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G_i Protein-mediated Functional Compartmentalization of Cardiac β_2-Adrenergic Signaling*

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In contrast to β_1-adrenoreceptor (β_1-AR) signaling, β_2-AR stimulation in cardiomyocytes augments L-type Ca^{2+} current in a cAMP-dependent protein kinase (PKA)-dependent manner but fails to phosphorylate phospholamban, indicating that the β_2-AR-induced cAMP/PKA signaling is highly localized. Here we show that inhibition of G_i proteins with pertussis toxin (PTX) permits a full phospholamban phosphorylation and a *de novo* relaxant effect following β_2-AR stimulation, converting the localized β_2-AR signaling to a global signaling mode similar to that of β_1-AR. Thus, β_2-AR-mediated G_i activation constricts the cAMP signaling to the sarcolemma. PTX treatment did not significantly affect the β_2-AR-stimulated PKA activation. Similar to G_i inhibition, a protein phosphatase inhibitor, calyculin A (3×10^{-8} M), selectively enhanced the β_2-AR but not β_1-AR-mediated contractile response. Furthermore, PTX and calyculin A treatment had a non-additive potentiating effect on the β_2-AR-mediated positive inotropic response. These results suggest that the interaction of the β_2-AR-coupled G_i and G_s signaling affects the local balance of protein kinase and phosphatase activities. Thus, the additional coupling of β_2-AR to G_i proteins is a key factor causing the compartmentalization of β_2-AR-induced cAMP signaling.

The classical view of β-AR[1] signal transduction is that agonist-bound β-AR selectively interact with stimulatory G proteins (G_s), which, in turn, activate adenylyl cyclase to enhance cAMP formation. Subsequently, PKA phosphorylates a multitude of regulatory proteins involved in cardiac excitation-contraction coupling, including L-type Ca^{2+} channels (1, 2), the sarcoplasmic reticulum Ca^{2+} pump regulator PLB (3, 4), and myofilament proteins (5, 6), producing positive inotropic and lusitropic effects. In addition, PKA also phosphorylates and activates the endogenous protein phosphatase inhibitor 1 (7), which further ensures the action of protein kinases by protein phosphatase inhibition.

Although cardiac β_1-AR signaling follows the scheme described above, recent studies have revealed a dissociation of β_2-AR-mediated positive inotropic as well as lusitropic effects from global cAMP accumulation in several mammalian species (8–11). In addition, it has been demonstrated that in contrast to β_1-AR, β_2-AR stimulation fails to induce a cAMP-dependent phosphorylation of non-sarcolemmal proteins involved in excitation-contraction coupling and energy metabolism (*e.g.* phospholamban, the myofilament proteins, troponin I, C protein, and the cytosolic protein glycogen phosphorylase kinase), but it does activate sarcolemmal L-type Ca^{2+} channels (10–12), suggesting that β_2-AR signaling is localized. More direct evidence supporting the localized β_2-AR signaling has emerged from single L-type Ca^{2+} channel recordings. Employing cell-attached patch clamp technique, the activity of single L-type Ca^{2+} channels has been measured in response to specific β-AR subtype agonist outside (remote) or inside (local) the patch pipette. In contrast to the diffusive effect of β_1-AR stimulation, β_2-AR stimulation by zinterol only locally activates the L-type Ca^{2+} channel but has no remote effect (13). Taken together, these previous studies have led to the hypothesis that the β_2-AR-induced cAMP signaling is compartmentalized to a subsarcolemmal space and cannot be transmitted to cytoplasmic and SR PKA target proteins. Alternatively, the signal is transmitted to cytosolic proteins but local inactivation occurs at these sites.

The mechanism for the local control of β_2-AR mediated signaling remains unclear. In many biological systems, G_s and G_i proteins cross-talk and operate as a complementary system. This balance system is usually regulated through different receptor families. For instance, activation of muscarinic receptors or adenosine receptors, prototypic G_i-coupled receptors, markedly antagonizes the positive inotropic effect of β-AR stimulation (14, 15). Interestingly, promiscuous G protein coupling of β_2-AR to both G_s and PTX-sensitive G proteins (G_{i2} and G_{i3}) has been demonstrated in intact cardiomyocytes (16, 17). This coupling of β_2-AR to G_i proteins and its downstream pathway might interplay with the β_2-AR/G_s signaling and contribute to the localization of β_2-AR signaling near the sarcolemmal membrane. Thus, in the present study, we intended to determine whether the localized β_2-AR signaling is mediated by the additional coupling of β_2-AR to G_i proteins and, if so, to elucidate potential underlying mechanisms.

EXPERIMENTAL PROCEDURES

Measurements of Cell Length—Single ventricular myocytes were isolated from rat hearts by a standard enzymatic technique (18). The cells were suspended in HEPES pH 7.4 buffer containing (in mmol/liter) 20 HEPES, 1 $CaCl_2$, 137 NaCl, 5 KCl, 15 dextrose, 1.3 $MgSO_4$, 1.2 NaH_2PO_4. In some experiments, cells were separately or simultaneously treated with 1.5 μg/ml PTX (Sigma) for 3 h at 37 °C to block G_i protein activation (15) or 3×10^{-8} M calyculin A (Calbiochem) for 20 min at 23 °C to inhibit protein phosphatases. Cells were stimulated at 0.5 Hz at 23 °C, and cell length was monitored from the bright-field image by an optical edge-tracking method using a photodiode array (model 1024 SAQ, Reticon) with a 3-ms time resolution (18).

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[1] The abbreviations used are: β_1-AR, β_1-adrenoreceptor; PLB, phospholamban; PTX, pertussis toxin; PKA, cAMP-dependent protein kinase; NE, norepinephrine.

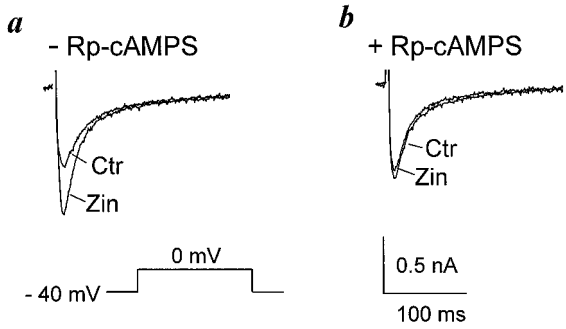

FIG. 1. **β_2-AR stimulation increases L-type Ca^{2+} current in rat cardiomyocytes by a cAMP-dependent mechanism.** Representative response of I_{Ca} (elicited by a depolarization from −40 to 0 mV) to the β_2-AR agonist zinterol (*Zin*, 10^{-5} M, 5 min) in the absence (*a*) and presence (*b*) of the inhibitory cAMP analog, (R_p)-cAMPs (10^{-4} M). The base line (*Ctr*) for I_{Ca} was 5.14±0.43 pA/pF ($n = 6$) and 6.44 ± 0.44 ($n = 7$) with and without (R_p)-cAMPs, respectively.

L-type Ca^{2+} Current Measurements—Whole cell L-type Ca^{2+} current (I_{Ca}) was measured via patch clamp technique using an Axopatch 1D amplifier (Axon Instruments Inc.) (12). To activate I_{Ca} selectively, cells were voltage-clamped at −40 mV to inactivate Na^+ and T-type Ca^{2+} channels. K^+ currents were inhibited by 4 mmol/liter 4-aminopyridine and 5.4 mmol/liter CsCl instead of KCl in the HEPES buffer and the pipette solution containing (mmol/liter) CsCl 100, NaCl 10, tetraethanolamine Cl 20, HEPES 10, MgATP 5, EGTA 5, pH 7.2, adjusted with CsOH. I_{Ca} was elicited by 300-ms pulses from a holding potential of −40 to 0 mV at 0.5 Hz at 23 °C. I_{Ca} was measured as the difference between the current at the peak and the end of the 300-ms pulse.

Site-specific Phospholamban Phosphorylation—The detection of site-specific PLB phosphorylation was performed as described recently (11). Briefly, cardiomyocytes were treated for 10 min with specific β-AR subtype agonists as indicated and solubilized prior to electrophoresis at 95 °C for 5 min to dissociate fully PLB into its monomeric form. Following electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Serva), which was probed with the phosphorylation site-specific Ser^{16} PLB antibody (PhosphoProtein Research). Following incubation with a peroxidase-conjugated antibody (Dianova), the immunoreaction was detected with ECL (Amersham Pharmacia Biotech) and quantified with a video documentation system (Bio-Rad).

Protein Kinase A Activity—The activation of PKA in soluble and particulate fractions was analyzed by a modified method of Murray *et al.* (19). Rat cardiomyocytes were homogenized and centrifuged at 6,000 × *g* for 5 min. The resulting supernatant was taken to represent the soluble protein kinase activity and the resuspended pellet, the particulate fraction. The PKA activity is expressed as the activity ratio of malantide (Bachem) ^{32}P incorporation in the absence and presence of cAMP (2.8 μmol/liter).

Statistics—Results are presented as means ± S.E. Statistical significance was determined by Student's *t* test or analysis of variance when appropriate. Values with $p < 0.05$ were considered to be statistically significant.

RESULTS

The β_2-AR agonist, zinterol (10^{-5} M), increased the whole cell L-type Ca^{2+} current (I_{Ca}) to 161 ± 8.8% ($n = 7$, $p < 0.05$) of control in single rat cardiomyocytes (Fig. 1*a*), which was completely abolished by the β_2-AR antagonist ICI 118,551 (10). To delineate a role of cAMP-dependent PKA activation in the modulation of L-type Ca^{2+} channels, an inhibitory cAMP analog, (R_p)-cAMPs (10, 11), was used to specifically block PKA activation. In the presence of (R_p)-cAMPs (10^{-4} M) zinterol failed to augment I_{Ca} (Fig. 1*b*), indicating that the cAMP-dependent PKA activation is obligatory for β_2-AR-mediated modulation of L-type Ca^{2+} channels.

PLB, the main modulator of cardiac relaxation, is phosphorylated following cardiac β_1-AR stimulation at two adjacent phosphorylation sites, Ser^{16} (Fig. 2*a*) and Thr^{17}, catalyzed by PKA and Ca^{2+}/calmodulin-dependent kinase, respectively (3, 4, 20). In contrast, the β_2-AR agonist, zinterol, even at a maximal concentration (10^{-5} M for 10 min), had only a very minor

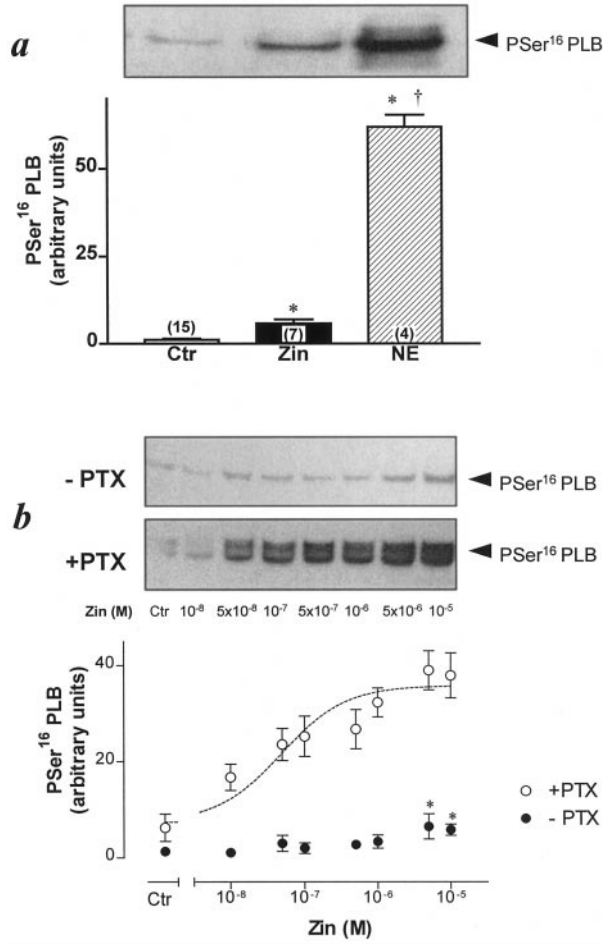

FIG. 2. **Western blot of phosphorylated Ser^{16} PLB in rat cardiomyocytes following β-AR subtype stimulation.** *a,* average effects of β_2-AR stimulation by zinterol (*Zin*, 10^{-5} M, 10 min) and β_1-AR stimulation by norepinephrine (*NE*, 10^{-7} M, 10 min, in the presence of 10^{-6} M prazosin) on Ser^{16} PLB phosphorylation (mean ± S.E., $p < 0.05$: * *versus* control, † *versus Zin*). *Inset* shows a representative Western blot. *b,* effect of PTX on the average dose-response relationship of the β_2-AR-mediated Ser^{16} PLB phosphorylation (mean ± S.E., $n = 4$–15 for each data point, * $p < 0.05$ *versus* control). Representative Western blots are shown as *inset*. Rat cardiomyocytes were incubated with different doses of zinterol (Zin, 10^{-8}-10^{-5} M, 10 min), as described under "Experimental Procedures."

effect on the PKA-mediated Ser^{16} phosphorylation of PLB, as detected with phosphorylation site-specific PLB antibodies (21) in the Western blot (Fig. 2, *a* and *b*). The dose-response relation and the time course of Ser^{16} PLB phosphorylation are shown in Fig. 2*b* and Fig. 3*c*, respectively. A maximal concentration (10^{-5} M) of the β_2-AR agonist, zinterol, only induced a minor increase in Ser^{16} PLB phosphorylation even if the incubation time was extended to 20 min (Fig. 3*c*). Concomitantly, the β_2-AR-mediated increase in contractility occurred in the absence of a significant relaxant effect (Fig. 3*a*). Thus, the failure of β_2-AR stimulation to induce PLB phosphorylation is the apparent mechanism for the absence of a lusitropic effect (Fig. 3*c*). These data illustrate that whereas both β_1- and β_2-AR share the common second messenger cAMP, they exhibit differences with respect to PKA-mediated protein phosphorylation and relaxant effects (8–12).

Based on our recent finding that β_2-AR dually couples to G_s and G_i proteins (16, 17), we hypothesized that the additional coupling of β_2-AR to G_i might interfere with the β_2-AR/G_s signaling, contributing to the restriction of β_2-AR-mediated cAMP signaling to a subsarcolemmal domain. To test this hypothesis, we examined the effect of G_i protein inhibition by PTX

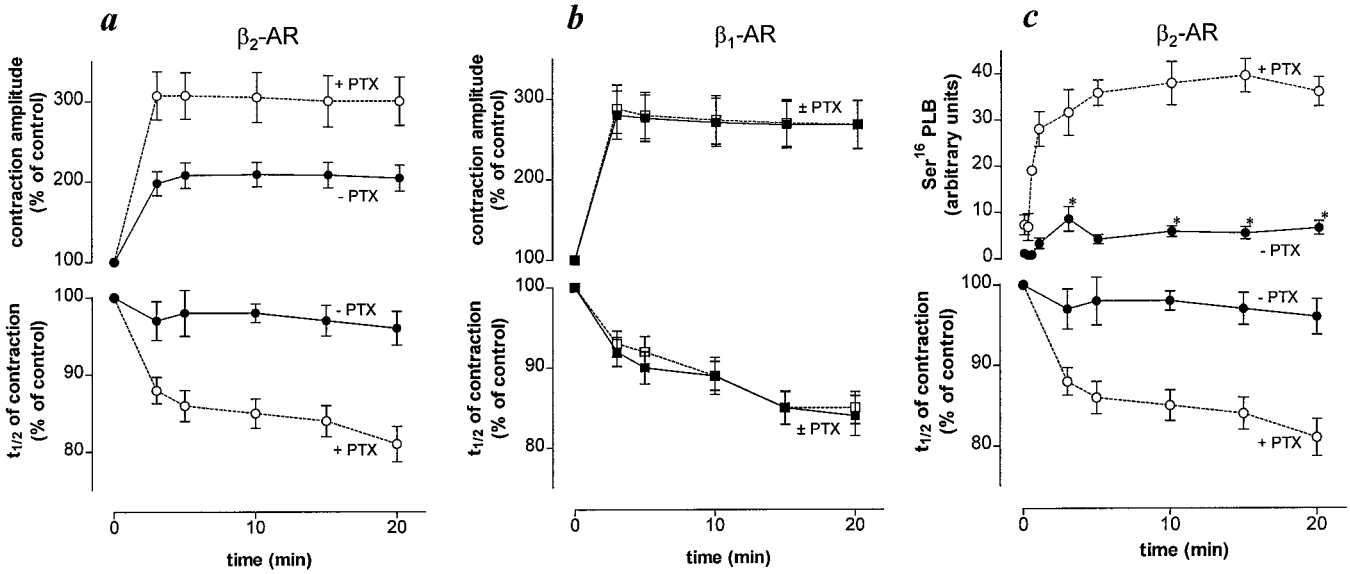

FIG. 3. **PTX treatment selectively enhances the β_2-AR-mediated augmentation of contraction amplitude, accelerates relaxation, and rescues Ser[16] PLB phosphorylation.** *a*, the time course of β_2-AR (zinterol (*Zin*), 10^{-5} M, 10 min)-mediated positive inotropic and relaxant effects in the presence (+*PTX*) and absence of PTX (−*PTX*) (n = 10 cells for each data point). *b*, β_1-AR stimulation (NE, 10^{-7} M and 10^{-6} M prazosin)-induced positive inotropic and relaxant effects are not affected by PTX (n = 10 cells for each data point). *c*, the time course of the β_2-AR (*Zin*, 10^{-5} M)-mediated *de novo* Ser[16] PLB phosphorylation (n = 4–15) correlates with the relaxation effect in PTX-treated cardiomyocytes. Base-line values for contraction amplitude are 5.9 ± 0.5 (n = 20) and 6.9 ± 0.5 (n = 20) and for $t_{½}$ are 378.9 ± 10.7 ms (n = 20) and 356.9 ± 19.4 ms (n = 20) in the absence or presence of PTX, respectively.

on the PLB phosphorylation following β_2-AR stimulation and its functional consequences. Whereas PTX itself had only a negligible effect on the basal Ser[16] PLB phosphorylation (Fig. 2*b*), β_2-AR stimulation with zinterol in PTX-treated cardiomyocytes markedly increased PLB phosphorylation in a dose-dependent manner (EC_{50} = 48.6 ± 1.8 nM) (Fig. 2*b*), with a maximal increase of 6.5-fold, comparable with that induced by the β_1-AR agonist norepinephrine (NE at 10^{-7} M) (37.9 ± 4.7 and 36.7 ± 7.7 in arbitrary units, respectively). Fig. 3*a* shows the time courses of β_2-AR effects on contraction amplitude and duration ($t_{½}$) in both PTX-treated and non-treated cells. In addition to the 1.5-fold potentiation of the β_2-AR inotropic response, inhibition of G_i function allowed zinterol to induce a *de novo* relaxant effect in rat cardiomyocytes. The β_2-AR-induced lusitropic effect in PTX-treated cells is highly comparable with that of β_1-AR stimulation in control cells (Fig. 3, *a* and *b*). Furthermore, the time course of the β_2-AR-induced Ser[16] PLB phosphorylation was tightly correlated to the time course of the relaxant effect in PTX-treated cardiomyocytes, both reaching a steady state within 5 min (Fig. 3*c*). In contrast, neither the β_1-AR-mediated contractile nor its relaxant response was affected by PTX (Fig. 3*b*). These results strongly suggest that the β_2-AR/G_i coupling functionally compartmentalizes the β_2-AR/G_s-mediated cAMP signaling, altering the quality as well as the magnitude of its cellular response.

To elucidate further the mechanism underlying the G_i-mediated spatial control of β_2-AR signaling, we measured the PKA activity following β-AR subtype stimulation. Similar to β_1-AR stimulation, β_2-AR activation also significantly increased the PKA activity ratio in both soluble and particulate fractions (Fig. 4). This suggests that, unlike β_1-AR, β_2-AR-mediated increases in cAMP accumulation (8, 10) and PKA activation (Fig. 4*a*) are dissociated from Ser[16] PLB phosphorylation (Fig. 2). Surprisingly, PTX did not significantly affect the response of PKA in either fraction (Fig. 4), suggesting that the cross-talk of G_s and G_i signaling following β_2-AR stimulation may occur downstream of PKA (see "Discussion"). We therefore examined the potential involvement of protein phosphatases in β_2-AR-mediated G_i signaling. Rat cardiomyocytes were treated with calyculin A (3×10^{-8} M) for 20 min to inhibit protein phosphatases. Control experiments showed that calyculin A at this concentration had only a marginal effect on contraction amplitude (127.1 ± 18.6% of control, n = 4), but induced a time- and dose-dependent augmentation in contraction at higher concentrations (data not shown). Interestingly, preincubation of cells with calyculin A at this near-threshold concentration markedly and selectively potentiated the submaximal contractile response to the β_2-AR agonist zinterol (10^{-6} M), whereas it had no effect on the submaximal β_1-AR (NE, 10^{-8} M)-stimulated contractile response (Fig. 5). Thus, the effects of protein phosphatase inhibition are similar to that of G_i inhibition by PTX, enhancing the contractile response in a β_2-AR-specific manner (Fig. 3). This result strongly suggests that protein phosphatases are likely to be involved in the β_2-AR/G_i-directed signaling. This conclusion was further substantiated by the observation that calyculin A failed to potentiate further the β_2-AR-mediated contractile response if the G_i pathway is disrupted by PTX treatment (Fig. 5). Therefore, protein phosphatases may serve as a key element of the β_2-AR-coupled G_i signaling cascade to spatially control the G_s-mediated cAMP/PKA signaling.

DISCUSSION

Recent advances in β_2-AR signaling have provided evidence for a novel subcellular compartmentalization of cAMP signaling. Specifically, although both β_1- and β_2-AR stimulation enhance cAMP accumulation (8, 10) and PKA activity and modulate I_{Ca} via cAMP/PKA-dependent signaling, the β_2-AR stimulation is uncoupled from the phosphorylation of more remote proteins (8, 9, 11). This indicates that the signaling may be highly localized to sarcolemmal microdomains or that it can be transmitted to cytoplasmic sites but locally inactivated there. The key question then is what mechanism enforces the tight spatial control of β_2-AR-mediated cAMP signaling. In principle, a localized cAMP signaling could arise from localization of signaling components, *e.g.* localization of cAMP by phosphodiesterases (22, 23) or PKA by specific anchoring proteins of PKA (24, 25). A close spatial association of Ca^{2+} channels with adenylyl cyclase and PKA (26, 27) could provide a structural

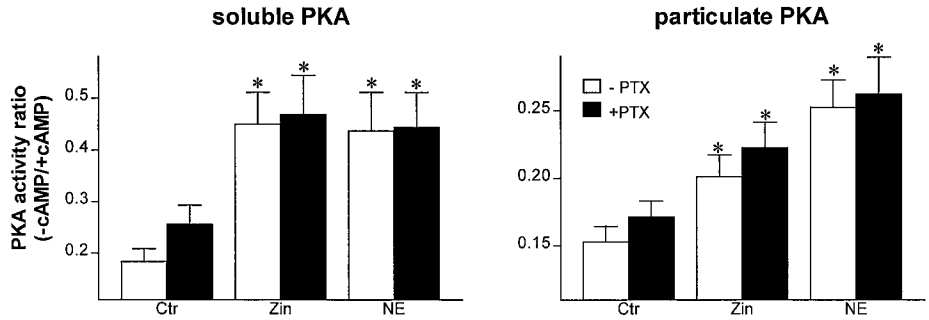

FIG. 4. **PTX treatment has no effect on β_2-AR stimulated protein kinase A activation.** Average effect of β_2-AR stimulation by zinterol (*Zin*, 10^{-6} M, 10 min) and β_1-AR stimulation by NE (10^{-7} M, 10 min, in the presence of 10^{-6} M prazosin) on PKA activity in soluble and particulate fractions in the presence (+*PTX*) or absence of PTX (−*PTX*). Data are shown as mean ± S.E. (n = 5–8), * $p < 0.05$ *versus* control.

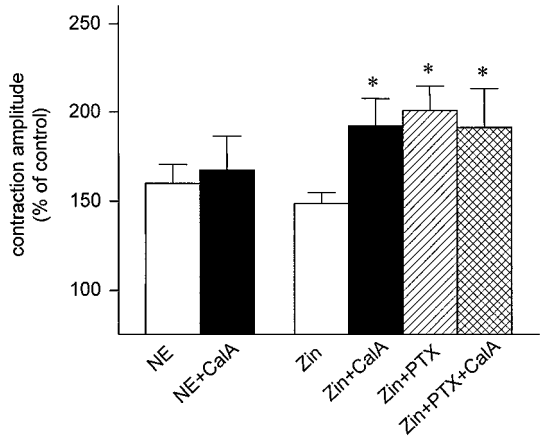

FIG. 5. **Involvement of protein phosphatases in the localization of β_2-AR signaling.** The protein phosphatase inhibitor, calyculin A (*CalA*, 3×10^{-8} M), selectively potentiates the β_2-AR agonist zinterol (*Zin*, 10^{-6} M)-induced inotropic effect but has no further potentiating effect on the β_2-AR contractile response in the presence of PTX pretreatment. The data (mean ± S.E., n = 7–19, * $p < 0.05$ *versus* zinterol) are expressed as percent change from values recorded before the application of the β-AR agonist. Base-line contractility is 5.98 ± 0.38 (n = 37), 5.20 ± 0.41 (n = 21), 5.04 ± 0.26 (n = 7), and 4.28 ± 0.48% (n = 7) of cell rest length for untreated, calyculin A-treated, PTX-treated, and PTX plus calyculin A-treated cells, respectively.

basis for the localized cAMP-dependent modulation of L-type Ca^{2+} channels following β_2-AR stimulation. However, since both cAMP and catalytic subunits of PKA (released following activation) are diffusive molecules, additional mechanisms should be involved to restrict actively their signaling to certain subcellular domains.

In the present study, we demonstrated that, apart from localization of signaling molecules of the cAMP/PKA cascade (22–25), an interaction between functionally opposing signal transduction pathways can also create compartmentalization of receptor-mediated signaling. In particular, following inhibition of G_i function by PTX treatment, β_2-AR stimulation markedly increased Ser^{16} PLB phosphorylation and elicited a *de novo* lusitropic effect in rat ventricular myocytes, which is highly comparable with that following β_1-AR stimulation. Thus, inhibition of G_i proteins converts the β_2-AR signaling mode to that of β_1-AR-like signaling. These results strongly suggest that the β_2-AR/G_i coupling effectively compartmentalizes the β_2-AR/G_s-mediated cAMP signaling, altering not only the magnitude but also the quality of its cardiac response.

It is well established that activation of protein phosphatases functionally counterbalances cellular effects of protein kinases. Recently, it has been reported that the inhibitory effect of the G_i protein-coupled muscarinic receptor stimulation on the β-AR-induced cAMP signaling is largely mediated by activation of protein phosphatases (28). Furthermore, angiotensin II increases protein phosphatase 2A activity in cultured neurons through a G_i protein-dependent mechanism (29). We therefore investigated the potential involvement of protein phosphatases in the cross-talk of the β_2-AR-stimulated G_s/G_i signaling. Interestingly, calyculin A, a non-selective protein phosphatase inhibitor, mimics the PTX effect. Both interventions, G_i inhibition by PTX or protein phosphatase inhibition by calyculin A, had a non-additive potentiating effect on β_2-AR-mediated contractile response when applied together, suggesting that PTX and calyculin A act on a common signaling pathway. Therefore, the negating and spatial restricting effects of β_2-AR-activated G_i proteins on the G_s-directed signaling might be mediated by a modulation of the protein phosphatase/kinase balance. For instance, a high dephosphorylation rate of non-sarcolemmal proteins following β_2-AR stimulation might negate the PKA-mediated phosphorylation of PLB (and other cytoplasmic proteins).

β_2-AR-mediated G_i protein activation could either directly modulate protein phosphatase activity or inhibit PKA activation at subcellular compartments and secondarily modulate the protein phosphatase inhibitor 1 and therefore protein phosphatase activity (7). In other words, the G_i protein activation could negate the β_2-AR/G_s-mediated PKA activation in certain subcellular microdomains, resulting in a higher protein phosphatase activity and a lower protein phosphorylation in those subcellular microdomains, which is functionally indistinguishable from a G_i-mediated direct activation of protein phosphatases. Previous studies in frog and canine hearts have provided evidence for the involvement of phosphodiesterases in the compartmentalization of β-AR signaling (22, 23). However, our previous studies have demonstrated that PTX treatment has no significant effect on β_2-AR-mediated global cAMP accumulation (10). The present results show further that β_2-AR-stimulated increase in PKA activity in two different subcellular fractions is insensitive to PTX. Although the non-additive effect of protein phosphatase and G_i protein inhibition suggests, but does not prove, that β_2-AR stimulation is modulating protein phosphatase activity through a PKA-independent pathway, these standard measures of global cAMP levels and PKA activity in cardiomyocytes provide no insight into the highly localized signaling. Although we are unable to distinguish if β_2-AR/G_i signaling modulates protein phosphatases PKA-independent or -dependent due to technical limitation, the present results strongly suggest that protein phosphatases are critically involved in the β_2-AR/G_i signaling, contributing to the functional compartmentalization of the β_2-AR/G_s signaling in rat ventricular myocytes.

It is noteworthy that the β_2-AR-mediated cardiac response and the extent of the G_i protein coupling may vary substantially among species, resulting in an enormous diversity in cardiac β_2-AR-mediated responses and its sensitivity to PTX. In mouse cardiomyocytes β_2-ARs are not functional unless G_i function is inhibited by PTX treatment, indicating a high level of G_i protein coupling (16). In contrast, β_2-AR stimulation does induce positive inotropic and lusitropic effects as well as phosphorylation of regulatory proteins in the failing human heart

(30, 31). Between these extremes, β_2-AR stimulation in rat cardiomyocytes induces significant increases in I_{Ca} and contractility in the absence of phosphorylation of cytoplasmic regulatory proteins. PTX treatment further enhances the β_2-AR contractile response (17) and restores its ability to phosphorylate cytoplasmic regulatory proteins in this species. The situation in canine myocytes is similar to that of rat myocytes, except that β_2-AR does induce lusitropic as well as inotropic effects in the absence of cytoplasmic protein phosphorylation (9, 11).

The aforementioned data also illustrate that the same signaling molecule, cAMP, mediates remarkably different cardiac functional responses following β_1- and β_2-AR stimulation (8–12). Analogously, it has been shown that intracellular Ca^{2+} located in different subcellular compartments may mediate distinctly different and sometimes even opposing cellular functions. For instance, a global elevation in cytosolic Ca^{2+} in arterial smooth muscle cells causes vasoconstriction, but Ca^{2+} sparks near the sarcolemma induce relaxation (32). In this case, local Ca^{2+} gradients are possible, because various endogenous binding sites buffer Ca^{2+} of discrete origins. Thus, physical or functional compartmentalization of ubiquitous intracellular messengers, such as cAMP and Ca^{2+}, creates specificity and diversity of a given receptor-mediated signaling.

In summary, we have demonstrated that, in addition to the potentiation of the inotropic response, inhibition of G_i function by PTX induces a *de novo* lusitropic effect and PLB phosphorylation following β_2-AR stimulation in rat ventricular myocytes. These results suggest a contribution of β_2-AR/G_i-coupled signaling to the compartmentalization of β_2-AR/G_s-stimulated cAMP/PKA signaling, possibly through a protein phosphatase-dependent mechanism. In addition, the present study demonstrates that compartmentalization of a common second messenger-directed signaling allows for selective modulation of a variety of target proteins and cellular processes, creating signaling specificity and versatility among closely related G protein-coupled receptors.

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