of adenylyl cyclase but also by inhibition of the membrane-associated, cGMP-inhibited, cAMP-phosphodiesterase (phosphodiesterase III) via activation of a PTX-sensitive G protein (7, 8). It has also been reported that an isoform of the prostaglandin E receptor subtype EP_{3C} is coupled to two different G proteins, G_o and G_s (9). Furthermore, in S49 lymphoma cells, in which G_s is absent, βARs are coupled to a PTX-sensitive G protein and its activation inhibits the activity of adenylyl cyclase (10). In C_6 glioma cells, the effect of βAR stimulation on adenylyl cyclase activity is potentiated by PTX pretreatment (11). The results of these studies suggest that the effects of βAR stimulation to activate adenylyl cyclase are at least partially antagonized by activation of a PTX-sensitive G protein. However, these studies do not distinguish which βAR subtype interacts with PTX-sensitive G proteins. Additionally, it has not yet been determined whether tonic activation of PTX-sensitive G proteins is involved in attenuation of the effect of βAR stimulation on

ABBREVIATIONS: AR, adrenergic receptor; Ca_i, intracellular Ca^{2+}; ISO, isoproterenol; CGP, CGP 20712A; ICI, ICI 118,551; I_{Ca}, Ca^{2+} channel current; NE, norepinephrine; SR, sarcoplasmic reticulum; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; PTX, pertussis toxin.

adenylyl cyclase. In the present study, we evaluate whether β_2AR couples to other G proteins in addition to G_s and, if so, whether this is physiologically or pathophysiologically relevant. We find that PTX treatment significantly enhances the β_2AR-stimulated increases in I_{Ca}, Ca_i transients, and contraction amplitudes, whereas neither the effects of β_1AR stimulation nor the base-line I_{Ca}, Ca_i transients, and contraction in the absence of βAR stimulation are affected by PTX pretreatment. These results indicate that, under physiological conditions, substantial coupling occurs between β_2AR and a PTX-sensitive G protein, exerting negative feedback on the cellular responses to β_2AR stimulation. Thus, distinct βAR subtype actions reside, at least in part, in different receptor-G protein interactions.

Materials and Methods

Simultaneous measurements of cell length and Ca_i transients. Single ventricular cardiac myocytes were isolated from 2–4-month-old rat hearts by a standard enzymatic technique (12). In some experiments, myocytes were loaded with the fluorescent Ca^{2+} probe indo-1/acetoxymethyl ester at room temperature, as described previously (12). After the indo-1 loading, cells were placed on the stage of a modified inverted microscope (Zeiss model IM-35) and perfused with HEPES buffer solution consisting of 1.0 mM $CaCl_2$, 137 mM NaCl, 5.0 mM KCl, 15 mM dextrose, 1.3 mM $MgSO_4$, 1.2 mM NaH_2PO_4, and 20 mM HEPES, pH 7.4, at 23°. The excitation wavelength was selected with a 350-nm interference filter. The ratio of emission intensity at 410 nm to that at 490 nm was computed off-line and the resulting waveform was used as an index of the Ca_i transient (13).

Each cell was simultaneously illuminated with red (650–750-nm) light through the normal bright-field path of the microscope. Cell length was monitored from the bright-field image of the cell by an optical edge-tracking method using a photodiode array (Reticon model 1024 SAQ) with a 5-msec time resolution (12).

Electrophysiological measurements. L-type I_{Ca} was measured via the whole-cell patch-clamp technique (14). To selectively examine L-type I_{Ca} in whole-cell current records, cells were voltage-clamped at −40 mV to inactivate the sodium channel current and T-type calcium current. The superfusion solution was the same as that used for cell length and Ca_i transient measurements, except that KCl was replaced by CsCl. Low-resistance (3–5 MΩ) microelectrodes were filled with a solution containing 120 mM CsCl, 20 mM HEPES, 5 mM $MgCl_2$, 10 mM NaCl, 10 mM EGTA, and 3 mM Mg-ATP; the pH was adjusted to 7.2 with CsOH. All of the experiments were performed with a discontinuous switch-clamp technique with an Axoclamp II amplifier (Axon Instruments) controlled by a specifically designed hardware/software system. The system was also used to acquire (at 2 kHz) and store I_{Ca}. Data analysis was performed on a VAX 3400 computer. I_{Ca} was measured as the difference between peak inward current and the current at the end of the 200-msec pulse. Because in some cells I_{Ca} kinetics could not always be fit by a single-exponential function, the current inactivation rate was indexed as 63% of the decay time.

Drugs. ISO, NE, adenosine, and PTX were obtained from Sigma, CGP was kindly supplied by Ciba-Geigy (Basel, Switzerland), ICI was kindly supplied by Imperial Chemical Industry (Macelesfield, UK), and zinterol was kindly supplied by Bristol-Myers (Evansville, IN).

Statistics. Data are reported as mean ± standard error. The effect of PTX treatment on Ca_i transient, contraction, and I_{Ca} characteristics was tested via paired *t* test or Student's *t* test when appropriate. Fisher's exact test was performed to determine the significance of the likelihood for spontaneous Ca^{2+} oscillation to occur. A *p* value of <0.05 was considered to be statistically significant.

Experimental protocols. Cells were incubated with PTX (0.75 μg/ml) at 37° for at least 3 hr. PTX-treated cells were compared with myocytes that had been kept at 37° in the absence of PTX for an equal time. Successful inactivation of inhibitory G proteins (G_i/G_o) in PTX-treated cells was routinely verified by a loss of the ability of adenosine to reverse the positive inotropic effect of β_1AR stimulation, because adenosine is generally thought to negatively modulate βAR stimulation by activating a PTX-sensitive G protein pathway. Fig. 1 shows the representative effects of PTX treatment to abolish the antagonistic effects of 10^{-6} M adenosine on βAR stimulation. Traces of Ca_i transients and contraction before and after exposure to NE and adenosine, recorded in a control cell and a PTX-treated cell, are shown in Fig. 1, which clearly illustrates that, in the absence of PTX treatment, the effects of NE (10^{-7} M) on Ca_i transient and contraction amplitudes were completely inhibited by adenosine. In contrast, after PTX treatment the effects of NE were not affected by adenosine. Similar results were obtained when cells were stimulated by the β_2AR agonist zinterol rather than NE. In addition, the antagonistic effects of acetylcholine (10^{-6} M) on βAR stimulation in rat ventricular myocytes were also blocked by the same PTX pretreatment.

Dose-response curves for βAR agonists were obtained in control cells and PTX-treated cells. A given individual myocyte was exposed to only one drug concentration. All measurements were obtained under steady

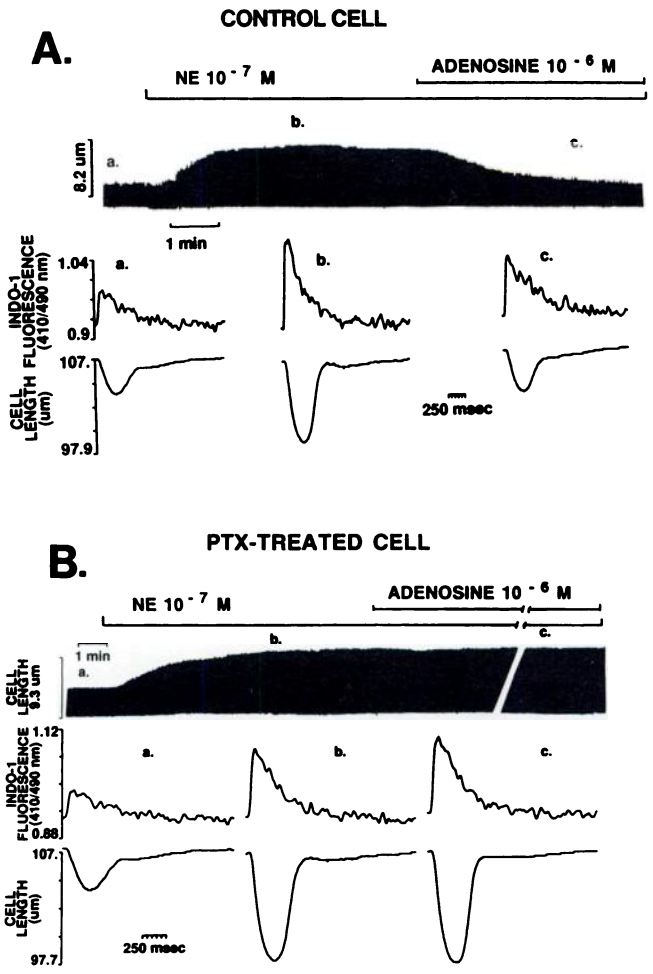

Fig. 1. Representative effects of adenosine (10^{-6} M) on the NE (10^{-7} M)-induced increases in Ca_i transient and contraction amplitudes. A, Antagonistic effects of adenosine in a myocyte not treated with PTX. *Continuous tracing at the top*, chart recording of the cell contraction. An upward deflection indicates cell shortening. *Traces beneath the continuous tracing*, obtained at times indicated in the top tracing, showing the simultaneously recorded Ca_i transient and contraction (plotted downward) before NE (a), after exposure to NE (b), and after adenosine addition (c). B, Typical result obtained in a PTX-treated cell. The experimental conditions are the same as in A. Note that the effects of NE on Ca_i transient and contraction amplitudes are not affected by adenosine.

state conditions after 10-min exposure to a designated agent at room temperature (23°).

Results

Effect of PTX treatment on βAR subtype-stimulated increases in cardiac contractility. Potential modulatory effects of PTX-sensitive G proteins on cellular responses to βAR subtype stimulations were initially assessed by measurements of cell contraction in non-indo-1-loaded cells. ISO (a nonselective βAR agonist) plus a highly selective β_1AR or β_2AR blocker was used to activate β_2ARs or β_1ARs, respectively. Fig. 2A illustrates that in PTX-treated cells the effect of ISO on contraction amplitude in the presence of the β_1AR-selective blocker CGP, i.e., ISO acting in the β_2 mode (6), was completely reversed by the β_2AR-selective antagonist ICI. The averaged data, presented in Fig. 2B, show that the increase in contraction amplitude induced by ISO plus CGP was about 2.5-fold greater in PTX-treated cells than in control cells. However, when ISO was acting in the β_1 mode, i.e., in the presence of ICI, its effect to increase contraction amplitude was not significantly altered after PTX treatment (Fig. 2B). These results indicate that PTX treatment potentiates the response to βAR stimulation and that this effect appears to be confined to the β_2AR subtype.

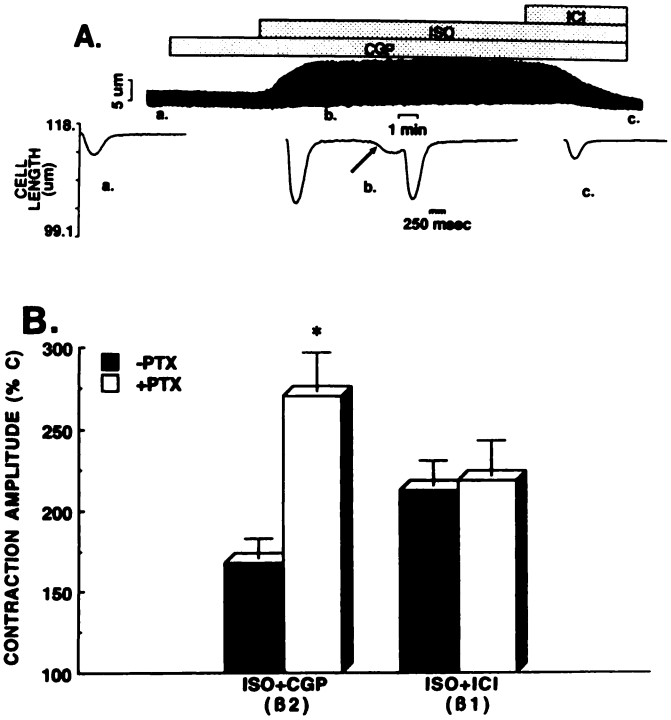

Fig. 2. Effect of β_2AR stimulation by ISO (10^{-6} M) plus CGP (3×10^{-7} M) to increase contraction amplitude. A, Representative result obtained in a PTX-treated cell. *Top*, continuous chart recording of cell contraction. Note that the effect of ISO plus CGP is blocked by the subsequent addition of the β_2AR blocker ICI (10^{-7} M). *Bottom*, contraction tracings obtained at the time points indicated, showing control (a), results obtained after addition of ISO with CGP (b), and the antagonistic effect of ICI (c). Note that the spontaneous contractile waves (*arrow*) that occur during β_2AR stimulation are also inhibited by ICI. B, Average effect of ISO (10^{-6} M) in the presence of CGP (3×10^{-7} M) or ICI (10^{-7} M) on contraction amplitude in non-PTX-treated cells (■) and in PTX-treated cells (□). Note that the effect of ISO acting in the β_2 mode (ISO plus CGP), but not in the β_1 mode (ISO plus ICI), is significantly enhanced by PTX treatment. *, $p < 0.001$ versus non-PTX-treated cells ($n = 10$ for both groups).

In addition, in more than half of the PTX-treated cells (eight of 15 cells) spontaneous contractile waves, i.e., a manifestation of spontaneous SR Ca^{2+} release, occurred concomitantly with the positive inotropic effect of β_2AR stimulation (Fig. 2A), whereas these were only occasionally (one of 11 cells) observed in the control cells ($p < 0.01$, PTX-treated cells versus control cells).

Sensitization by PTX treatment of cellular responses to β_2AR stimulation. The potentiating effect of PTX was manifested not only as an increase in the maximum response but also as an increase in the sensitivity of cardiac myocytes to respond to β_2AR stimulation. Fig. 3A illustrates that the dose-response curve for the effects of the selective β_2AR agonist zinterol on contraction amplitude was shifted markedly leftward and upward. The maximal increase in contraction amplitude after zinterol was enhanced from 201 ± 23.0% of control in non-PTX-treated cells to 307 ± 29.8% of control in treated cells. The EC_{50} was markedly decreased by PTX pretreatment, from about 1.0 μM to 70 nM. In contrast, the dose-response curve for the β_1AR agonist NE to increase contraction was not significantly altered by PTX treatment (Fig. 3B). These results further confirm the observation that only the effects of β_2AR stimulation, and not the effects of β_1AR stimulation, are significantly potentiated by PTX treatment.

Effect of PTX treatment on βAR subtype-induced increases in Ca_i transients. In additional studies, myocytes were loaded with the fluorescent Ca^{2+} probe indo-1 and changes in contraction and Ca_i transients were simultaneously recorded. In the absence of βAR stimulation, PTX had no significant effects on the cell contraction or Ca_i transient (Table 1). Fig. 4 illustrates the effect of PTX treatment on the Ca_i transient and contraction responses to the β_2AR agonist zinterol. Fig. 4, A and B, shows representative examples of the effects of zinterol (10^{-5} M) on the Ca_i transients and contraction in a PTX-treated cell and a control cell, respectively. The effects of zinterol to increase the Ca_i transient and contraction amplitudes were enhanced by PTX. On average, PTX treatment enhanced the zinterol-induced increase in Ca_i transient amplitude by about 1.7-fold (Fig. 4C). This effect was associated with an increase in the contraction amplitude (Fig. 4D). Also, note that zinterol markedly accelerated the kinetics of the Ca_i transient and contraction in the PTX-treated cells, as shown in the normalized and superimposed tracings in Fig. 4, A and B. On average, the effect of zinterol to reduce the $t_{1/2}$ of the Ca_i transient was markedly increased (99.1 ± 2.6% of control, $n = 9$, versus 85.7 ± 4.6% of control, $n = 15$, for control cells and PTX-treated cells, respectively; $p < 0.05$ for PTX-treated cells versus control cells). Concomitantly, the effect of zinterol to reduce the $t_{1/2}$ of contraction was also enhanced from 91.3 ± 1.4% of control in control cells to 82.8 ± 2.6% of control in PTX-treated cells ($p < 0.01$ versus control cells). Additionally, spontaneous Ca_i oscillations and contractile waves were frequently observed in PTX-treated cells (five of 12 cells) but not in control cells (none of 12 cells) during exposure to zinterol ($p < 0.01$ for PTX-treated cells versus control cells). These results with zinterol as the β_2AR agonist are in accord with those obtained with ISO plus a β_1AR blocker described above.

In contrast to the marked effects of PTX to potentiate the cell responses to β_2AR stimulation, the effects of β_1AR stimulation induced by NE on contraction and Ca_i transients were not significantly affected by PTX treatment (Fig. 4, C and D).

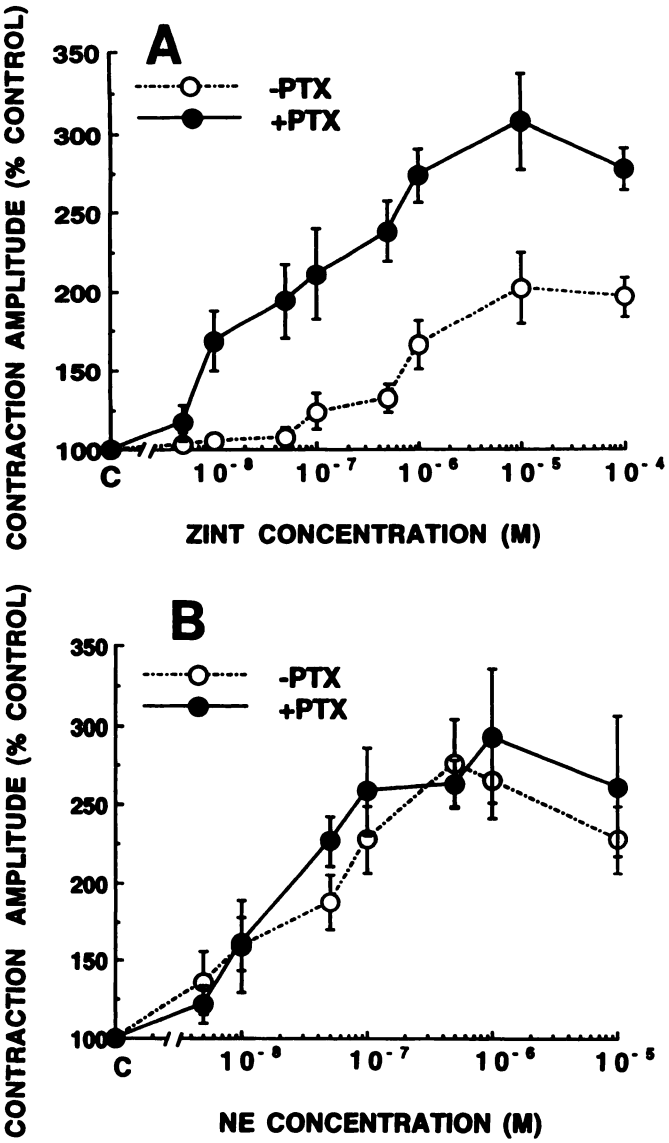

Fig. 3. Average dose-response curves for effects of the β_2AR agonist zinterol (*ZINT*) and the β_1AR agonist NE on contraction amplitude in the presence or absence of PTX pretreatment. A, The dose-response curve for the effect of zinterol was shifted markedly leftward, by approximately 1 order of magnitude, after PTX pretreatment. B, In contrast, the dose-response curve of the effect of NE was not altered by PTX pretreatment. Myocytes were exposed to only one concentration of agonist, and each *point* represents the mean ± standard error of seven to 10 cells. Results are expressed as percentage of the control value. Control values of contraction amplitude for zinterol experiments are 6.89 ± 0.31 and 6.86 ± 0.24% of the resting cell length for control cells (n = 62) and PTX-treated cells (n = 58), respectively. Control values of contraction amplitude for NE experiments are 6.93 ± 0.33 and 6.28 ± 0.26% of the resting cell length for control cells (n = 50) and PTX-treated cells (n = 50), respectively.

Because the potentiating effects of NE on Ca_i transient and contraction amplitude reached a maximum at 10^{-7} M, the failure of PTX to potentiate the responses to NE could be masked by a saturation effect. This was not the case, however, because the effects of lower concentrations of NE (10^{-8} M) on contraction amplitude were also not significantly affected by PTX treatment (Fig. 3B). As has been already demonstrated, NE likely induces its positive inotropic effects almost exclusively via β_1AR stimulation in rat cardiac myocytes (neither the α_1AR blocker prazosin nor the β_2AR blocker ICI affects the responses of the Ca_i transients or contraction to NE) (6, 15). We conclude that the potentiating effect of PTX is confined to β_2AR stimulation.

TABLE 1

Base-line contraction, Ca_i transient, and I_{CA} parameters in PTX-treated and untreated cells

Values are mean ± standard error.

	Contraction amplitude	IFTA[a]	I_{Ca}
	% of resting cell length	*410/490 nm*	*nA*
Non-PTX-treated cells (n = 15)	4.96 ± 0.29	1.046 ± 0.023	−1.04 ± 0.12[b]
PTX-treated cells (n = 18)	4.89 ± 0.27	1.042 ± 0.022	−1.11 ± 0.15

[a] IFTA, Ca_i transient amplitude, indexed as the indo-1 fluorescence transient ratio (410/490 nm).
[b] n = 6.

Modulation of I_{Ca} by β_2AR stimulation in PTX-treated cells. It is well known that βAR stimulation augments Ca^{2+} influx via L-type sarcolemmal Ca^{2+} channels (16), which triggers SR Ca^{2+} release during excitation-contraction coupling in cardiac myocytes (17, 18). To further investigate the cellular mechanism for the potentiation of β_2AR stimulation by PTX treatment, we examined the I_{Ca} response to βAR subtype agonists. Fig. 5A shows the typical effect of zinterol at 10^{-5} M on I_{Ca} in a cell not treated with PTX. The I_{Ca} amplitude was increased about 2-fold. In a PTX-treated cell, the effect of zinterol on I_{Ca} was further potentiated to about 3.5-fold (Fig. 5B), whereas the base-line I_{Ca} was comparable to that in control cells (see also Table 1). Fig. 5, C and D, shows representative current-voltage relationships for responses of I_{Ca} to zinterol in a non-PTX-treated cell and a PTX-treated cell. Note that nifedipine (2 μM), a specific L-type Ca^{2+} channel blocker, inhibited the recorded current. The effect of the β_2AR agonist zinterol to enhance peak I_{Ca} was markedly voltage dependent, i.e., the effect of zinterol was greater at more negative test potentials than at positive ones (in both PTX-treated and untreated cells). This result is similar to those obtained with the nonselective βAR agonist ISO (19) and is probably accounted for by a voltage dependence of Ca^{2+} channel phosphorylation. The average effect of zinterol on I_{Ca} amplitude at a test potential of 0 mV was enhanced 2.8-fold after PTX treatment (Fig. 5E). In contrast, I_{Ca} responsiveness to NE in non-PTX-treated cells was not significantly different from that in PTX-treated cells (Fig. 5E).

In addition to increasing the I_{Ca} amplitude, zinterol prolonged the I_{Ca} inactivation time in non-PTX-treated cells (from 16.9 ± 1.3 msec to 25.0 ± 1.8 msec, $p < 0.01$, n = 6), in agreement with our previous observation (6). However, in PTX-treated cells the I_{Ca} decay time was not significantly altered by zinterol (18.3 ± 2.0 msec and 19.4 ± 1.4 msec, n = 6, before and after superfusion with zinterol, respectively).

Discussion

Previous studies have shown that β_2ARs, like β_1ARs, are coupled to G_s and that their activation leads to an increase in cell cAMP content (1–5). The present results demonstrate, for the first time, that the β_2AR in rat cardiac myocytes is simultaneously coupled to a PTX-sensitive G protein and to G_s.

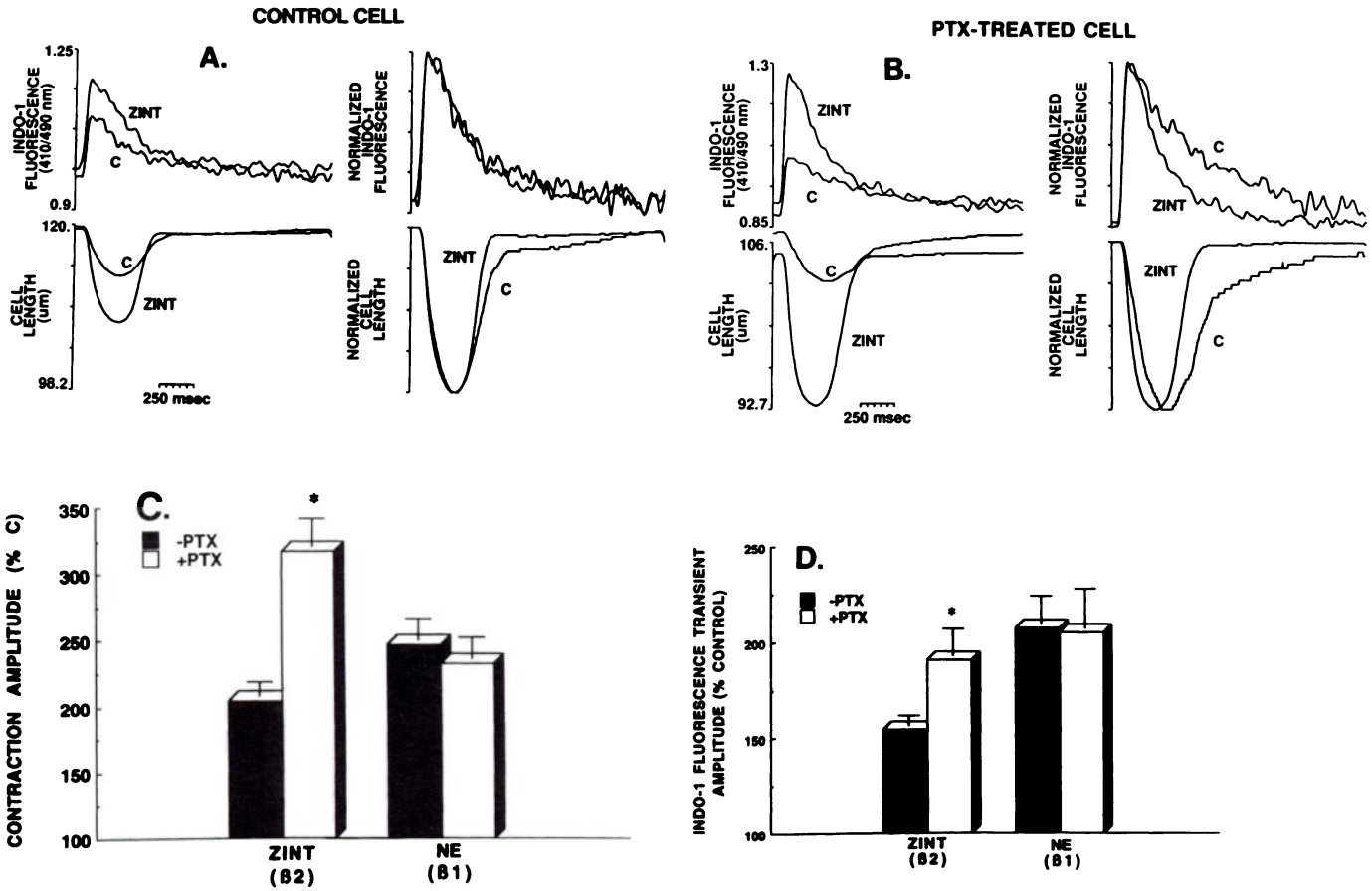

Fig. 4. Effects of β_2AR or β_1AR stimulation on Ca_i transients and contraction in non-PTX-treated and PTX-treated cells. A, *Left traces*, simultaneously recorded Ca_i transient and contraction signals, in the presence (*ZINT*) and absence (*C*) of zinterol (10^{-5} M), in a non-PTX-treated cell. *Right traces*, Ca_i transient and contraction normalized to their peaks. B, Signals measured in a PTX-treated cell. C, Bar graph showing the average effects of zinterol and NE to increase contraction amplitude in non-PTX-treated cells (n = 15 for zinterol and n = 20 for NE) and PTX-treated cells (n = 9 for zinterol and n = 10 for NE). Data are presented as percentage of control. *, $p < 0.001$ versus non-PTX-treated cells. D, Effects of zinterol and NE on Ca_i transient amplitude in the absence and presence of PTX treatment. Data were obtained in the same cells as in C and are presented as percentage of control. *, $p < 0.001$ versus non-PTX-treated cells.

After PTX treatment, Ca_i transient and contraction responses to β_2AR stimulation were markedly enhanced, as manifested by greater maximum responses and increased sensitivity to the β_2AR agonist zinterol. These can be accounted for, at least in part, by an increased I_{Ca} response to β_2AR stimulation in PTX-treated cells versus control cells. The potentiating effects of PTX were robustly and consistently observed during β_2AR stimulation elicited either by the selective β_2AR agonist zinterol (Figs. 3–5) or by ISO acting in the β_2 agonist mode (Fig. 2). Because the effects of both zinterol and ISO plus β_1AR blockade in the PTX-treated cells are antagonized by the β_2AR-selective blocker ICI, the potentiated actions after PTX pretreatment were still mediated by β_2AR activation. These results suggest that a PTX-sensitive G protein is activated during β_2AR stimulation of normal rat ventricular myocytes, which produces negative feedback on the β_2AR effects to augment I_{Ca}, Ca_i, and contraction responses.

To exclude the possible involvement of a tonically activated PTX-sensitive G protein in our experimental preparation, we examined the response of the β_1AR to PTX treatment. In contrast to its effect on the β_2AR response, PTX treatment failed to potentiate the effects of β_1AR stimulation induced by NE (Figs. 3 and 4) or by ISO plus a β_2AR blocker (Fig. 2B). The distinctly different effects of PTX pretreatment on β_1AR and β_2AR actions strongly support the idea that activation of the PTX-sensitive G protein described above is a consequence of β_2AR stimulation rather than basal activation of the G protein. Additional support is provided by the observation that the I_{Ca}, Ca_i transients, and contraction in the absence of βAR stimulation are not affected by PTX treatment (Table 1). This result also argues that the effects of tonic PTX-sensitive G protein activation on contractility and Ca^{2+} homeostasis in rat ventricular cardiac cells, under the present conditions, are negligible. Furthermore, the potentiating effect of PTX on β_2AR stimulation is not unique to rat cardiac myocytes, because similar results have been observed in canine ventricular myocytes.[1] Taken together, the results of the present study indicate that β_2AR stimulation can lead to the activation of a PTX-sensitive G protein, in parallel with the activation of G_s. Most of the known G proteins have been detected in myocardial tissues (20). The PTX-sensitive G protein family includes three G_i subtypes, G_{i1}, G_{i2}, and G_{i3} (21). This family also includes at least two G_o subtypes, G_{oA} and G_{oB} (22). The present study cannot discriminate which specific PTX-sensitive G protein is activated by β_2AR stimulation.

Cell surface membrane receptors were first thought to be

[1] R. P. Xiao, E. G. Lakatta, personal communication.

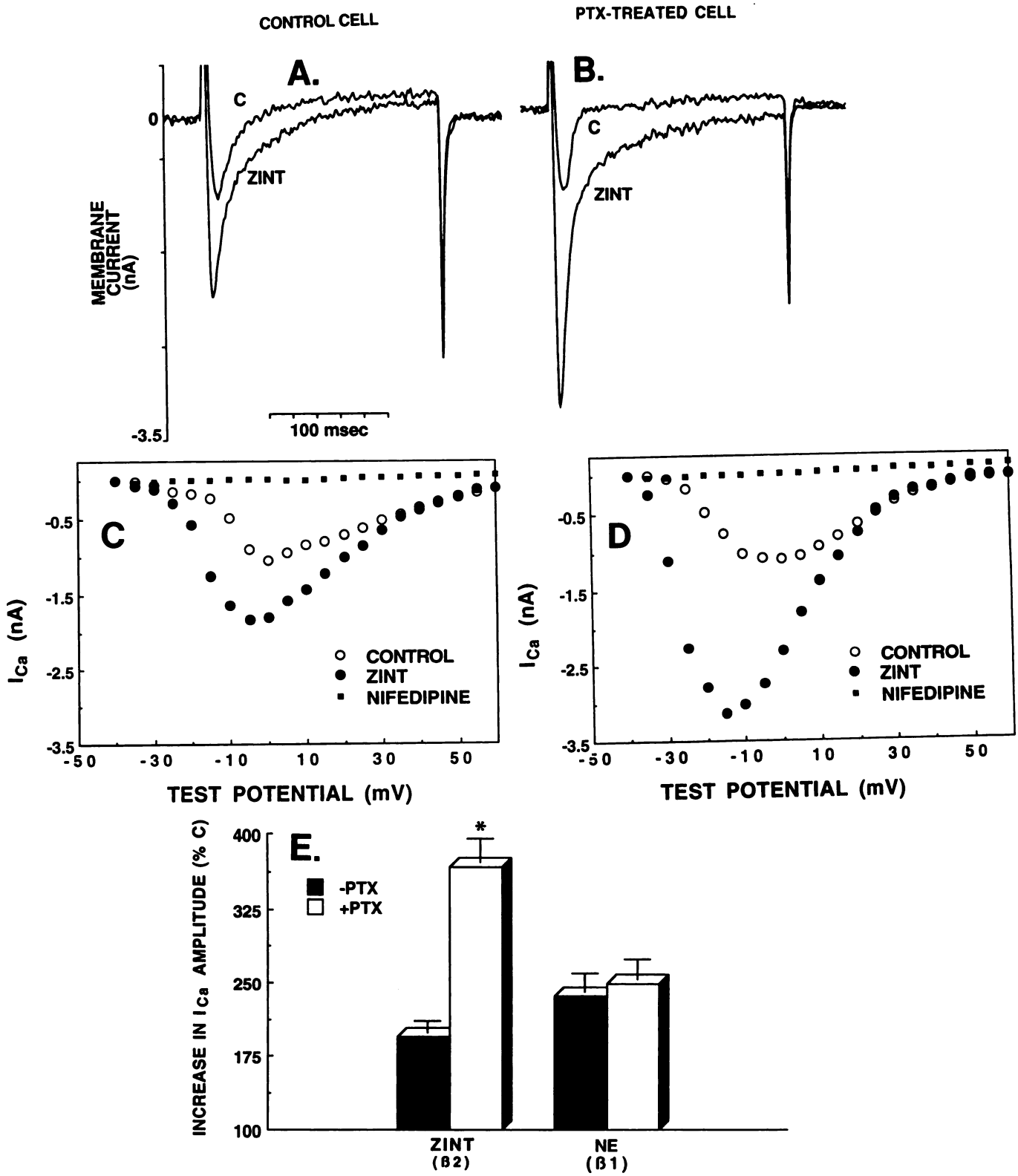

Fig. 5. Effect of zinterol (10^{-5} M) on I_{Ca}. A, Superimposed current tracings recorded before (*C*) and after (*ZINT*) zinterol in a non-PTX-treated cell. Currents were elicited by depolarizing test pulses from a holding potential of −40 to 0 mV for 200 msec at 0.5 Hz. B, Same experiment as in A, except in a PTX-treated cell. Note that the augmentation of I_{Ca} induced by zinterol is enhanced, compared with that in the non-PTX-treated cell in A. C, Current-voltage relations of I_{Ca} under control conditions, with zinterol, and with zinterol plus nifedipine (2 μM) in a cell that was not treated with PTX. The cell was depolarized from a holding potential of −40 mV to test potentials from −35 to +60 mV in 5-mV increments. D, Current-voltage plots for a PTX-treated cell, measured under the same experimental conditions as in C. E, Average effects of zinterol (10^{-5} M) and NE (10^{-7} M) to increase I_{Ca} amplitude at a test potential of 0 mV in the absence and presence of PTX treatment. *, $p < 0.001$ versus non-PTX-treated cells ($n = 6$ for both zinterol groups, $n = 4$ for both NE groups).

selective for individual G proteins. However, it is now apparent that a signal can be routed from one receptor to multiple G proteins. Specifically, several other G_s-coupled cellular receptors have the ability to couple to additional G proteins. For example, stimulation of EP_{3C} oppositely regulates the activities of G_o and G_s, causing inhibition of G_o and activation of G_s (9). This pattern of simultaneously coupled G proteins likely produces a functionally synergistic effect. In contrast, the results reported here suggest a unique mode of receptor-G protein interaction, i.e., the simultaneous activation of more than a single G protein routing to functionally opposite pathways. Removal of the inhibitory pathway by PTX pretreatment, as demonstrated above, results in significantly potentiated stimulatory pathway performance. This surprising finding not only reveals a new level of complexity in receptor-G protein interactions but also provides new insight for understanding the distinct βAR subtype actions in cardiac myocytes (5, 6).

Although β_1AR and β_2AR are closely related G protein-coupled receptors that contain seven transmembrane domains, they are genetically distinct (23–26). The sequences of human β_1AR and β_2AR have only 71 and 54% amino acid identity in the transmembrane domains and in overall sequence, respectively (24). Studies on chimeric or mutated G protein-coupled receptors (including the major subtypes of ARs) have demonstrated that the cytoplasmic loop that connects transmembrane domains V and VI of those receptors is an important determinant for G protein coupling (3, 27–30). Thus, the differences between β_1AR and β_2AR in G protein coupling demonstrated by the present results could be related to some critical differences in the sequences of these βAR subtypes in the cytoplasmic loop. However, additional studies are required to determine the molecular and structural bases that mediate the coupling of different G proteins to β_2ARs versus β_1ARs.

Unlike β_1AR stimulation, β_2AR stimulation does not abbreviate the duration of the Ca_i transients and contraction, nor does it induce spontaneous Ca^{2+} oscillations and contractile waves (5, 6). These are probably due to the inability of β_2AR stimulation to cause significant phosphorylation of phospholamban (5), resulting in an acceleration of Ca^{2+} sequestration and accumulation by the SR. Moreover, the dose-response relation for cAMP levels after β_2AR stimulation is shifted to the left of the dose-response relations for the Ca_i transients and contraction (6). The present results illustrate that, after removal of the PTX-sensitive G protein inhibitory pathway, β_2AR stimulation not only is more potent in its effects to increase I_{Ca}, Ca_i transient, and contraction amplitudes but also accelerates the Ca_i and contraction kinetics and increases the occurrence of Ca^{2+} and contractile waves. Thus, the effects of β_2AR stimulation after PTX treatment appear to be qualitatively similar to those of β_1AR stimulation. It is possible that the dissociation of the cAMP- and β_2AR-mediated effects observed in the absence of PTX (5) might also be attributable to the PTX-sensitive mechanism(s). Indeed, recent studies suggest that the G_i/G_o that couple adenosine or muscarinic receptors to cAMP also couple to cAMP-independent pathway(s), such as stimulation of protein phosphatases, resulting in an antagonism of the βAR-induced positive inotropic effects (31, 32). However, the PTX-sensitive pathway demonstrated in the present results does not necessarily inhibit cAMP production after β_2AR stimulation, because β_2AR-stimulated cAMP elevation is similar to that induced by β_1AR stimulation in the absence of PTX pretreatment (5). Furthermore, the results of a recent study show that the dose-response curves for the β_2AR agonist zinterol to increase total or particulate (membrane-bound) cellular cAMP production are not significantly affected by PTX pretreatment.[2] This suggests that the β_2AR-coupled PTX-sensitive G protein has no effect on adenylyl cyclase or cellular cAMP metabolism. This result is in contrast to the results obtained in S49 lymphoma cells and C_6 glioma cells (10, 11). We tentatively postulate that the effect of the β_2AR-coupled, PTX-sensitive, G protein might be mediated by some cAMP-independent mechanisms, such as activation of protein phosphatases. An understanding of the exact mechanisms requires further study.

The present results show that, in the presence of PTX, activation of the β_2AR increases the likelihood of the SR-generated spontaneous Ca^{2+} oscillations and contractions. This observation is not trivial, because spontaneous oscillations in Ca_i can activate depolarizing membrane currents, which may lead to a disturbance of the electrical behavior of the heart and lead to arrhythmia (33–36). Thus, in the absence of PTX, the interplay of the two functionally opposite G protein pathways after β_2AR stimulation may be beneficial, because it provides inotropic support without promoting SR Ca^{2+} overload and spontaneous SR Ca^{2+} release. The cellular logic for the multiplicity of βARs may partly reside in the different arrhythmigenic properties of βAR subtypes. It is well established that, in human heart failure, the efficacy of βAR stimulation to increase cardiac contractility is significantly reduced (37–39). However, the responses to the stimulation of the two βAR subtypes in heart failure are not symmetrically reduced. In failing hearts, the β_1AR-stimulated positive inotropic effect is markedly diminished, which may be due, in part, to down-regulation of β_1AR density (40–43). In contrast, β_2AR density is relatively increased during heart failure (43–45). It has also been reported that the PTX-sensitive G protein G_i is increased by about 35–40%, whereas the $G_{s\alpha}$ level and activity remain constant, resulting in a decrease in the ratio of G_s/G_i in human heart failure (39, 43, 46). More recent studies have shown that similar changes can occur in nonfailing aged hearts (47) (see Ref. 48 for review). In light of the present findings, the up-regulation of β_2ARs and G_i might reflect important adaptive changes associated with heart failure and aging of the heart.

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Send reprint requests to: Edward G. Lakatta, Laboratory of Cardiovascular Science, Gerontology Research Center, 4940 Eastern Ave., Baltimore, MD 21224.