Ca\(^{2+}\) Inhibition of Type III Adenylyl Cyclase in Vivo*

(Received for publication, January 31, 1995, and in revised form, June 13, 1995)

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Type III adenylyl cyclase is stimulated by β-adrenergic agonists and glucagon in vitro and in vivo, but not by Ca\(^{2+}\) and calmodulin. However, the enzyme is stimulated by Ca\(^{2+}\) and calmodulin in vitro when it is concomitantly activated by the guanyl nucleotide stimulatory protein Gs (Choi, E. J. et al., 1992). Each of the eight adenylyl cyclases that have been cloned (Krupinski et al., 1989; Feinstein et al., 1991; Bakalyar and Reed, 1990; Gao and Gilman, 1991; Ishikawa et al., 1989; Katsushika et al., 1992; Yoshimura and Cooper, 1992; Krupinski et al., 1992; Cali et al., 1994; Waterton et al., 1994) has distinct regulatory properties. For example, I-AC (Tang et al., 1991; Choi et al., 1992a), III-AC (Choi et al., 1992a), and VII-AC (Cali et al., 1994) are stimulated by Ca\(^{2+}\) and calmodulin (CaM) in vitro but II-AC, IV-AC, V-AC, VI-AC, and VII-AC are not.

In contrast to I-AC and VII-AC which are directly stimulated by Ca\(^{2+}\) and CaM in vitro, III-AC is not stimulated by Ca\(^{2+}\) and CaM unless it is also activated by GppNHp or forskolin (Choi et al., 1992a). Furthermore, the concentrations of free Ca\(^{2+}\) for half-maximal stimulation of I-AC and III-AC are 150 nM and 5.0 μM Ca\(^{2+}\), respectively. These data suggested that III-AC might be synergistically stimulated by intracellular Ca\(^{2+}\) and Gs-coupled receptors in intracellular Ca\(^{2+}\) in vivo. Surprisingly, intracellular Ca\(^{2+}\) inhibited hormone-stimulated type III adenylyl cyclase activity. Submicromolar concentrations of intracellular free Ca\(^{2+}\) which stimulated type I adenylyl cyclase, inhibited glucagon- or isoproterenol-stimulated type III adenylyl cyclase. Inhibition of type III adenylyl cyclase by intracellular Ca\(^{2+}\) was not mediated by Gs, cAMP-dependent protein kinase, or protein kinase C. However, an inhibitor of CaM kinases antagonized Ca\(^{2+}\) inhibition of the enzyme, and coexpression of constitutively activated CaM kinase II completely inhibited isoproterenol-stimulated type III adenylyl cyclase activity. We propose that Ca\(^{2+}\) inhibition of type III adenylyl cyclase may serve as a regulatory mechanism to attenuate hormone-stimulated cAMP levels in some tissues.

Adenylyl cyclases are regulated by extracellular and intracellular signals including neurotransmitters, hormones, and intracellular Ca\(^{2+}\) (reviewed in Tang and Gilman, 1992) and Choi et al. (1993a). Cell Culture—Human embryonic kidney 293 cells were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in a humidified 95% air, 5% CO\(_2\) incubator. Unless otherwise noted, components for cell culture were from Life Technologies, Inc.

Expression of III-AC and the Glucagon Receptor in HEK-293 Cells—The I-AC cDNA clone was isolated from a bovine brain cDNA library as described by Xia et al. (1991), and the III-AC cDNA clone (Bakalyar and Reed, 1990) was generously provided by R. R. Reed (The Johns Hopkins University, Baltimore, MD). The coding sequence of III-AC and I-AC were ligated into CDM-8 for expression in HEK-293 cells. HEK-293 cells stably expressing I-AC (CDM-I-AC) or III-AC (CDM-III-AC) and neomycin resistance have been characterized previously (Choi et al., 1992a, 1992b, 1993a, 1993b; Wu et al., 1993) and were used for co-transfection with the rat glucagon receptor cDNA vector (J. Eink et al., 1993). Both I-AC and III-AC cell lines were stably transfected with a hygromycin resistance vector and either the pZCEP expression vector encoding the rat glucagon receptor (pl 4) or pZCEP alone. For DNA transfections, cells were plated on 100-mm dishes at a density of 2 × 10\(^6\) cells/plate, grown overnight, and transfected with the pZCEP control vector (1 μg of DNA/plate) and a hygromycin resistance vector (1 μg DNA/plate) by the calcium phosphate method (Chen and Okayama, 1987). Hygromycin-resistant cells were selected in culture medium containing hygromycin B (Sigma, 460 units/ml) and 300 μg/ml G418. Multiple hygromycin/neomycin-resistant clones of each cell type, expressing the rat glucagon receptor (Glur) and III-AC or I-AC were isolated.

Adenylyl Cyclase Assay—Membrane preparations were isolated from HEK-293 cells, and the adenylyl cyclase assay was carried out as described previously (Choi et al., 1992a). Assay solutions contained 1 mM [\(^{32}\)P]ATP (500 cpm/μmol), \(^{3}H\)-labeled cyclic AMP (20,000 cpm/μM), 5 mM MgCl\(_2\), 0.2 mM EGTA, 1 mM EDTA, 2 μM cAMP, 5 mM theophylline, 0.5% bovine serum albumin, 20 mM creatine phosphate, and 100 units/ml creatine phosphokinase in 20 mM Tris-HCl, pH 7.4, unless otherwise specified. When CaCl\(_2\) and EGTA were included in the assay, the concentration of free Ca\(^{2+}\) was calculated by the method of Brooks and Storey (1992). Adenylyl cyclase activities are the mean of triplicate determinations.

CAMP Accumulation—Changes in intracellular cAMP were measured by determining the ratio of \(^{3}H\)-cAMP to total ATP, ADP, and AMP pool in \(^{3}H\)-adenine-loaded cells (Wong et al., 1991). Absolute numbers for cAMP accumulation generally show some variation between experiments using different sets of cells (Federman et al., 1992; Dittman et al., 1994). However, relative changes in cAMP were highly consistent between experiments. Confluent cells in 6-well plates were initially incubated in DMEM containing \(^{3}H\)-adenine (2.0 μCi/ml, ICN) for 16–20 h, washed once with 150 mM NaCl, and incubated at 37 °C for 30 min in Dulbecco's modified Eagle's media (DMEM, Life Technologies, Inc.) containing 1.0 mM isobutylmethylxanthine and various effectors as indicated. Reactions were terminated by aspiration, washing cells once with 150 mM NaCl, and addition of 1.0 ml of ice-cold 5% trichlo-
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RESULTS

Synergistic Stimulation of III-AC by Ca\textsuperscript{2+} and Activated G\textsubscript{s} in Membranes—To examine the Ca\textsuperscript{2+} sensitivity of III-AC in vitro and in vivo, III-AC and glucagon receptors were stably expressed in HEK-293 cells. The sensitivity of III-AC to Ca\textsuperscript{2+} and hormones was examined in isolated membranes or intact cells. HEK-293 cells do not express I-AC or VIII-AC, and endogenous adenylyl cyclase activity is not stimulated by Ca\textsuperscript{2+} and CaM. In the absence of other effectors, Ca\textsuperscript{2+} and CaM did not significantly stimulate III-AC in isolated membranes (Fig. 1A). However, III-AC was stimulated by Ca\textsuperscript{2+} and CaM when the enzyme was activated by GppNHz, a nonhydrolyzable GTP analogue that activates the guanyl nucleotide stimulatory protein Gs. In the presence of 100 \mu M GppNHz, Ca\textsuperscript{2+} and CaM stimulated III-AC activity 2.1 \pm 0.1-fold.

To determine if Ca\textsuperscript{2+} and receptor-activated Gs will also synergistically stimulate III-AC in membranes, the sensitivity of the enzyme to CaM and Ca\textsuperscript{2+} was analyzed in the presence of glucagon (Fig. 1B). In membrane preparations, III-AC was stimulated 4.1 \pm 0.1-fold by glucagon with an EC\textsubscript{50} of 7 nM. Glucagon-stimulated III-AC activity was enhanced 45 \pm 6.1\% by CaM and Ca\textsuperscript{2+}; however, the EC\textsubscript{50} for glucagon was not significantly affected by CaM. These data suggested that Ca\textsuperscript{2+} stimulation of III-AC is conditional upon Gs activation, and that Ca\textsuperscript{2+} and hormones might synergistically activate the enzyme in vivo.

Ca\textsuperscript{2+} Inhibition of Glucagon-stimulated III-AC Activity in Vivo—Glucagon stimulated III-AC 222 \pm 16 fold in vivo, but I-AC was insensitive to glucagon (Fig. 2A), consistent with previous data reporting that I-AC is not stimulated by Gs-coupled receptors in vivo (Wayman et al., 1994). The slight stimulation of CAMP levels seen with I-AC-G cells was due to glucagon stimulation of endogenous adenyl cyclase activity. Although endogenous adenyl cyclases in HEK-293 cells have
which stimulated I-AC, and the curves were almost mirror images of each other. The concentration of free intracellular Ca$^{2+}$ for half-maximal inhibition of glucagon-stimulated III-AC activity was estimated at 150 to 200 nM using Fura-2 imagining.

**Inhibition of Isoproterenol-stimulated III-AC Activity in Vivo**—To address the generality of the phenomenon described above, we also examined the effect of intracellular Ca$^{2+}$ increase on isoproterenol stimulated III-AC activity. HEK-293 cells express endogenous $\beta$-adrenergic receptors that are coupled to stimulation of III-AC in vivo (Fig. 4). Isoproterenol-stimulated III-AC activity was inhibited 41±6% by A23187 indicating that Ca$^{2+}$ inhibition of III-AC activity was not a unique property of glucagon-stimulated activity.

In the experiments described above, intracellular Ca$^{2+}$ was elevated using A23187 and it was of interest to determine if Ca$^{2+}$ generated by physiologically relevant signals would also inhibit hormone stimulated III-AC. HEK-293 cells contain muscarinic receptors coupled to the mobilization of intracellular Ca$^{2+}$. Treatment of HEK-293 cells with 10 $\mu$M carbachol elevates intracellular Ca$^{2+}$ to approximately 300 nM free Ca$^{2+}$ and stimulates I-AC (Choi et al., 1992b). Carbachol alone did not significantly affect III-AC activity but did inhibit isoproterenol-stimulated activity 43±5% (Fig. 5). Inhibition of isoproterenol-stimulated III-AC activity by carbachol was insensitive to pertussis toxin and therefore not due to endogenous muscarinic receptors coupled to III-AC through G$i$ (data not shown). These data indicate that physiologically relevant concentrations of intracellular Ca$^{2+}$ inhibit hormone-stimulated III-AC activity in vivo.

**Ca$^{2+}$ Inhibition of Forskolin-stimulated III-AC Activity in Vivo**—Since inhibition of hormone-stimulated III-AC activity was not receptor-specific, the effect of Ca$^{2+}$ could occur through G$s$, G$i$, or the catalytic subunit. Therefore, we examined the influence of Ca$^{2+}$ on forskolin-stimulated III-AC activity since forskolin interacts directly with the catalytic subunit of adenylyl cyclases. Forskolin stimulation is not dependent upon the presence of G$s$ or receptors for its actions (Seamon and Daly, 1981). Cells expressing III-AC were treated with increasing concentrations of forskolin in the presence or absence of 10 $\mu$M A23187 and 1.8 mM CaCl$_2$. In the absence of A23187, maximal forskolin stimulation of III-AC was 753±20 fold with an EC$_{50}$
of approximately 10 μM (Fig. 6). A23187 inhibited forskolin-stimulated III-AC activity 53 ± 5%. These data indicate that Ca\(^{2+}\) inhibition of III-AC may be due to modifications of the catalytic subunit that affect its stimulation by forskolin or activated Gs.

Inhibition of Gs-stimulated III-AC Activity Is Not Due to G Activation—One of the major mechanisms for inhibition of adenylyl cyclases is by activation of Gs. For example, stimulation of Gs by activation of M4 muscarinic receptors inhibits III-AC activity in vivo (Dittman et al., 1994). To address the role of Gs for Ca\(^{2+}\) inhibition of III-AC, cells expressing III-AC were pretreated with pertussis toxin, an agent which ADP-ribosylates Gs-α and blocks Gs-mediated inhibition of adenylyl cyclases (Katada and Ui, 1982; Bokoch et al., 1983). M4 muscarinic receptor inhibition of III-AC in HEK-293 cells is prevented by pertussis toxin treatment (Dittman et al., 1994). Pertussis toxin-treated cells were analyzed for isoproterenol-stimulated III-AC activity in the presence and absence of 10 μM A23187 and 1.8 mM CaCl\(_2\). cAMP accumulations were monitored as described under “Experimental Procedures.” The data are the mean ± S.D. of triplicate assays.

Fig. 3. Ca\(^{2+}\) concentration dependence for inhibition of glucagon-stimulated III-AC activity in vivo. HEK-293 cells stably expressing I-AC (I-AC-G) or III-AC (III-AC-G) were treated with glucagon (100 nM), 10 μM A23187, and increasing concentrations of CaCl\(_2\) as described under “Experimental Procedures.” Relative cAMP accumulations were determined as described under “Experimental Procedures.” The I-AC and I-AC data are presented as percentage of the ratio (cAMP/[ATP + ADP + AMP]) \(\times 100\) with no added CaCl\(_2\) and are the mean ± S.D. of triplicate assays.

Fig. 4. Ca\(^{2+}\) inhibits isoproterenol-stimulated III-AC activity in vivo. HEK-293 cells expressing III-AC were exposed to increasing concentrations of the β-adrenergic agonist isoproterenol, in the absence or presence of 10 μM A23187 and 1.8 mM CaCl\(_2\) as described under “Experimental Procedures.”

Fig. 5. Inhibition of isoproterenol-stimulated type III adenylyl cyclase by carbachol. HEK-293 cells expressing III-AC (III-AC-G) were exposed to increasing concentrations of the β-adrenergic agonist isoproterenol in the presence or absence of 10 μM carbachol. Under these conditions, carbachol increased intracellular free Ca\(^{2+}\) from approximately 50 nM to 300 nM. cAMP accumulations were monitored as described under “Experimental Procedures,” and the data are the mean ± S.D. of triplicate assays.

Fig. 6. Ca\(^{2+}\) inhibits forskolin-stimulated III-AC activity in vivo. HEK-293 cells stably expressing III-AC (III-AC-G) were treated with increasing concentrations of forskolin in the presence or absence of 10 μM A23187 and 1.8 mM CaCl\(_2\). cAMP accumulations were monitored as described under “Experimental Procedures.” The data are the mean ± S.D. of triplicate assays.
CaM kinase II Inhibits Isoproterenol and Forskolin-stimulated Type III Adenylyl Cyclase Activity in Vivo—Ca²⁺ inhibition of Type III-AC activity might be due to the action of one of the Ca²⁺-sensitive protein kinases. This question was initially addressed by examining the effect of several protein kinase inhibitors on Ca²⁺ inhibition of III-AC. The cAMP protein kinase inhibitors H89 and Rp-cAMP (Rothermel et al., 1988) as well as calphostin C, an inhibitor of protein kinase C, did not affect Ca²⁺ inhibition of III-AC (data not shown). We are confident that H89 inhibits the activity of cAMP-dependent protein kinase in HEK-293 cells because this inhibitor blocked cAMP stimulation of CRE-mediated transcription in these cells (Impey et al., 1994). Furthermore, we have determined that calphostin C inhibits phorbol ester stimulation of adenylyl cyclase activity in HEK-293 cells.² KN-62, a specific inhibitor of CaM kinases (Enslen et al., 1994), blocked Ca²⁺ inhibition of glucagon-stimulated III-AC activity (Fig. 7). Ten μM KN-62 almost completely abolished Ca²⁺ inhibition of glucagon-stimulated III-AC activity. Calmidazolium, a CaM antagonist, also blocked Ca²⁺ inhibition of III-AC. These data suggest that Ca²⁺ activation of CaM kinases may contribute to Ca²⁺ inhibition of III-AC.

To determine if CaM kinase II inhibits the activity of III-AC activity in vivo, we made stable transfectants in HEK-293 cells expressing CaM kinase II under the control of a metallothionein promoter. The CaM kinase II used in this experiment (KII-290) contains a point mutation that truncates the protein, removes its autoinhibitory domain, and makes it constitutively active (Matthews et al., 1994). These cells were then transiently transfected with a construct encoding III-AC, and the sensitivity of the adenylyl cyclase to CaM kinase II was evaluated by inducing the expression of the kinase with Zn²⁺. Zn²⁺ treatment of cells not expressing KII-290 had no effect on basal, isoproterenol, or forskolin-stimulated III-AC activities. However, induction of CaM kinase II activity in KII-290 cells expressing III-AC completely inhibited isoproterenol (Fig. 8A) and forskolin (Fig. 8B) III-AC activities. These data suggest that Ca²⁺ inhibition of III-AC in vivo may be mediated by CaM kinase II. Thus far, we have been unable to inhibit hormone stimulation of III-AC in membrane preparations using purified CaM kinase II suggesting this kinase may not directly phosphorylate III-AC. However, further experimentation is required to elucidate the mechanism for CaM kinase II regulation of adenylyl cyclase activity.

**DISCUSSION**

The adenylyl cyclases exhibit diverse regulatory properties that provide a number of interesting mechanisms for regulation of intracellular cAMP by extracellular and intracellular
signals. Several of the adenylyl cyclases are synergistically stimulated by signals arising from different pathways and therefore can generate enhanced cAMP signals in response to signal convergence. For example, the βγ complex from G proteins stimulates Gαi-activated II-AC and IV-AC (Tang and Gilman, 1992) providing a mechanism for signal integration. I-AC is synergistically activated by Ca2+ and neurotransmitters in vivo (Wayman et al., 1994), a regulatory property that may be important for some forms of synaptic plasticity and spatial learning of adenylyl cyclases by two or more signals may be a new mechanism for regulation of adenylyl cyclase activity; inhibition of adenylyl cyclase activity and optimization of cAMP levels may be equally important. The data in this study identify a new mechanism for regulation of adenylyl cyclase activity; physiologically significant levels of intracellular Ca2+ attenuate hormone stimulation of III-AC.

III-AC is stimulated by Ca2+ and CaM when it is activated by Gαi in vitro, but hormone-stimulated III-AC is inhibited by Ca2+ in vivo. Glucagon, isoproterenol, and forskolin-stimulated III-AC activities were all partially inhibited by physiologically relevant concentrations of intracellular Ca2+ (100 to 300 nM free Ca2+). The mechanism for Ca2+ inhibition of III-AC activity was not dependent upon the activity of CAMP-dependent protein kinase, protein kinase C, or Gαi. However, KN-62, an inhibitor of CaM kinases, blocked Ca2+ inhibition suggesting the interesting possibility that Ca2+-activation of CaM kinases may directly or indirectly inhibit III-AC activity in vivo. Furthermore, expression of constitutively active CaM kinase II completely blocked hormone stimulation of III-AC activity in vivo.

To date, five Ca2+-regulated adenylyl cyclases have been identified: I-AC, III-AC, V-AC, VI-AC, and VIII-AC. I-AC and VIII-AC are stimulated by intracellular Ca2+ in vivo (Choi et al., 1992b; Cali, et al., 1994) and mutagenesis of the CaM binding domain of I-AC has established that Ca2+ stimulation is mediated by CaM (Wu et al., 1993). Neither I-AC nor VIII-AC is stimulated by Gαi-coupled receptors in vivo (Wayman et al., 1994; Cali et al., 1994). Although I-AC is synergistically regulated by intracellular Ca2+ and hormones in vivo, VIII-AC is not (Cali et al., 1994). In contrast, V-AC and VI-AC are directly inhibited by Ca2+ in membranes (Yoshimura and Cooper, 1992; Katsushika et al., 1992), and VI-AC is inhibited by submicromolar Ca2+ in vivo (Cooper et al., 1994). Regulation of III-AC by Ca2+ and hormones is distinct from all of the other adenylyl cyclases characterized thus far; it is stimulated by hormones in vivo, and increases in intracellular Ca2+ inhibit this response.

Although in vitro studies using isolated membrane preparations or purified recombinant adenylyl cyclases and G proteins have provided valuable insight concerning mechanisms for regulation of adenylyl cyclases, it is becoming increasingly evident that conclusions drawn from in vitro data do not necessarily apply in vivo. For example, purified I-AC or I-AC in membranes is stimulated by addition of relatively high levels of activated recombinant Gαi2, demonstrating that this enzyme has a Gαi2 interaction domain (Tang et al., 1991). However, I-AC is not stimulated by activation of Gαi-coupled receptors in HEK-293 cells (Wayman et al., 1994) or in cultured neurons.5 VIII-AC is synergistically stimulated by CaM and recombinant Gαi5 in vitro, but it is not synergistically stimulated by Ca2+ and Gαi activation in vivo (Cali et al., 1994). Characterization of mechanisms for regulation of III-AC described in this study also demonstrates the importance of defining the regulatory properties of each adenylyl cyclase in vivo.

The physiological significance of Ca2+ inhibition of hormone-stimulated I-AC activity remains to be established. Adenylyl cyclase activity in most tissues is inhibited by millimolar levels of Ca2+ which has been attributed to formation of complexes between ATP and Ca2+, or binding of Ca2+ to a Mg2+ regulatory site on adenylyl cyclases (Steer and Levitzki, 1975). Several tissues including heart muscle (Potter et al., 1980) have been reported to contain adenylyl cyclase activity that is inhibited by submicromolar Ca2+. It is therefore possible that III-AC (Xia et al., 1992) and VI-AC (Yoshimura and Cooper, 1992; Katsushika et al., 1992) are both expressed in heart. The presence of III-AC activity in heart may provide a mechanism whereby the positive ionotropic and chronotropic effects of β-adrenergic agonists are attenuated by increased intracellular Ca2+. The development of transgenic mice strains deficient in III-AC should provide valuable information concerning the physiological functions of the enzyme and the significance of this regulatory mechanism for specific physiological processes including heart muscle contractility and rafactory signal transduction.

In summary, this study describes a novel mechanism for regulation of adenylyl cyclase activity and is the first report showing that CaM kinases can regulate adenylyl cyclase activity in vivo. This regulatory mechanism may be important for a variety of physiological processes including heart muscle contractility and attenuation of neurotransmitter-stimulated cAMP levels in neurons.

Acknowledgments—We thank Dr. Randy Reed for providing the III-AC cDNA clone; Dr. Zhengxi Xia for providing the I-AC clone, and Dr. E. J. Choi and Dr. Andy Dittman for providing HEK-293 cells stably expressing I-AC and III-AC. The construct encoding CaM kinase II was generously provided by Dr. P. R. Matthews and G. S. McKnight. We also thank Dr. Enrique Villarecis, Dr. Lauren Baker, Dr. Wenhui Hua, Dr. Guy Chan, Mark Nielsen, Soren Impye, and Scott Wonn for critical reading of this manuscript.

REFERENCES

Baksal, B. J., Hochner, B., Mahaut-Smith, M., Adams, S. R., Kaang, B. K., Kandel, E. R., and Tsien, R. Y. (1983) Science 260, 222–226

Bakalyar, H. A., and Reed, R. R. (1990) Science 250, 1403–1406

Brooks, S. P. J., and Storey, K. B. (1992) Anal. Biochem. 203, 119–126

Choi, E. J., Zwaagstra, J. C., Morel, C., Cooper, D. M. F., and Krupinski, J. (1994) J. Biol. Chem. 269, 12190–12195

Chen, C., and Okayaama, H. (1987) Mol. Cell. Biol. 7, 2745–2752

Choi, E. J., Xia, Z., and Storm, D. R. (1992a) Biochemistry 31, 6492–6498

Choi, E. J., Wong, S. T., Hinds, T. J., and Storm, D. R. (1992b) J. Biol. Chem. 267, 12440–12442

Choi E. J., Xia, Z., Villarecis, E. C., and Storm, D. R. (1993a) Curr. Opin. Cell Biol. 5, 269–273

Choi, E. J., Wong, S. T., Dittman, A. H., and Storm, D. R. (1993b) Biochemistry 32, 1891–1894

Cooper, D. M., Yoshimura, M., Zhang, Y., Chiono, M., and Mahey, R. (1994) Biochim. J. 297, 437–440

Dittman, A. H., Weber, J. P., Hinds, T. J., Choi, E. J., Migeon, J. C., and Storm, D. R. (1994) Biochemistry 33, 943–951

Enslin, S., Sun, P., Brückle, D., Soding, T. H., Klamo, E., and Soderling, T. R. (1994) J. Biol. Chem. 269, 15520–15527

Federman, A. D., Conkin, B. R., Schrader, K. A., Reed, R. R., and Bourne, H. R. (1992) Nature 356, 159–161

Feinstein, P. G., Schrader, K. A., Bakalyar, H. A., Tang, W. J., Krupinski, J., Gilman, A. G., and Reed, R. R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10173–10177

Gao, B., and Gilman, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10178–10182

Hill, D. H., and Straka, J. G. (1988) Anal. Biochem. 170, 203–208

Impye, S., Wayman, G. W., and Storm, D. R. (1994) Mol. Cell. Biol. 14, 5272–5281

Ishikawa, Y., Katsushika, S., Chen, L., Halnon, N. J., Kawabe, J., and Homcy, C. J., and Smith, R. A., Grant, F. J., Biggs, S., Bensch, P. A., Kuijper, J. L., Sheppard, P. O., Sprecher, C. A., and Kinosvog, W. (1993) Science 259, 1614–1616

Katsushika, S., and Ut, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3219–3223

Katsushika, S., Chen, L., Kawabe, J., Nilakantan, R., Halnon, N. J., Homcy, C. J., and Ishikawa, Y. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8774–8778

Krupinski, J., Coussei, F., Bakalyar, H. A., Tang, W. J., Feinstein, P. G., Orth, K., Slaughter, C., Reed, R. R., and Gilman, A. G. (1989) Science 244, 1558–1564

Krupinski, J., Lehman, T. C., Frankenfield, C. D., Zwaagstra, J. C., and Watson, P.
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A. (1992) J. Biol. Chem. 267, 24858–24862
Masure, H. R., Head, J. R., and Tice, H. M. (1984) Biochem. J. 218, 691–696
Matthews, R. P., Guthrie, C. R., Wailes, L. M., Zhao, X., Means, A. R., and
McKnight, G. S. (1994) Mol. Cell. Biol. 14, 6107–6116
Potter, J. D., Piascik, M. T., Wisler, P. L., Robertson, S. P., and Johnson, C. L.
(1980) Ann. N. Y. Acad. Sci. 356, 220–231
Rothermel, J. D. (1988) J. Biochem. (Tokyo) 251, 757–762
Salomon, Y., Londo, C., and Rodbell, M. (1979) Anal. Biochem. 85, 541–548
Seamon, K., and Daly, J. W. (1981) J. Biol. Chem. 256, 9799–9801
Steer, M. L., and Levitzki, A. (1975) J. Biol. Chem. 250, 2080–2084
Tang, W. J., and Gilman, A. G. (1991) Cell 70, 869–872
Tang, W. J., Krupinski, J., and Gilman, A. G. (1991) J. Biol. Chem. 266, 8595–8603
Watson, P. A., Krupinski, J., Kempinski, A. M., and Frankenfield, C. D. (1994)
J. Biol. Chem. 269, 28893–28898
Wayman, G. A., Impey, S., Wu, Z., Kinsvogel, W., Prichard, L., and Storm, D. R.
(1994) J. Biol. Chem. 269, 25400–25405
Wong, Y. H., Federman, A., Pace, A. M., Zachary, I., Evans, T., Pouyssegur, J., and
Bourne, H. R. (1991) Nature 351, 63–65
Wu, Z., Wong, S. T., and Storm, D. R. (1993) J. Biol. Chem. 268, 23766–23768
Wu, Z., Thomas, S. A., Xia, Z., Villacres, E. C., Palmiter, R. D., and Storm, D. R.
(1995) Proc. Natl. Acad. Sci. U. S. A. 92, 220–224
Xia, Z., Cheryl, D. R., Merchant, K. M., Dorsa, D. M., and Storm, D. R. (1991)
Neuron 6, 431–443
Xia, Z., Choi, E. J., Wang, F., and Storm, D. R. (1992) Neurosci. Lett. 144, 169–173
Yoshimura, M., and Cooper, D. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89,
6716–6720