β-Adrenergic Receptor Activation Induces Internalization of Cardiac CaV1.2 Channel Complexes through a β-Arrestin 1-mediated Pathway*§

Received for publication, March 17, 2008, and in revised form, April 21, 2008. Published, JBC Papers in Press, May 5, 2008, DOI 10.1074/jbc.C800061200

Rachele Lipsky†, Essie M. Potts†, Sima T. Tarzami†, Akil A. Puckerin†, Joanne Stocks‡, Alison D. Schecter§, Eric A. Sobie‡, Fadi G. Akar‡, and Maria A. Diversé-Pierluissi‡

From the †Department of Pharmacology and Systems Therapeutics and the ‡Cardiovascular Research Institute, Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029

Voltage-dependent calcium channels (VDCCs) play a pivotal role in normal excitation-contraction coupling in cardiac myocytes. These channels can be modulated through activation of β-adrenergic receptors (β-ARs), which leads to an increase in calcium current (I_Ca-L) density through cardiac CaV1 channels as a result of phosphorylation by cAMP-dependent kinase A. Changes in I_Ca-L density and kinetics in heart failure often occur in the absence of changes in CaV1 channel expression, arguing for the importance of post-translational modification of these channels in heart disease. The precise molecular mechanisms that govern the regulation of VDCCs and their cell surface localization remain unknown. Our data show that sustained activation of β-ARs induces internalization of a cardiac macromolecular complex involving VDCC and β-arrestin 1 (β-AR, CaV1.2, and Cav1.2). Pretreatment with pertussis toxin prevents the internalization of CaV1.2 channels. β-Arrestin mediates this response, disrupting the interaction between CaV1.2 and Cav1.2 and tyrosine kinase inhibitors readily prevent agonist-induced VDCC internalization. These observations suggest that VDCC trafficking is mediated by Gi/o, which disrupts the interaction with this domain accelerated the rate of trafficking of CaV1.2 channels to distal regions of the dendritic arbor. CaM imparts CaV1.2 channel activity to become a target for arrestin (4), which mediates the recruitment of the receptor into clathrin-coated vesicles (5).

Regulation of voltage-dependent calcium channels (VDCCs) plays a pivotal role in excitation-contraction coupling in cardiac myocytes. During the action potential upstroke, membrane depolarization causes the opening of VDCCs, encoded by the pore-forming α subunit, CaV1.2 (1). Ca2+ entry through VDCCs triggers the release of Ca2+ from the sarcoplasmic reticulum via ryanodine receptors. Although the regulation of VDCCs in the heart has been extensively studied, key molecular mechanisms underlying channel function, trafficking, membrane targeting, retention, and internalization remain unknown. Activation of the β-AR, a G protein–coupled receptor (GPCR), leads to multiple inotropic effects mediated by phosphorylation (3, 11), which are transient phenomena since persistent activation of the receptor causes its subsequent desensitization (8). This desensitization is caused by rapid recycling of the effector, the G protein-coupled receptor kinase; CaM, calmodulin; ISO, isoproterenol; PTx, pertussis toxin; PP2, 4-amino-5-(4-chlorophenyl)-7-(t-buty)pyrazolo[3,4-d]pyrimidine; MAPK, mitogen-activated protein kinase; aa, amino acid(s).

* This work was supported, in whole or in part, by National Institutes of Health Grant NS37443 (to M. D.-P.). This work was also supported by American Heart Association AHA0830126N (to F. G. A.), HL076230 (to E. A. S.), AHA0735576T (to S. T. T.), and HL7458 (to A. D. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The on-line version of this article (available at http://www.jbc.org) contains a supplemental figure.

These authors contributed equally to this work.

§ To whom correspondence should be addressed: Mount Sinai School of Medicine, Dept. of Pharmacology and Systems Therapeutics, One Gustave L. Levy Place, Box 1603, New York, NY 10029. Tel.: 212-241-5569; Fax: 212-996-7214; E-mail: maria.diverse@mssm.edu.

†‡ The abbreviations used are: VDCC, Voltage-dependent calcium channels; β-AR, β-adrenergic receptors; GPCR, G-protein-coupled receptor; GRK, GPCR kinase; CaM, calmodulin; ISO, isoproterenol; PTx, pertussis toxin; PP2, 4-amino-5-(4-chlorophenyl)-7-(t-buty)pyrazolo[3,4-d]pyrimidine; MAPK, mitogen-activated protein kinase; aa, amino acid(s).
bility that the internalization of calcium channels is a result of β-AR switch in coupling from Gs to Gi. Our results represent a new mechanism of cellular adaptation during hyperadrenergic simulation, which might have implications to a host of cardiac pathologies, including hypertrophic remodeling.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following primary antibodies were used: rabbit polyclonal anti-Ca2.2α, Subunit(Alomone Labs, Jerusalem, Israel), anti-Ca1.2 (NeuroMab); anti-β-Arr1 (BD Biosciences); and polyclonal anti-clathrin heavy chain (BD Biosciences). The following secondary antibodies were used: Oregon Green Alexa Fluor 488-conjugated goat anti-rabbit IgG (H+L) (Invitrogen); and Cy3-conjugated goat anti-mouse IgG (H+L) (Jackson Laboratories). For live cell imaging experiments, calcium channels were visualized by incubation of the cells for 5 min at 37 °C with 2.5 μM DM-BODIPY (-)-dihydropyridine high affinity enantiomer (Invitrogen).

Fluoresceinlabeled 894–929 and 920–944 peptides used in this study were based on the Ca2.2α sequence from chick dorsal root ganglion neurons (CDB1, GenBankTM AAD51815). Peptides were synthesized by FastMoc chemistry at the Tufts University Core Facility (Boston, MA) and purified by high-pressure liquid chromatography with >97% purity as determined by mass spectrometry. The N terminus included the sequence of the Penetratin domain of the Drosophila protein Antennapedia. Peptides were dissolved in 5 mM acetic acid.

**Cardiomyocyte Isolation**—Cardiomyocytes were isolated from adult rat hearts as described previously in Schiff et al. (11). Cardiomyocytes were plated on laminin-coated dishes as described above, were used for each condition. Cells were exposed to control solution (culture medium) or control solution containing 100 μM ISO plus ascorbic acid. After treatment with agonist, cells were lysed. VDCC was immunoprecipitated from 2 mg of lysate as previously in Schiff et al. (11).

**RESULTS**

**Cardiac Ca1.2/β-Arr1 Complexes Are Internalized upon Sustained β-AR Activation**—To directly determine if sustained conditions (treatment with saline), the change in Ca1.2 receptor activity signal from the cell membrane of the adult heart, we used EYFP-Ca1.2 transgenic rat cardiomyocytes to report the localization of both receptor complexes. Cells were treated with a green fluorescent probe specific to Cav1.2/Ca1.2 (with a red fluorescent probe), yielding a yellow signal, indicating co-localization of the two proteins. The relative abundance of the merged signal in the top of the cell is higher than its relative absence except for a discrete ring around the cell periphery in deeper layers further underscores the selective localization of both Ca1.2/Ca1.2 and β-Arr1 at the cell membrane (Fig. 1A).

Upon β-AR stimulation with ISO (100 μM) for 5 min, the individual green and red fluorescence signals become distinct and more intense in the middle slices, suggesting dissociation of the Ca1.2/β-Arr1 complex and movement of each component to deeper layers away from the cell surface (Fig. 1, C, E, and F). The ISO-mediated internalization of the channels was observed in 95% of the cells tested with a 40 ± 5% mean loss of fluorescent signal in the top optical slice. This pattern of Ca1.2/β-Arr1 separation and internalization is further amplified following 15 min of exposure to ISO (Fig. 1, D and F). Exposure of cells to a lower concentration of ISO (100 nM) yielded qualitatively similar results, with 55.0 ± 2.0 of the signal associated with the cytosolic region observed in 93% of the cardiac myocytes.

Dynamic changes in the association of the two proteins were quantitatively measured using the Pearson’s correlation coefficient in saline-treated myocytes (sham, control) and following ISO exposure for 20 s, 5 min, and 15 min. In accordance with the qualitative images, the Pearson’s coefficient was significantly reduced from 0.89 ± 0.05 in saline-treated cardiomyocytes to 0.77 ± 0.02 (20 s ISO), 0.69 ± 0.03 (5 min ISO), and 0.67 ± 0.05 (15 min ISO) (n = 10). Biochemical experiments provided further evidence of the association between β-Arr1...
Internalization of VDCCs

FIGURE 1. Activation of β-ARs regulate channel–β-Arr1 interaction. A–D, adult rat cardiac myocytes were treated with saline (A) or 100 μM isoproterenol for 20 s (B), 5 min (C), and 15 min (D). Calcium channels and β-Arr1 were detected by indirect immunofluorescence using anti-pan α1 antibody followed by Oregon Green-conjugated anti-rabbit IgG and anti-β-Arr1 followed by Cy-3 anti-mouse IgG, respectively. Series of merged images (Ca_{1.2} channel/β-Arr1, yellow signal) of X-Y optical slices acquired at 0.2 μm intervals are shown from the top to bottom of each cell. The scale bar represents 10 μm. Data are representative of five independent experiments. E, close view (zoom 150%) of the membrane-associated channel/β-Arr1 clusters. F, pie charts representing the subcellular distribution of calcium channels expressed as the percentage of total fluorescence signal. Integrated fluorescence was calculated for membrane and cytoplasmic pools of calcium channels. Data represent the mean value of 25 cells. G, calcium channels from adult rat cardiac myocytes were precipitated using anti-pan α1 antibody, and the precipitate was probed for β-Arr1. Lane 1 is the precipitate, and lane 2 is the total lysate. To avoid the signal from the antibody heavy and light chains, peroxidase-conjugated protein A/G were used. H, calcium channels were precipitated from pig atria and ventricle using anti-Ca_{1.2}, and the precipitate was probed for β-Arr1. IP, immunoprecipitate; IB, immunoblot. I, adult rat cardiac myocytes were treated with saline or 100 μM ISO and then lysed. Ca_{1.2} channel was precipitated, and the precipitate was probed for β-Arr1. The membrane was stripped and probed with anti-Ca_{1.2} channel antibodies for normalization. J, the histogram shows quantitation of the density of the β-Arr1 band from three independent experiments. Protein density was normalized using the density for the calcium channel band. Error bars represent S.D. Analysis between saline and 5 min of ISO was significant at p = 0.037.
FIGURE 2. β-Arr1 and tyrosine kinase activity are required for channel internalization. A, indirect immunofluorescence was used to detect clathrin (green signal) and β-Arr1 (red signal). Merged X-Y optical slices are shown. B, cardiac myocytes were incubated for 4 h with 100 ng/ml pertussis toxin or control culture medium. Cells were exposed to ISO for the times indicated in the figure. Calcium channels (green) and β-Arr1 (red) were detected by indirect immunofluorescence. X-Y optical slices of the merged images are shown. C, histogram shows the integrated fluorescence values for the top optical slices. D, cells were incubated with an arrestin-binding peptide (aa 894–920), a peptide containing aa sequence from the adjacent region of the Cav2.2 channel (aa 920–944) and control vehicle. Fluorescence as a function of time was measured in the top optical slice in the presence of ISO. Each data point represents the median value of 10 random cells. E, VDCCs in adult rat cardiomyocytes were labeled by incubation with 1.5 μM DM-BODIPY, a fluorescent dihydropyridine, for 5 min. Cells were preincubated with saline, 10 μM genistein, a tyrosine kinase inhibitor, and 10 μM daidzein, an inactive analog. Fluorescence as a function of time was measured in the top optical slice in the presence of ISO. Each data point represents the median value of seven random cells. F, Cav1.2 channels from rat liver (negative control) or cardiomyocytes were immunoprecipitated with anti-Cav1.2 antibody and immunoblotted with a monoclonal antibody raised against p60 Src kinase. IP, immunoprecipitate; WB, immunoblot. G, scheme showing proposed model. Upon sustained receptor activation, the β-AR switches and couples to G protein. The activation of Gi results in an increase in Src kinase activity. The VDCC/β-Arr1 complex is internalized. AC, adenylyl cyclase.
stimulation results in the switch of $\beta_2$ARs to $G_\text{i/o}$ protein. To
determine whether activation of heterotrimeric $G_{i/o}$ proteins is
required for ISO-induced internalization of VDCC/ $\beta$-Arr1 complexes
in cardiac myocytes, cells were pretreated with 100 ng/ml pertussis
toxin (PTx) for 4 h and then exposed to either agonist (ISO, 100 $\mu$m) or
saline. PTx treatment effectively blocked the internalization of VDCC/ $\beta$-Arr1 complexes in 95% of
the cells tested. Confocal X-Y optical slices of cells pretreated
with PTx showed that the VDCC complexes were retained at
the plasma membrane. In fact, the yellow signal representing
the co-localization of Cav1.2 and $\beta$-Arr1 was limited to the top
optical slice and the periphery of the myocyte in the middle
slices. Integrated fluorescence signals for cells pretreated with
PTx and exposed to ISO for 5 min demonstrate that Cav1.2 is
located predominantly at the plasma membrane in sharp con-
trast to the internalized channel distribution in myocytes
pretreated with ISO for 5 min (Fig. 2B). Pretreatment of myocytes
with PTx prevents ISO-induced internalization of Cav1.2 as the
fluorescent signal remains membrane bound, even following 15
min of ISO treatment (Fig. 2, B and C). In addition to its potent
inhibitory effect upon channel internalization, PTx also prevents
the dissociation between Cav1.2 and $\beta$-Arr1, as evident by a high
Pearson coefficient measured in myocytes following exposure to
ISO for 5 (0.81 ± 0.04) and 15 (0.82 ± 0.01) min, indicating a high
degree of co-localization between these two proteins. These results
indicate that ISO-induced internalization of Cav1,2/ $\beta$-Arr1 com-
plexes requires the activation of $G_{i/o}$ proteins.

**$\beta$-Arr1 and Tyrosine Kinase Activity Are Required in Agonist-mediated Internalization of Cardiac VDCCs**

We tested whether arrestin plays a role in agonist-induced internalization of VDCCs. Myocytes were labeled for 5 min with
fluorescent dihydropyridine. Because dihydropyridines bind to
the extracellular domain of Cav1.2 channels, which is at the
plasma membrane at rest, and arrestin binds to Cav1.2 cardiac
myocytes with unlabeled dihydropyridine prevents the binding of
DM-BODIPY, demonstrating that this probe binds selectively
(data not shown). The number of VDCC subcellular
distribution and the extent of ISO-induced internalization were
not different in myocytes in which the channels were labeled
with anti-Cav1,2 antibodies when compared with the labeling
by fluorescent dihydropyridine (data not shown). Image acquisi-
tion at 5-s intervals for 15 min produced <5% loss of signal,
suggesting that any loss of fluorescence signal observed in our
experiments was not caused by photobleaching.

In live cell imaging experiments, exposure of cardiac myocytes
to ISO produced a decrease in the fluorescence signal in the top
surface of the cell (Fig. 2D), concurrent with an increase in fluores-
cence in the middle optical slices. Washout of the agonist (ISO)
resulted in the recovery of the fluorescent signal at the top slice and
the cell periphery of deeper optical slices to a level comparable with
that prior to ISO exposure (supplemental Fig. 1).

To test whether Cav1,2/ $\beta$-Arr1 interaction is required for
ISO-induced internalization of VDCCs, we used cell-permeant
peptides based on the sequence of the $\beta$-Arr1-binding site in
Cav1,2 channel (Gallus gallus CDB1, aa 894−944) (15). We have
previously used this peptide to interfere with $\beta$-Arr1-channel
interaction in dorsal root ganglion neurons and block agonist-
induced VDCC internalization (15). This peptide works as a
“sponge” to bind the endogenous $\beta$-Arr1, which should result
in the blockade of responses mediated by this molecule. In con-
trol experiments, a wide range of concentrations (100 ng/ml to
100 $\mu$g/ml) and incubation times (5 min to 1 h) was tested. Data
shown in Fig. 2D were obtained by incubating rat cardiac myo-
cytes with a saturating concentration of peptide (1.4 $\mu$g/ml) for
5 min. In time-lapse experiments, the aa 894−929 peptide pre-
vents agonist-induced Cav1,2 channel internalization without
altering their basal distribution. A structurally similar but inac-
active peptide containing aa 920−944 was without effect (Fig. 2D).

$G_i$-coupled receptors are known to activate Src kinase (16, 17). To test whether tyrosine kinase activity plays a role in ISO-
induced, pertussis toxin-sensitive VDCC internalization, we
pretreated adult rat cardiac myocytes with genistein (10 $\mu$M, 10
min), a tyrosine kinase inhibitor (Fig. 2E). ISO failed to induce
VDCC internalization in cells pretreated with genistein. Daidzein,
an inactive analog of genistein, was without an effect. Internaliza-
tion was prevented by an Src kinase inhibitor, 4-amino-5-(4-chloro-
phenyl)-7-(t-buty1)pyrazolo[3,4-d]pyrimidine (PP2, 50 $\mu$M). In
the presence of PP2, ISO induced 6.4 ± 5% channel internaliza-
tion ($n = 5$) whereas ISO induced 16.8 ± 8% internalization ($n = 5$) in control cells. Indole analog, and a protein kinase
C inhibitor (H11005, 50 $\mu$M), were without effect.

Peptides derived from the cell surface of deeper optical slices to a level comparable with
the cell periphery of deeper optical slices to a level comparable with
that prior to ISO exposure (supplemental Fig. 1).

To test whether Cav1,2/ $\beta$-Arr1 interaction is required for
ISO-induced internalization of VDCCs, we used cell-permeant
peptides based on the sequence of the $\beta$-Arr1-binding site in
Cav1,2 channel (Gallus gallus CDB1, aa 894−944) (15). We have
previously used this peptide to interfere with $\beta$-Arr1-channel
interaction in dorsal root ganglion neurons and block agonist-
induced VDCC internalization (15). This peptide works as a
“sponge” to bind the endogenous $\beta$-Arr1, which should result
in the blockade of responses mediated by this molecule. In con-
trol experiments, a wide range of concentrations (100 ng/ml to
100 $\mu$g/ml) and incubation times (5 min to 1 h) was tested. Data
shown in Fig. 2D were obtained by incubating rat cardiac myo-
cytes with a saturating concentration of peptide (1.4 $\mu$g/ml) for
5 min. In time-lapse experiments, the aa 894−929 peptide pre-
vents agonist-induced Cav1,2 channel internalization without
altering their basal distribution. A structurally similar but inac-
active peptide containing aa 920−944 was without effect (Fig. 2D).

$G_i$-coupled receptors are known to activate Src kinase (16, 17). To test whether tyrosine kinase activity plays a role in ISO-
induced, pertussis toxin-sensitive VDCC internalization, we
pretreated adult rat cardiac myocytes with genistein (10 $\mu$M, 10
min), a tyrosine kinase inhibitor (Fig. 2E). ISO failed to induce
VDCC internalization in cells pretreated with genistein. Daidzein,
an inactive analog of genistein, was without an effect. Internaliza-
tion was prevented by an Src kinase inhibitor, 4-amino-5-(4-chloro-
phenyl)-7-(t-buty1)pyrazolo[3,4-d]pyrimidine (PP2, 50 $\mu$M). In
the presence of PP2, ISO induced 6.4 ± 5% channel internaliza-
tion ($n = 5$) whereas ISO induced 16.8 ± 8% internalization ($n = 5$) in control cells. Indole analog, and a protein kinase
C inhibitor (H11005, 50 $\mu$M), were without effect.

Peptides derived from the cell surface of deeper optical slices to a level comparable with
the cell periphery of deeper optical slices to a level comparable with
Internalization of VDCCs

G_{i/o}-coupled receptors such as the muscarinic receptor does not modulate Ca_{1.2} channels by themselves but prevents β-AR-induced enhancement of I_{Ca-L} (18). Interestingly, a “rebound” effect has been described in contractility assays following activation of G_{i/o}-coupled receptors (19). In the experimental paradigm used in these studies, contraction evoked by ISO was measured, followed by the simultaneous application of ISO with a muscarinic agonist in which no ISO effect was observed. After washout of the muscarinic agonist, the effect of ISO on contractility was larger than in the previous applications (19, 20). This rebound effect has been consistently observed. These results suggest a modulatory effect of the adrenergic activity for G_{i/o}-coupled receptors. A similar rebound effect was also observed in electrophysiological experiments in which I_{Ca-L} was measured (18), and hence our findings potentially offer a mechanism for this phenomenon, linking alterations in current density and kinetics to channel internalization.

Several mechanisms could potentially account for the β-AR-mediated VDCC internalization. The β-AR in the G_{i}-coupled state can activate Src kinase, and subsequently the GTPase dynamin, which is known to play a role in the budding of vesicles from the plasma membrane during internalization (21, 22). In addition, it has been shown that the β-AR can activate Src kinase in a G protein-independent manner (16, 17). The ISO-induced internalization of VDCC complexes observed in our studies is G protein-dependent as it is prevented by pertussis toxin.

Another potential mechanism mediating the β-AR-induced regulation of Ca_{1} channel and β-Arr1-mediated activation of a mitogen-activated protein kinase (MAPK) pathway has been suggested (23). MAPKs are known to translocate to the cytosol, and it has been shown that activation of the pathway by G_{i} protein migrates VDCCs from the plasma membrane to endosomes (16, 17).

Finally, it has been suggested that the β-Arr1 mediated cardioprotective effect could be attributed to Src kinase activation (26). Our present findings regarding the internalization of Ca_{1.2} channels through a β-Arr1 and tyrosine kinase-mediated pathway could represent a new mechanism by which β-Arr1 can exert its cardioprotective effects, in effect limiting the surface expression of channels and thereby reducing calcium entry.

Acknowledgements—Confocal laser microscopy was performed at the Mount Sinai - School of Medicine-Microscopy Shared Facility, supported with funding from the NCI National Institutes of Health Shared Resources Grant 1R24 CA095823 and National Science Foundation Major Research Instrumentation Grant DBI-9724504.

REFERENCES

1. Molkentin, J. D. (2006) J. Clin. Investig. 116, 623–626
2. Osterrieder, W., Brum, G., Hescheler, J., Trautwein, W., Flockerzi, V., and Hofmann, F. (1984) Nature 298, 576–578
3. Benovic, J. L., Kuhn, H., Weyand, I., Codina, J., Caron, M. G., and Lefkowitz, R. J. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8879–8882
4. Lefkowitz, R. J., and Shenoy, S. K. (2005) Science 308, 512–517
5. Goodman, O. B. Jr., Krupnick, J. G., Gurevich, V. V., Benovic, J. L., and Keen, J. H. (1997) J. Biol. Chem. 272, 15017–15022
6. Green, E. M., Barrett, C. F., Bulymyk, G., Shamah, S. M., and Dolmetsch, R. E. (2007) Neuron 55, 615–632
7. Hudson, A., Schulman, H., Kim, J., Maltez, M. J., Tsien, R. W., and Pitt, G. S. (2005) J. Cell Biol. 171, 537–547
8. Wang, H. G., George, M. S., Kim, J., Wang, C., and Pitt, G. S. (2007) J. Neurosci. 22, 9086–9093
9. Pyo, R. T., Sui, J., Dhume, A., Palomeque, J., Blaxall, B. C., Diaz, G., Tunstead, J., Logothetis, D. E., Hajjar, R. J., and Schecter, A. D. (2006). J. Mol. Cell. Cardiol. 41, 834–844
10. Tombler, E., Cabanilla, N. J., Carman, P., Hall, J. I., Richman, R., Rodriguez, J., Lee, J., Felsenfeld, D. P., Hennigan, R. F., and Diverse-Pierluissi, M. A. (2006). J. Biol. Chem. 281, 1827–1839
11. Schiff, M. L., Siderovski, D. P., Batters, G., Snow, B., Jordan, J. D., De Vries, L., Ortiz, D. F., and Diverse-Pierluissi, M. (2000) Nature 408, 723–727
12. Xiao, K., Moeller, S., Zhao, Y., Chen, M., Shenoy, S. K., Yates, J. R., and Lefkowitz, R. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2485–2489
13. Benovic, J. L., Strasser, R. H., Caron, M. G., and Lefkowitz, R. J. (1986) J. Biol. Chem. 261, 15017–15022
14. Gong, H., D. L. Adamson, Ranu, H. K., Koch, W. J., Heubach, J. F., Ravens, J. J., Lee, J., Felsenfeld, D. P., Hennigan, R. F., and Diverse ´-Pierluissi, M. A. (2000) Cell. Cardiol. 723–727
15. Puckerin, A., Liu, L., Permaul, N., Carman, P., Lee, J., and Diverse ´-Pierluissi, M. A. (1998) J. Biol. Chem. 273, 15017–15022
16. Luttrell, L. M., Daaka, Y., and Lefkowitz, R. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 505–508
17. Pyo, R. T., Sui, J., Dhume, A., Palomeque, J., Blaxall, B. C., Diaz, G., Tunstead, J., Logothetis, D. E., Hajjar, R. J., and Schecter, A. D. (2006). J. Mol. Cell. Cardiol. 41, 834–844
18. Nagata, K., Ye, C., Jain, M., Milstone, D. S., Liao, R., and Mortensen, R. M. (2000) Circ. Res. 87, 903–909
19. Wang, Y. G., and Lipsius, S. L. (1995) Circ. Res. 76, 634–644
20. Hou, Z.-Y., Lin, C. L., Vasalle, M., Chiang, B. N., and Cheng, K. K. (1989) Am. J. Physiol. 256, H74–H84
21. Herrings, D., Huang, R., Singh, R., Robinson, L. C., Dillon, G. H., and Lei, J. F., Lee, J., Felsenfeld, D. P., Hennigan, R. F., and Diverse ´-Pierluissi, M. A. (2000) Cell. Cardiol. 723–727
22. Koppen, C. J. (2001) Biochem. Soc. Trans. 29, 505–508
23. Crespo, P., Cachero, T. G., Xu, N., and Gutkind, J. S. (1995) J. Biol. Chem. 270, 25259–25265
24. Lyon, A. R., Rees, P. S., Prasad, S., Poole-Wilson, P. A., and Harding, S. E. (2008) Nat. Clin. Pract. Cardiovasc. Med. 5, 22–29
25. Ho, P. D., Fan, J. S., Hayes, N. L., Nada, N., Palade, P. T., Gembotski, C. C., and McDonough, P. M. (2001). Cir. Res. 88, 62
26. Noma, T., Lemaire, A., Naga, Prasad, S. V., Barki-Harrington, L., Tildey, D. G, Chen, J., Le Corvoisier, P, Violin, J. D., Wei, H., Lefkowitz, R. J., and Rockman, H. A. (2007) J. Clin. Investig. 117, 2445–2458

17226 JOURNAL OF BIOLOGICAL CHEMISTRY VOLUME 283 • NUMBER 25 • JUNE 20, 2008