Increasing evidence shows that stimulation of β-adrenergic receptor (AR) activates mitogen-activated protein kinases (MAPKs), in addition to the classical Gs-adenyllyl cyclase-cAMP-dependent protein kinase (PKA) signaling cascade. In the present study, we demonstrate a novel β2-AR-mediated cross-talk between PKA and p38 MAPK in adult mouse cardiac myocytes expressing β2-AR, with a null background of β1β2-AR double knockout. β2-AR stimulation by isoproterenol increased p38 MAPK activity in a time- and dose-dependent manner. Inhibiting Gs with pertussis toxin or scavenging Gβγ with βARK-ct overexpression could not prevent β2-AR-induced p38 MAPK activation. In contrast, a specific peptide inhibitor of PKA, PKI (5 μM), completely abolished the stimulatory effect of β2-AR, suggesting that β2-AR-induced p38 MAPK activation is mediated via a PKA-dependent mechanism, rather than by Gs or Gβγ. This conclusion was further supported by the ability of forskolin (10 μM), an adenyllyl cyclase activator, to elevate p38 MAPK activity in a PKI-sensitive manner. Furthermore, inhibition of p38 MAPK with SB203580 (10 μM) markedly enhanced the β2-AR-mediated contractile response, without altering base-line contractility. These results provide the first evidence that cardiac β2-AR activates p38 MAPK via a PKA-dependent signaling pathway, rather than by Gs or Gβγ, and reveal a novel role of p38 MAPK in regulating cardiac contractility.

Mitogen-activated protein kinases (MAPKs)1 have been implicated in the regulation of multitude vital cellular processes, including cell differentiation, proliferation, cell growth, and cell death (1–4). There are three major subgroups identified, including the extracellular signal-regulated kinase (ERK1/2), c-jun-NH2-terminal kinase (JNK), and p38 MAPK. Various extracellular stimuli, such as growth factors, cytokines, mechanical stress, UV light, osmotic stress, and heat shock, can activate MAPK signaling cascades (5). Increasing evidence has shown that G protein-coupled receptors (GPCRs), e.g. β-adrenergic receptor (AR), also regulate MAPKs, particularly ERK1/2 MAPK. One major pathway of GPCR-mediated activation of MAPKs is dependent on “transactivation” of a panel of receptor tyrosine kinases (such as epidermal growth factor and insulin-like growth factor) (6–8). Specifically, stimulation of GPCRs leads to the release of free Gβγ dimers, which, in turn, activate these receptor tyrosine kinases by unidentified mechanisms, resulting in activation of ERK1/2 MAPKs (9, 10).

A large body of evidence has demonstrated that activation of p38 MAPK, also called a stress-activated protein kinase, is associated with the onset of cardiac hypertrophy and cell death in response to in vivo pressure overload or ischemic/reperfusion injury (11–13). In Gs transgenic mice, the transition of hyper trophy to apoptosis is coincident with activation of p38 MAPK (14). This paradigm has been further manifested by p38 MAPK-induced apoptosis in cultured neonatal rat cardiac myocytes overexpressing p38 MAPK (15). These previous studies suggest an involvement of p38 MAPK in cardiac apoptosis. However, more recent studies have proposed that in cultured adult rat myocytes, p38 MAPK is activated by β2-AR stimulation via a Gβγ-dependent mechanism, protecting myocytes against β-AR/Gs-mediated apoptosis (16). Thus, in contrast to the wealth of knowledge as to GPCR-mediated ERK1/2 activation, the role of GPCRs in regulating p38 MAPK activity and its physiological relevance remains controversial.

This study seeks to determine whether cardiac β2-AR stimulation regulates p38 MAPK signaling and, if so, to explore underlying mechanisms and examine the possible interaction between the concurrent PKA and p38 MAPK signaling pathways. To selectively stimulate cardiac β2-AR, we expressed the human β2-AR in ventricular myocytes isolated from adult β1β2-AR double knockout (DKO) mice using adenoviral gene transfer (17). Here we demonstrate that “pure” β2-AR stimulation activates p38 MAPK via a PKA-dependent mechanism, but independent of Gs and Gβγ, and that the activated p38 MAPK provides a novel negative feedback to the PKA-mediated contractile response in adult mouse ventricular myocytes.

**EXPERIMENTAL PROCEDURES**

Isolation and Culture and Adenoviral Infection of Adult Mouse Cardiomyocytes—The investigation conforms to National Institutes of Health guiding principles in the care and use of animals. Single mouse cardiac myocytes were isolated from the hearts of 2–3-month-old β2-AR DKO mice with an enzymatic technique, then cultured and infected with β2-AR-adenoviral vector, adeno-β2-AR at a multiplicity of 410-558-8150; E-mail: xiaor@grc.nia.nih.gov.

1 The abbreviations used are: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; GPCR, G protein-coupled receptor; AR, adrenergic receptor; DKO, double knockout; SB, SB203580; PKA, cAMP-dependent protein kinase; m.o.i., multiplicity of infection; PKI, a specific peptide inhibitor of PKA; MEM, minimal essential medium; FBS, fetal bovine serum; PTX, pertussis toxin; ISO, isoproterenol; FSK, forskolin.
infection (m.o.i.) of 100, as described previously (17). In a subset of experiments, myocytes were co-infected with adenovirus-β-AR and adenovirus-βARK-et (adenovirus vector carrying a βARK carboxy-terminal fragment) or infected with adenovirus-β-Gal (adenovirus vector with reporter gene lacZ) as negative control, all at m.o.i. of 100. Before culture, myocytes were suspended in minimal essential medium (MEM) containing 1.2 mM Ca²⁺, 2.5% fetal bovine serum (FBS) and 1% penicillin-streptomycin and then plated at 0.5 × 1 × 10⁶/cm² with the same medium in the culture dishes precoated with 10 μg/ml mouse laminin. Following 1 h of culture (to achieve attachment), the culture medium was aspirated along with unattached cells. Adenovirus-mediated gene transfer was implemented by adding a minimal volume of the FBS-free MEM containing an appropriate titer of gene-carrying adenovirus. The full volume of FBS-free MEM was supplied after culture for another 1–2 h. All experiments were performed after 24 h of adenoviral infection. In a subset of experiments, myocytes were treated with pertussis toxin (PTX, 0.5 μg/ml) to inhibit Gi/Go proteins. The efficacy of PTX was routinely examined by the abolition of β2-AR-stimulated cAMP formation.

β-AR Stimulation by ISO Activates p38 MAPK in a Time- and Dose-dependent Manner—To define the effect of β2-AR stimulation on p38 MAPK, both the phosphorylation status and the activity of p38 MAPK in response to a β-AR agonist were examined in adult β1β2 DKO mouse cardiac myocytes expressing β2-AR (450 ± 48 fmol/mg protein, n = 4). Stimulation of β2-AR by ISO (1 μM) increased p38 MAPK phosphorylation (an index of the kinase activation) in a time-dependent manner, as shown by the typical Western blot (Fig. 1A, top band) and the average data (Fig. 1B). Phosphorylation of p38 MAPK was transiently elevated following β2-AR stimulation; it occurred significantly as early as 5 min, reached the peak of 1.7-fold augmentation at 15 min, and then gradually declined to the basal level at 60 min (Fig. 1, A and B). Concomitantly, phosphorylation of its substrate protein, ATF-2, was also enhanced by 1.8-fold in response to β2-AR stimulation, with a similar temporal profile to that of p38 MAPK phosphorylation (Fig. 1, A and B). Pretreatment of cells with a β-AR antagonist, propranolol (10 μM), 15 min prior to ISO, completely ablated β2-AR-stimulated p38 MAPK activation (Fig. 1C).

Further, a p38 MAPK inhibitor, SB203580, antagonized a β2-AR-induced increase in p38 MAPK activity in a dose-dependent fashion (Fig. 1D). Fig. 2 shows the average dose-response curve of p38 MAPK phosphorylation to β2-AR stimulation; ISO dose-dependently increased p38 MAPK phosphorylation with an EC₅₀ of 10 nM and a maximal response at concentrations ≥ 1 μM. These results indicate that ISO-induced β2-AR stimulation activates p38 MAPK in a time- and dose-dependent fashion.

β2-AR-induced p38 MAPK Activation Does Not Depend on Gᵢ or Gᵦβγ Signaling—Since β2-AR couples to both Gᵢ and Gᵦ proteins (19–21), we first asked whether Gᵢ signaling is essentially involved in the β2-AR-induced p38 MAPK activation. To test this hypothesis, we treated cells with PTX to disrupt Gᵢ signaling. While PTX treatment completely ablated the inhibitory effect of M₂-muscarinic stimulation on β2-AR-mediated positive contractile response in all of the cells examined (data not shown), it could not prevent the effect of β2-AR stimulation to activate p38 MAPK (Fig. 3, A and B). Next, we determined whether the β2-AR-stimulatory effect is dependent on Gᵦβγ, as often is the case for GPCR-mediated ERK1/2 MAPK activation (9, 10). As shown in Fig. 3, A and B, inhibiting Gᵦβγ subunits by overexpressing Gᵦβγ scavenger, βARK-et, could not abolish β2-AR-induced p38 MAPK activation. In contrast, overexpression of βARK-et fully prevented β2-AR-mediated anti-apoptotic effect (data not shown), indicating it is effective in blocking Gᵦβγ. Thus, the effect of β2-AR stimulation on p38 MAPK is independent of either Gᵢ or Gᵦβγ signaling.

We next examined the possible role of the classic G₁α-adenyl cyclase-PKA signaling pathway in β2-AR-induced p38 MAPK activation. Cells were treated with PKI (5 μM), a specific peptide inhibitor of PKA, 15 min prior to ISO application. Fig. 4 shows that PKI fully prevented the effect of β2-AR stimulation (1 μM ISO for 15 min) on p38 MAPK. If PKA is sufficient to activate p38 MAPK, receptor-independent reagents induced PKA activation should also increase this MAPK activity. Indeed, direct stimulation of adenyl cyclase by forskolin (FSK, 10 μM), similar to β2-AR stimulation by ISO, markedly increased p38 MAPK phosphorylation. Therefore, we conclude that the β2-AR-mediated p38 MAPK activation is independent of either Gᵢ or Gᵦβγ signaling.
phorylation. Moreover, PKI completely abolished the response of p38 MAPK to forskolin (Fig. 4, A and B). As a negative control, we further demonstrated that in cells transfected with adeno-βGal, ISO had no effect on p38 MAPK activation, whereas FSK did increase the p38 MAPK activity in a PKI-sensitive manner (Fig. 4C). These results indicate that β2-AR stimulation activates p38 MAPK via a PKA-dependent pathway.

p38 MAPK Activation Inhibits β2-AR-mediated Contractile Response—A predominant functional role of acute β-AR stimulation in cardiac myocytes is to increase contractility. To investigate the possible physiological relevance of β2-AR-stimulated p38 MAPK activation, we measured cell contractile response to β2-AR stimulation by ISO at a submaximal concentration (10⁻⁹ M) in the presence or absence of the p38 MAPK inhibitor, SB203580. Since our data showed that SB203580, the p38 MAPK inhibitor, at 10 μM effectively antagonized β2-
AR-mediated p38 MAPK activation (Fig. 1D), we chose this concentration of SB203580 to determine the possible cross-talk between p38 MAPK and PKA in regulating cardiac contractility. SB203580 at 10 μM markedly enhanced β2-AR-induced contractile response, although SB alone at the concentration employed had no detectable effect on cell base-line contractility (Fig. 5, A and B). On average, the positive inotropic effect of β2-AR stimulation was enhanced by ~60% (Fig. 5B). In fact, SB203580 even at a lower concentration (5 μM) also significantly enhanced ISO-induced positive inotropic effect. Thus, inhibition of p38 MAPK potentiates β2-AR-stimulated contractile response in adult mouse cardiac myocytes, suggesting that p38 MAPK provides a negative feedback to β2-AR-stimulated, PKA-mediated contractile response.

**DISCUSSION**

There are three major findings in this study. First, we have demonstrated that in adult mouse cardiac myocytes, β2-AR stimulation by ISO induces a time- and dose-dependent increase in p38 MAPK activation (Fig. 1). Second and most importantly, we have shown that the stimulatory effect of β2-AR on p38 MAPK is mediated by a PKA-dependent pathway, rather than by Gα or Gβγ signaling (Fig. 2). Finally, the present results elucidate a novel role of p38 MAPK in regulating cardiac contractility, in addition to its chronic functional role in regulating cell growth and cell death (11–16). Thus, the present study not only shows that β2-AR sequentially activates the Gα-adenylyl cyclase-PKA and p38 MAPK signaling pathways, but also documents an intriguing forward and retrograde cross-talk between those signaling pathways.

**β2-AR Activates p38 MAPK via PKA**

While previous studies have demonstrated that Gβγ and Gαq/11 increase p38 MAPK activity (22, 23), more recent studies showed that in rat cardiac myocytes both β1-AR and β2-AR subtypes activate p38 MAPK in a PTX-sensitive manner (16). We determined the possible role of Gα in β2-AR-mediated p38 MAPK activation in adult mouse ventricular myocytes. Surprisingly, inhibition of Gα with PTX cannot prevent the β2-AR-induced p38 MAPK activation, although PTX treatment fully abolishes M2-mediated anti-adrenergic effect under the same experimental conditions. In sharp contrast, inhibition of PKA by a specific peptide inhibitor, PKI, completely abrogates the stimulatory effect of β2-AR on p38 MAPK activation, strongly suggesting that PKA plays an essential role in β2-AR-induced p38 MAPK activation. This conclusion is reinforced by the fact that direct stimulation of adenylyl cyclase by forskolin also markedly increases p38 activity in a PKI-sensitive manner, resembling β2-AR stimulation.

**FIG. 4.** β2-AR- and forskolin-stimulated p38 MAPK activation is fully abolished by a peptide PKA inhibitor, PKI. After 24-h adenovirus infection, cells were treated with the membrane-permeable PKA inhibitor, PKI (5 μM), 30 min prior to ISO (1 μM) or FSK (10 μM) stimulation. A illustrates typical Western blots for phosphorylated or total p38 MAPK. Stimulation effects of ISO and FSK on p38 phosphorylation are completely prevented by inhibiting PKA with PKI (A and B) (n = 3 independent experiments; *, p < 0.05 versus control + PKI, or ISO and FSK in the presence of PKI. C shows, in cells infected with adenovirus, forskolin but not ISO increased p38 MAPK phosphorylation, and PKI abolished the effect of FSK on p38 MAPK.

**FIG. 5.** Inhibition of p38 MAPK enhances β2-AR-mediated contractile response. A shows the potentiating effect of SB203580 (SB, 10 μM) on ISO (10−9 M) induced contractile response in a representative DKO mouse myocyte expressing β2-AR. B summarizes the average ISO-induced contractile responses in the presence or absence of SB. Note that SB alone has no detectable effect on base-line contractility. (n = 6–14 cells; *, p < 0.05 versus control, SB, and ISO + SB; #, p < 0.01 versus control, SB, and ISO).
Thus, β2-AR-stimulated p38 MAPK activation is largely attributable to the Gs-adenylyl cyclase-PKA, rather than a Gi signaling pathway. The reason caused the different outcomes of the present study from that of the previous work in adult rat cardiac myocytes (16) is presently unclear. However, it might be related to the different species used. It has been shown that there are six p38 MAPK isoforms (24). Both α and β p38 MAPK isoforms coexist in cardiac myocytes and elicit divergent functional roles (25, 26). Whether rat and mouse cardiac myocytes have different predominant isoform(s) of p38 MAPK awaits further studies.

In principal, a dynamic interplay of protein kinases and protein kinases may be applicable to the cross-talk between PKA and p38 MAPK. Various protein kinases may regulate p38 MAPK activity by modulating the phosphorylation status of this MAPK. It has been well accepted that that MKK7 and MKK3 activate p38 MAPK activity by modulating the phosphorylation status of this MAPK. It has been well accepted that that MKK kinase is involved in the MAPK signaling cascade in regulating cardiac contractility, i.e. cardiac β2-AR-PKA activated p38 MAPK provides a negative feedback to the PKA-mediated contractile response. These findings shed new light on understanding the close association of p38 MAPK activation with the onset of cardiac dysfunction and markedly diminished β-AR-cardiac contractile support in response to pressure overload and ischemia or reperfusion injury (11–13).

Acknowledgments—We thank Dr. Walter J. Koch and Robert J. Lefkowitz for kindly providing adeno-β-AR and adeno-βARKct and Drs. Edward G. Lakatta, Heping Cheng, Yuesen Liu, and Yibin Wang for critical comments.

REFERENCES

1. Marshall, C. J. (1995) Cell 80, 179–185
2. Cobb, M. H., and Goldsmith, E. J. (1995) J. Biol. Chem. 270, 14843–14846
3. Minder, A., and Karin, M. (1997) Biochim. Biophys. Acta 1333, F85–F104
4. Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 24313–24316
5. Christian, W., Spencer, G., Matthew, B. J., and Gary, L. J. (1999) Physiol. Rev. 79, 143–180
6. Roudabush, F. L., Pierce, K. L., Maudsley, S., Khan, K. D., and Luttrell, L. M. (2000) J. Biol. Chem. 275, 22583–22589
7. Luttrell, L. M., van Biesen, T., Hawes, B. E., Koch, W. J., Touhara, K., and Lefkowitz, R. J. (1995) J. Biol. Chem. 270, 16496–16498
8. Maudsley, S., Pierce, K. L., Zamah, A. M., Miller, W. E., Ahn, S., Daaka, Y., Lefkowitz, R. J., and Luttrell, L. (2000) J. Biol. Chem. 275, 9572–9580
9. van Biesen, T., Hawes, B. E., Luttrell, D. K., Krueger, K. M., Touhara, K., Porfiri, E., Sakaue, M., Luttrell, L. M., and Lefkowitz, R. J. (1995) Nature 378, 781–784
10. Koch, W. J., Hawes, B. E., Allen, L. F., and Lefkowitz, R. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12706–12710
11. Bogoyevitch, M. A., Gillespie-Brown, J., Ketterman, A. J., Fuller, S. J., Ben-Levy, R., Ashworth, A., Marshall, C. J., and Sugden, P. H. (1996) Circ. Res. 79, 162–173
12. Yin, T., Sandhu, G., Wolfgang, C. D., Burrier, A., Webb, R. L., Rigel, D. F., Haisch, M. A., and Whelan, J. (1997) J. Biol. Chem. 272, 19945–19958
13. Clerk, A., Fuller, S. J., Michael, A., and Sugden, P. H. (1998) J. Biol. Chem. 273, 7228–7234
14. Adams, J. W., Sakata, Y., Davis, M. G., Sah, V. P., Wang, Y., Liggett, S. B., Chien, K. R., Brown, J. H., and Dorn, G. W., Jr. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10140–10145
15. Wang, Y., Su, B., Sah, V. P., Brown, J. H., Han, J., and Chien, K. R. (1998) J. Biol. Chem. 273, 5423–5429
16. communal, C., Colucci, W. S., and Singh, K. (2000) J. Biol. Chem. 275, 19395–19400
17. Zhu, Y. Y., Wang, S. Q., Zhu, W. Z., Chruscielski, A., Koblika, B., Bimar, Z., Wang, S., Lakatta, E. G., Cheng, H., and Xiao, R. P. (2000) Am. J. Physiol. 279, H429–H436
18. Kochetelov, M., Zhou, Y. Y., Cheng, H., Zhang, S. J., Chen, Y., Lakatta, E. G., and Xiao, R. P. (1999) J. Biol. Chem. 274, 22948–22952
19. Xiao, R. P., Xi, J., and Lakatta, E. G. (1995) Mol. Pharmacol. 47, 322–329
20. Xiao, R. P., Cheng, H., Zhou, Y. Y., Kochetelov, M., and Lakatta, E. G. (1999) Circ. Res. 85, 1092–1100
21. Xiao, R. P., Avdonin, P., Zhou, Y. Y., Cheng, H., Akhter, S. A., Eschenhagen, T., Lefkowitz, R. J., Koch, W. J., and Lakatta, E. G. (1999) Circ. Res. 85, 1101–1107
22. Nagao, M., Yamauchi, J., Nakao, M., Kanzo, Y., and Itoh, H. (1999) J. Biol. Chem. 274, 27711–27717
23. Nagao, M., Yamauchi, J., Kanzo, Y., and Itoh, H. (1998) J. Biol. Chem. 273, 22889–22896
24. Jiang, X., Gram, H., Zhao, M., New, L., Gu, J., Feng, L., Di, Padova, F., Ulevitch, R. J., and Han, J. (1997) J. Biol. Chem. 272, 30122–30128
25. Kumar, S., McDowell, P. C., Gunn, B. J., Hand, A. T., Lee, J. C., and Young, P. R. (1997) Biochem. Biophys. Res. Commun. 235, 533–539
26. Wang, Y., Huang, S., Sah, V. P., Ross, J., Jr., Brown, J. H., Han, J., and Chien, K. R. (1998) J. Biol. Chem. 273, 2161–2168

2 Y. Wang and P. Liao, personal communication
27. Lim, R., and Zaheer, A. (1996) J. Biol. Chem. 271, 22953–22956
28. Daaka, Y., Luttrell, L. M., and Lefkowitz, R. J. (1997) Nature 390, 88–91
29. Gupta, R. C., Neumann, J., Watanabe, A. M., Lesch, M., and Sabbah, H. N. (1996) Am. J. Physiol. 270, H1159–H1164
30. Saxena, M., Williams, S., Brockdorff, J., Gilman, J., and Mustelin, T. (1999) J. Biol. Chem. 274, 11693–11700
31. Saxena, M., Williams, S., Taske´n, K., and Mustelin, T. (1999) Nat. Cell Biol. 1, 305–310
32. Mackay, K., and Mochly-Rosen, D. (1999) J. Biol. Chem. 274, 6272–6279
33. Kuschel, M., Zhou, Y. Y., Spurgeon, H. A., Bartel, S., Karczewski, P., Zhang, S. J., Krause, E. G., Lakatta, E. G., and Xiao, R. P. (1999) Circulation 99, 2458–2465
34. Zhou, Y. Y., Cheng, H., Bogdanov, K. Y., Hohl, C., Altschuld, R., Lakatta, E. G., and Xiao, R. P. (1997) Am. J. Physiol. 273, H1611–H1618
35. Skeberdis, V. A., Jurevicius, J., and Fischmeister, R. (1997) J. Pharmacol. Exp. Ther. 283, 452–461
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J. Biol. Chem. 2000, 275:40635-40640.
doi: 10.1074/jbc.M006325200 originally published online October 3, 2000

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