Identification of G protein-coupled signaling pathways in cardiac fibroblasts: cross talk between \( G_\text{q} \) and \( G_\text{s} \)

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Meszaros, J. Gary, Annette M. Gonzalez, Yuka Endo-Mochizuki, Sonia Villegas, Francisco Villareal, and Laurence L. Brunton. Identification of G protein-coupled signaling pathways in cardiac fibroblasts: cross talk between \( G_\text{q} \) and \( G_\text{s} \). Am. J. Physiol. Cell Physiol. 278: C154–C162, 2000.—Cardiac fibroblasts (CFs) are an important cellular component of myocardial responses to injury and to hypertrophic stimuli. We studied G protein-coupled receptors to understand how CFs integrate signals that activate \( G_\text{q} \), \( G_\text{s} \), and \( G_i \). We predicted that the second messenger pathways present in CFs were distinct from those in cardiac myocytes and that unique signaling interactions existed in the CFs. ANG II, bradykinin, ATP, and UTP stimulated inositol phosphate (IP) production 2.2- to 7-fold. Each of these agonists elevated intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\( i \)) via release from the intracellular Ca\(^{2+}\) storage compartment. Endothelin-1 (ET-1), carbachol, and norepinephrine failed to increase either IP production or [Ca\(^{2+}\)]\( i \). Although agonists that activated IP and Ca\(^{2+}\) transients had no effect on cAMP production when administered alone, these agents potentiated the \( \beta_2 \)-adrenergic response two- to fourfold. Hormones known to inhibit adenyl cyclase activity in cardiac myocytes, such as ET-1 and carbachol, failed to lower the \( \beta_2 \)-adrenergic response in fibroblasts. Order of potency and inhibitor data indicate that the functional receptor subtypes in these cells are \( \beta_2 \), P2Y\(_2\), and AT\(_1\) for isoproterenol, ATP, and ANG II, respectively. We conclude that CFs express functional G protein-linked receptors that couple to \( G_\text{q} \) and \( G_\text{s} \), with little or no coupling to \( G_i \). The expression of receptors and their coupling to \( G_\text{q} \) but not to \( G_i \) linked responses distinguishes the signaling in CFs from that in myocytes. Furthermore, agonists that activate \( G_\text{q} \) in CFs potentiate stimulation of \( G_\text{s} \), an example of signaling cross talk not observed in adult myocytes. These data suggest that G protein-mediated signaling in CFs is unique and may contribute to the specificity of hormone and drug action on individual cell types within the heart.

FIBROBLASTS COMPRISE AS MUCH as two-thirds of cell number in cardiac tissue (9) and play an active role in wound healing, hypertrophy, and fibrosis in the heart. All of these processes are thought to be regulated by hormones and paracrine factors in the adult myocardium. Studies in cardiac fibroblasts (CFs) have focused on gene regulation and secretion of various extracellular matrix (ECM) proteins, most notably collagen, and on the hormones that regulate these ECM proteins (reviewed in Ref. 42). Few reports to date have thoroughly examined G protein-coupled second messenger systems in CFs. Direct ligand binding studies and functional studies indicate the presence of receptors for endothelin (ET-1) and ANG II (16, 23, 42). ANG II promotes proliferation and collagen deposition in cultured CFs (3, 8). Fibroblasts also produce ANG II and other putative growth factors that may act as autocrine and paracrine modulators of cell function and growth within the heart (9, 25, 28). Understanding which hormones and signaling pathways control the proliferative and synthetic capacities of the CFs is necessary to assess their contribution to cardiac remodeling, fibrosis, and hypertrophy.

This laboratory has previously studied cardiac signal transduction using whole ventricles excised from isolated perfused adult rat hearts as well as isolated ventricular myocytes (5, 6, 20, 31). Many differences in transmembrane signaling events have been observed in whole ventricles vs. isolated ventricular myocytes, indicating that nonmyocytes play a significant role in the hormonal responsiveness of intact cardiac tissue. For instance, adult rat ventricular myocytes have relatively few \( \beta_2 \) receptors (\( \beta_2 \) receptors predominate), whereas the intact ventricle has an abundance of \( \beta_2 \) receptors (6, 26). Additionally, elevation of inositol phosphates (IPs) is negligible in isolated adult rat ventricular myocytes following exposure to ANG II (20), whereas the intact ventricle has a prominent response to ANG II (22). These studies suggest that components of signal-transducing pathways are not uniformly distributed among different cell types in the heart. The cell-cell communication that exists between myocytes and nonmyocytes is physiologically important, since these cells interact via paracrine mechanisms to produce the hormonal responses characteristic of intact cardiac tissue. For example, conditioned medium from nonmyocyte cultures (the majority of which were CFs) induced neonatal cardiac myocyte hypertrophy in vitro (28). In a similar study, conditioned medium from neonatal CFs treated with ANG II stimulated hypertrophic growth of neonatal myocytes (25). Mechanical and other cell-cell interactions may also exist among cells of...
the myocardium (fibroblasts, myocytes, endothelial cells, and smooth muscle cells), adding to the complexity of cellular communication and signaling in the heart. Identifying signaling components in each cardiac cell type is essential to understanding the basis of hormonal effects on cardiac function.

In this study, we examine G protein-mediated second messenger production in the major nonmyocyte cell, the CF. We hypothesized that the G protein-coupled second messenger pathways present in CFs were distinct from those known to exist in cardiac myocytes. The data support the existence of unique G protein-coupled receptors in CFs: strong coupling to Gq [ANG II, bradykinin (BK), and purinergic agonists] and Gs (isoproterenol and epinephrine), with little or no functional coupling to Gi (carbachol, ET-1, and norepinephrine). We also observed a potentiative interaction between the Gq- and Gs-linked signaling in which activation of Gs-phospholipase C (PLC)-IP signaling enhanced the β-adrenergic response to isoproterenol.

MATERIALS AND METHODS

Preparation and culture of adult rat CFs. Fibroblasts were prepared by the methods of Eghbali et al. (11) and Villarreal et al. (43). Briefly, the ventricles of three to five hearts from adult male 300- to 350-g Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were minced, pooled, and placed in a collagenase-pancreatin digestion solution. After four digestions, cells were pooled and debris and myocytes were removed by unit gravity sedimentation. Fibroblasts were isolated from enriched fractions on Percoll and suspended in DMEM (GIBCO BRL, Grand Island, NY) supplemented with penicillin, streptomycin, fungicide, and 10% fetal bovine serum (FBS; Gemini Bio-Products, Calabasas, CA). After a 30-min period of attachment to uncoated culture plates, cells that were weakly attached or unattached were rinsed free and discarded. After 48–72 h, confluent cultures were passaged by trypsinization. For signaling assays, only cells that were weakly attached or unattached were removed by unit gravity sedimentation. Fibroblasts were grown on 105 cells/plate or 60-mm plates (1.5–2.0 × 10^5 cells/plate) or 35-mm plates (0.5–1.0 × 10^5 cells/plate) or 60-mm plates (1.5–2.0 × 10^5 cells/plate) and grown to 80–90% confluency (3–4 days). The purity of these cultures was >95% CFs as measured by vimentin and collagen (types I and III) expression as previously described (43, 44).

Phosphoinositide hydrolysis. Cells in 35-mm dishes were washed with DMEM and labeled for 18 h with myo-[3H]inositol (5 µCi/ml; Amersham, Arlington Heights, IL) in DMEM without serum. Free [3H]inositol was removed by rinsing, after which cells were equilibrated with DMEM containing 10 mM LiCl, 10 mg/ml leupeptin, and 1 mg/ml BSA. After 10 min, agonists were added, and incubation continued at 37°C for 15 min. Incubations (performed in duplicate) were terminated by aspiration of medium and the addition of cold 5% TCA to a volume of 2 ml during the entire experiment. Fluorescence measurements were performed on 8–12 fields of cells in the presence of 1 mM Ca^2+ and on 3–5 fields in the absence of extracellular Ca^2+. In all cases, the existence of releasable Ca^2+ stores was confirmed at the end of the recording by the addition of 1 µM thapsigargin and the R_max and R_min were determined by ionomycin and EGTA additions, respectively.

Assay of cAMP accumulation. Cells in 60-mm dishes were incubated with DMEM without serum for 2 h and then equilibrated with DMEM containing 0.1 mM IBMX. After 15 min, agonist or diluent was added, and the incubations continued at 37°C for 10 min. Incubations were terminated by aspiration of the medium and the addition of cold 5% TCA to the adherent cells. TCA extracts were collected, purified, and assayed for cAMP as previously described (20). Data, corrected for recovery, are expressed as picomoles of cAMP per dish or per milligram protein.

Assay of cGMP accumulation. Experiments to assess cGMP were similar to those for cAMP. Briefly, fibroblasts were pretreated with 0.5 mM IBMX for 15 min before 6-min incubations with agonists; 5% TCA supernatants were extracted four times with water-saturated ether, and the cGMP content was determined by RIA using the method of Harper and Brooker (17). Data are expressed as picomoles of cGMP per dish.

Estimation of protein content. When necessary, acid-precipitable material was suspended in 0.4 N NaOH and the protein content was estimated by the method of Bradford (4).

Analysis of data. Statistical comparisons (t-tests and single-factor ANOVA) were performed with the program InStat, and curve fitting was performed with the program GraphPad Prism 2.0 (GraphPad Software, San Diego, CA).

Materials. Unless otherwise noted, all chemicals were reagent grade and were purchased from Sigma (St. Louis, MO).

RESULTS

IP production. We tested a variety of drugs and hormones for their capacity to stimulate IP production (Fig. 1). Hormones that we expected to enhance IP production, on the basis of previous studies using intact ventricles, failed to do so: muscarinic cholinergic agonists (carbachol) and α-adrenergic agonists (norepinephrine and phenylephrine) were without effect. These results suggest an absence of functional (G protein-coupled) muscarinic and α₁-adrenergic receptors on the cells. Of the agents that did stimulate IP production, ATP, UTP, and ANG II were the most efficacious.
producing increases of three- to sevenfold, and BK doubled IP production. Each of these effective agonists produced a statistically significant increase over control [3H]phosphoinositide hydrolysis ($P_{0.05}$). Surprisingly, ET-1 elicited a small and statistically insignificant increase ($<30\%$) in IP production over basal.

Concentration dependence curves demonstrated that ANG II enhances IP production with an EC 50 of 1 nM (Fig. 2A), in agreement with the affinity determined by Villarreal et al. (43), whose ligand binding studies indicated that the ANG II receptor of the fibroblasts is of the AT1 subtype. The AT1-specific antagonist losartan (at 0.1 µM) shifted the concentration dependence curve to the right by two orders of magnitude, indicating AT1 receptor involvement. Dose-shift analysis (35) yields a calculated value for the apparent inhibition constant ($K_i$) for losartan of 1 nM, an appropriate affinity for losartan binding at an AT1 receptor.

The efficacy of the purinergic receptor agonists UTP and ATP in the IP assays demanded further studies to determine the identity of the purinergic receptors present in the CFs. Because purinergic receptors may be distinguished by the order of potency of various nucleotides, we tested the effect of ATP, ADP, and UTP over a concentration range of $10^{-7}$ to $10^{-2}$ M (Fig. 2B). UTP was the most potent, with an EC 50 of 5 µM. The rank order of potency, UTP > ATP > ADP, indicates that the functional purinergic receptor linked to IP production in adult CFs is a P2Y2 receptor.

Intracellular Ca$^{2+}$ measurements. To confirm the existence of a hormone-sensitive Ca$^{2+}$ release system in adult CFs, we employed the Ca$^{2+}$-sensitive dye indo 1-AM to make fluorometric measurements in multiple fields of five to eight adherent cells. We first sought to extend the data from the IP assays to demonstrate a measurable increase in [Ca$^{2+}$], following administration of those agonists that were effective agonists of IP accumulation (see Fig. 1). Indo 1 emission ratios (405 nm/495 nm) were collected in the presence (Fig. 3) and absence (Fig. 4) of extracellular Ca$^{2+}$ to determine whether the source of the Ca$^{2+}$ increase was extracellular or internal stores. The actual [Ca$^{2+}$], values were calculated and are summarized in Table 1; they range from 81 to 1,260 nM (1 mM extracellular Ca$^{2+}$) and from 47 to 1,197 nM (0 extracellular Ca$^{2+}$). The agonists that were most effective in increasing IP production (ANG II, UTP, ATP, and BK) also elevated [Ca$^{2+}$]. Hormones that elicited little or no significant stimulation of IP production, such as carbachol and ET-1, failed to increase [Ca$^{2+}$], (data not shown). We conclude that the agonist-induced Ca$^{2+}$ transients were evident only in response to agents most efficacious in producing IPs. Furthermore, experiments performed in the absence of extracellular Ca$^{2+}$ (by chelation with EGTA; Fig. 4) indicated that the Ca$^{2+}$ transients were mainly due to release from internal stores, since they were similar in magnitude to those performed in 1 mM extracellular Ca$^{2+}$.
Ca^{2+}. Taken together, the IP and Ca^{2+} data strongly suggest that the IP₃ receptor plays a major role in Ca^{2+} mobilization and signaling in CFs.

cAMP production. We tested the agonists used in the IP studies to assess their capacity to influence cAMP metabolism. These experiments were conducted in the presence of a phosphodiesterase (PDE) inhibitor, 0.1 mM IBMX, so that changes in cellular cAMP content could be ascribed to enhanced activation of Gₛ-adenylyl cyclase and not to altered PDE activity. Under these conditions, the basal content of cAMP in control cells was 20 pmol/mg protein. The β agonist isoproterenol stimulated a large increase in cAMP accumulation, to 500 pmol/mg protein. This robust β-adrenergic response indicates that CFs possess a complete and functional β receptor-Gₛ-adenylyl cyclase pathway. Order of potency data (epinephrine > norepinephrine; Fig. 5) indicate that the β receptor in these cells is of the β₂-subtype. PGE₂ modestly stimulated cAMP accumulation (fourfold over basal) indicating the presence of a PG receptor linked to Gₛ. No agents that stimulated IP production increased cAMP levels when added alone.

Agents known to activate the Gₛ pathway and to inhibit adenylyl cyclase in cardiac myocytes and other cell types were tested for their capacity to reduce the β-adrenergic response of isoproterenol (Fig. 6). ET-1, phenylephrine, norepinephrine, and carbachol did not significantly reduce isoproterenol-induced cAMP production. Carbachol reduced cAMP production by 18%, a reduction that was not statistically significant. These data suggest that CFs do not express ETA, a₂-adrenergic, or muscarinic receptors that functionally couple to
adenylyl cyclase via G<sub>i</sub>. This was surprising, since ET and carbachol are potent activators of G<sub>i</sub> in adult rat cardiac myocytes, reducing G<sub>s</sub>-linked cAMP production by 50–65% (18, 20).

Synergistic interaction of G<sub>i</sub>-linked IP pathway with G<sub>s</sub>-cyclase pathway. Frequently, agents that stimulate IP production via G<sub>i</sub>-linked pathways also act at receptors linked to G<sub>i</sub> to inhibit adenylyl cyclase. Thus we tested the effects of agonists that act through the G<sub>i</sub> signaling pathway on isoproterenol-induced cAMP production. None of the agonists produced the anticipated reduction of the β-adrenergic response. Rather, hormones that were strong stimulators of IP production acted synergistically with isoproterenol to potentiate cAMP production in CFs (Fig. 6). These agonists included ANG II, BK, UTP, and ATP, none of which increased cAMP when added alone (see above). The synergism was observed both in the absence and presence of IBMX, suggesting that alterations in PDE activity are not involved in the cross talk (data not shown). ET, which had a statistically insignificant effect on IP production, did not potentiate the β-adrenergic response.

We examined the synergistic effects of ANG II and of P2Y agonists in more detail to determine whether the same receptor type that couples to the G<sub>i</sub>-PLC pathway is responsible for the synergy with the β-adrenergic response. The synergistic effect of ANG II on isoproterenol-induced cAMP production was inhibited by losartan (0.1 mM) with an apparent affinity (K<sub>i</sub> = 1 nM) consistent with interaction at an AT<sub>1</sub> receptor (Fig. 7). Figure 7 shows that 10<sup>-5</sup> to 10<sup>-6</sup> ANG II causes a substantial enhancement of the β-adrenergic response and that losartan (constant at 0.1 mM) blocks the enhancement. Losartan also inhibited ANG II-induced IP production with a similar affinity. Additionally, potentiation of isoproterenol-induced cAMP production by UTP is greater than that of ATP, suggesting that the G<sub>i</sub>-linked P2Y<sub>2</sub> receptor mediates the synergistic effect of the purines in the β-adrenergic response. These data, along with those observed with BK, suggest that activation of G<sub>i</sub>-linked IP production and intracellular Ca<sup>2+</sup> release are required to potentiate cAMP accumulation by G<sub>i</sub>.

Production of cGMP. In the presence of 0.5 mM IBMX, basal cGMP content of cultured CFs is 0.7–1.0 pmol/60-mm dish. Nitroprusside and atrial natriuretic factor increased cGMP content by 3.8- to 6-fold, indicating that the cells contain both the soluble and membrane-bound forms of guanylyl cyclase. The presence of a nitroprusside-sensitive guanylyl cyclase suggested that hormones that stimulate IP production and elevate [Ca<sup>2+</sup>] and cAMP (Fig. 1–4) would elevate cellular cGMP via Ca<sup>2+</sup>/calmodulin stimulation of soluble NO synthase, with the resultant NO stimulating the soluble guanylyl cyclase. Surprisingly, no agonists that stimulated IP production or elevated [Ca<sup>2+</sup>] altered cellular cGMP content (Fig. 8). The addition of the Ca<sup>2+</sup> ionophore...
Ca^{2+} accumulation causes mobilization of intracellular in CFs. The ANG II receptor of the rat CFs is the AT1 receptor, in duplicate.

Response to 1 mM isoproterenol alone is shown as a dashed line. Data are means ± SE of 3 replicate experiments, each performed in duplicate.

A-23187 (10 µM) likewise caused no accumulation of cGMP. Thus it appears that soluble NO synthase is absent from CFs or is inactive; as a result, the hormones that elevate [Ca^{2+}], do not increase cGMP levels in CFs.

**DISCUSSION**

In this report, we show evidence that a number of functional receptors in the CFs, including β-adrenergic, ANG II, BK, and purinergic P2Y2 receptors, couple to G protein-linked signaling pathways in a pattern that is distinct from that of cardiac myocytes. All of these receptors, except the β2 receptor, couple to stimulate phosphoinositide hydrolysis via Gq and PLC; sufficient IP accumulation causes mobilization of intracellular Ca^{2+}. A number of these putative Gq-linked receptors can be subtyped on the basis of our data. The functional ANG II receptor of the rat CFs is the AT1 receptor, in agreement with the binding data of Villarreal et al. (43) and other investigators (9, 12). The identification of this receptor is based on the effectiveness and appropriate affinity of the AT1 antagonist losartan. Villarreal et al. (43) found that stimulation of this receptor increases expression of collagen and fibronectin mRNAs and increases collagen production. Activation of AT receptors appears to influence or cause proliferative changes associated with cardiac hypertrophy and failure in the rat model (9, 14, 17, 36). Stimulation of AT receptors in cardiac myocytes causes hypertrophy (27), increased mitogen-activated protein (MAP) kinase activity (14, 36), p21 Ras activation (33), and activation of signal transducers and activators of transcription (2). The transmembrane signaling that we describe for AT in the CF distinguishes the AT receptor of the fibroblasts from that of the cardiac myocyte, since ANG II does not activate significant phosphoinositide hydrolysis in freshly isolated adult rat ventricular myocytes (20). Other cardiac cell types express AT receptors as well (32, 39) but do not comprise a major fraction of the cardiac mass. From our data and those of the studies discussed above, we conclude that CFs likely represent a major site of ANG II action in the heart, an action mediated at least in part by the Gq-PLC-IP pathway.

We also tested two other peptides that have been associated with proliferative changes in the myocardium, ET-1 and BK. ET-1 causes increased CF proliferation and stimulates production of ECM proteins (15, 16), whereas BK appears to inhibit ECM production (24). Both of these peptide hormones normally couple to Gq-PLC-linked signaling pathways. BK modestly stimulated IP production in the current study; the effect of ET-1 did not reach statistical significance. This small effect of ET-1 did not result in the mobilization of Ca^{2+} as measured by indo 1 fluorescence. This may be due to the low level of IP production in response to ET-1; even in the presence of 10 mM LiCl we observed only a 30% increase over basal. One other study demonstrated that ET-1 modestly increased [Ca^{2+}] in CFs isolated from Wistar-Kyoto rats (41), a discrepancy that we attribute to differences in rat strains and/or differences in cell isolation techniques. In our case, ET-1-induced IP production in CFs appears insufficient to elicit a measurable Ca^{2+} transient. Consistent with the lack of Ca^{2+} response is the failure of ET to potentiate the β-adrenergic response (see below).

ET receptor coupling in the fibroblasts differs from that in the myocyte. Our data indicate negligible coupling of fibroblast ET receptors to Gq-PLC and no coupling to Gs-adenylyl cyclase. One binding study found roughly equal expression of ETa and ETb subtypes in adult rat CFs (13); another found predominantly ETb receptors (23). It is surprising that cells with significant numbers of ETa receptors (~10,000 per cell, from Ref. 13) do not show the expected coupling of the ETA receptor to Gs-linked inhibition of adenylyl cyclase. This distinguishes the fibroblast ET receptor and its coupling from that of the adult cardiac myocyte, in which we find a single population of ETa receptors that couples strongly to both Gs and Gi (19, 20).
CFs express functional receptors for the major transmitters released from adrenergic storage vesicles, catecholamines and ATP. The catecholamine receptors of CFs couple to stimulate cAMP predominantly through the β2 receptor subtype, on the basis of the order of potency data. The predominance of β2 receptors in CFs is consistent with earlier observations that these receptors exist in membranes prepared from whole heart and ventricles but appear to be only a small proportion (~15%) of β receptors in purified cardiac myocyte preparations (6, 26). Additionally, it has been observed that β2 receptors may couple to G1 (in addition to Gs) in isolated cardiac myocytes, which may suggest that these receptors have differential effects on cAMP production over that of Gs (largely 15%).

When we stimulated the β2 and P2Y2 receptors of CFs simultaneously, the result was a potentiation of cAMP production with an order of potency indicative of a P2Y2 receptor. The potentiation of cAMP production by activated phosphoinositide hydrolysis was not restricted to the interaction of purinergic and β2 agonists. ANG II and BK also caused a synergistic accumulation of cAMP; the interaction extends to other agonists of adenylyl cyclase (PGE2 and forskolin) as well. A number of mechanisms are possible. Bell et al. (1) first noted that a stimulation of protein kinase C (PKC) by phorbol esters led to an enhancement of adenyl cyclase activity in 549 lymphoma cells, a finding that may reflect the capacity of types II and V adenylyl cyclase to function as a substrate for PKC (38). Houssay (21) reviewed the possible targets for regulation of cAMP metabolism by PKC, including reduction of inhibitory tone by Gα2 and the modulation of activities of PDE isozymes. Houssay also stressed the role of PKC in the interaction of signal transduction pathways through phospholipases and eicosanoid production, on the activity of the tyrosine kinase activities of growth factor receptors, and on the regulation of ion channels. Mobilization of Ca2+ could stimulate Ca2+/calmodulin-sensitive adenyl cyclase (40). Recent work indicates that cross talk between Gs and Gα signaling pathways may also involve βγ-subunits released when Gα is activated, with either direct action on adenyl cyclase or actions mediated by other effectors such as growth factors, receptor tyrosine kinases, and MAP kinases (37). The precise mechanisms by which cross talk occurs between Gs and Gα in CFs are not known and require further study, as do the long-term or “downstream” consequences of Gs/Gα cross talk. We have preliminary evidence that the interaction of the Gα1PLC with Gs-stimulated cAMP production produces significant effects on immediate early gene activation: treatment of CFs with ANG II in combination with isoproterenol reduces c-jun mRNA expression compared with that of ANG II alone (Endo-Mochizuki and Brunton, unpublished observations). These responses to ANG II may involve not only the Gs pathway but also a number of Ca2+-dependent steps, including activation of PKC, tyrosine kinases, MAP kinases, and S6 kinases (34).

The β-adrenergic responses may involve multiple effects of protein kinase A and interactions of stimulatory and inhibitory transcriptional control factors with the cAMP response elements of target genes.

In summary, this study demonstrates that adult rat CFs contain G protein-coupled signaling systems that are distinct from those of adult rat cardiac myocytes. A comparison of functional G protein-coupled signaling in adult rat CFs and myocytes is shown in Table 2. CFs have receptors for the peptide growth factors, ANG II and BK, that link to Gs-PLC. These cells also respond to the two major components of the adrenergic storage vesicle, norepinephrine and ATP, with β2 receptors linking to adenyl cyclase via Gs and P2Y2 receptors linking to IP production and Ca2+ mobilization. Surprisingly, we found no evidence of muscarinic receptor coupling to Gs or Gα, linkages that are prominent in cardiac myocytes. ET does not appear to couple to Gs or Gα in fibroblasts, also in contrast to the ET-A receptor coupling observed in myocytes. Last, we describe an intriguing Gs/Gα cross talk phenomenon not yet described in myocardial cells, which may be important in specific signaling events controlling the function of CFs within the myocardium.

### Table 2. Functional G protein-coupled signaling in isolated adult rat cardiac fibroblasts vs. myocytes

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<tr>
<th>Receptor</th>
<th>Fibroblasts</th>
<th>Myocytes (Ref.)</th>
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<tbody>
<tr>
<td>BK</td>
<td>Moderate</td>
<td>Strong (30)</td>
</tr>
<tr>
<td>ET-1</td>
<td>Negligible</td>
<td>Strong(Gs) (20)</td>
</tr>
<tr>
<td>AT1</td>
<td>Strong</td>
<td>Negligible (20*)</td>
</tr>
<tr>
<td>P2Y</td>
<td>Strong</td>
<td>Negligible*</td>
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<td>Muscarinic</td>
<td>None</td>
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<tr>
<td>α1-Adrenergic</td>
<td>None</td>
<td>Strong (7)</td>
</tr>
<tr>
<td>β-Adrenergic</td>
<td>Strong (largely β2)</td>
<td>Moderate (6, 26)</td>
</tr>
<tr>
<td>PGE2</td>
<td>Moderate</td>
<td>Negligible*</td>
</tr>
<tr>
<td>Muscarinic</td>
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<tr>
<td>α2-Adrenergic</td>
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<td>None (7)</td>
</tr>
<tr>
<td>ET-1</td>
<td>None</td>
<td>Strong (20)</td>
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Gs signaling was assessed as agonist-stimulated inositol phosphate production; presence of Gs was inferred from agonist-stimulated cAMP accumulation; Gi activity was assessed as agonist-induced inhibition of Gs-stimulated cAMP production. *Meszaros and Brunton, unpublished observations. †J. R. Kanter and L. L. Brunton, unpublished observations.
REFERENCES


