Nitric Oxide Activation of p38 Mitogen-activated Protein Kinase in 293T Fibroblasts Requires cGMP-dependent Protein Kinase*

(Received for publication, June 24, 1999, and in revised form, October 19, 1999)

Darren D. Browning‡, Marisa P. McShane, Caroline Marty, and Richard D. Ye§

From the Department of Pharmacology, MC868, University of Illinois at Chicago, Chicago, Illinois 60612

Nitric oxide (NO) is an important signaling molecule that can affect many tissues to regulate diverse physiological processes, including smooth muscle relaxation, inflammation, platelet activation, apoptosis, and neuronal function (1–5). Several mechanisms have been described for NO function, including nitrosylation of key thiol groups of susceptible proteins, formation of reactive peroxynitrite, stimulation of ADP-ribosylation, and activation of soluble guanylyl cyclase (6–12). Guanylyl cyclase converts available GTP into cGMP that behaves as a second messenger by activating cyclic nucleotide dependent protein kinases (12). Increases in either cAMP or cGMP are capable of activating both protein kinase A (PKA)1 and protein kinase G (PKG), but the former is responsive to lower levels of cAMP and the latter is more sensitive to cGMP. In the past, the lower expression relative to PKA has made it difficult to identify processes regulated specifically by PKG, although specific inhibitors such as KT5823 have often been used to suggest the importance of this enzyme.

It has been reported that some effects of NO, including activation of gene expression and apoptosis, may be mediated by mitogen activated kinases (MAPK) (12–20). The MAP-kinases including extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK) and p38 represent a family of proteins that are activated by phosphorylation by upstream MAPK kinases (MEKs), which are activated in response to growth factors, cytokines, or various forms of cellular stress (21–23). MAPKs regulate fundamental cellular processes such as growth and differentiation by activating transcription factors (24, 25). Several studies have addressed the mechanism of NO-mediated activation of the ERK pathway. In Jurkat and murine neuronal cells activation of ERK is cGMP-independent, and there is evidence for nitrosylation of p21Waf1, which can then activate the c-Raf/MEK1,2 pathway (20, 26, 27). More recently it has been shown that activation of ERK in endothelial cells by NO required cGMP formation (28). Furthermore, using KT5823, these authors demonstrated a role for cGMP-dependent protein kinase (PKG) in a process involving phosphorylation and activation of c-Raf. Of interest, the PKG mediated phosphorylation of c-Raf has also been shown to prevent its coupling to Ras and therefore inhibits growth factor-induced ERK activation in baby hamster kidney cells (29). Thus it is likely that NO can activate the ERK pathway using a combination of both events.

Despite several reports of the phenomenon in different cell types, the events underlying the activation of JNK or p38 MAP-kinase by NO stimulation remain unclear (14, 15, 18, 20, 30). Using peroxynitrite to stimulate rat liver epithelial cells, it has been suggested that protein nitrosylation might be important to activation of p38 MAPK. Recently we reported that NO could specifically activate MEK3 and p38 MAPK in human neutrophils (15). The importance of PKG in this system has been suggested by the ability of increased cGMP levels to mimic the effect of NO and that KT5823 was able to block p38 MAPK activation by lipopolysaccharide.

This study investigated the mechanism of NO induced activation of p38 MAPK. We report that exogenous NO leads to phosphorylation of p38 MAPK in 293T fibroblasts and that this results in ATF-2-dependent gene expression. In addition, it was found that cGMP can mimic this response, and using a catalytic mutant of PKG we demonstrate that this enzyme is essential for both of these functions.

EXPERIMENTAL PROCEDURES

Materials—BPDEtide, H-89 and, 8-Br-cGMP were obtained from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). Protease inhibitor mixture, sodium nitroprusside (SNP-1), and Nonidet P-40 were from Calbiochem. p81 phosphocellulose was obtained from Up-
state Biotechnology Inc. (Lake Placid, NY), and \( \gamma^{32}\text{P}\)ATP was from Amersham Pharmacia Biotech, Inc. All other chemicals were obtained from Sigma. The PKG constructs were cloned as detailed below. Antibodies raised against PKG and PKA-R were purchased from Upstate Biotechnology Inc. (Lake Placid, NY). The murine FLAG epitope-tagged VASP protein was a gift from Dr. Michael Uhler (University of Michigan). Wild-type ASK-1 was a gift from Dr. Tatjana Vyoyn-Yasenetskaya (University of Illinois, Chicago). The dominant negative regulatory subunit for murine PKA (S81a) was a gift from Dr. Stanley McNight (University of Washington, Seattle, WA).

Measurement of Cellular cGMP Levels—The cGMP accumulation in response of 293T cells with nitric oxide was determined using an competitive enzyme-linked immunosorbent assay-based kit (BIOMOL Research Laboratories, Inc., Plymouth Meeting, PA). For these assays the cells from 10-cm dishes (approximately 2 \( \times 10^6 \)) were treated with SNP-1, and at various times the cells were washed in ice-cold phosphate-buffered saline and divided into two fractions for duplicate measurements. The cell extracts were prepared with 0.1 M HCl, and the cGMP was acylated and quantitated according to the manufacturer’s instructions.

Cell Culture and Transfection—The human embryonic kidney fibroblast cell line 293T was maintained in RPMI 1640 medium (Life Technologies, Inc.) containing 10% fetal bovine serum (Omega Scientific). The cells were grown on 10-cm tissue culture plates and split by repeated pipetting to achieve the desired cell density. Cells were transfected using exogenous proteins and reporter constructs using the calcium phosphate precipitation method. In all cases the DNA concentration was kept to 10 \( \mu \text{g} \) in 1 ml of precipitate using empty pRK7 vector. DNA precipitate (1 ml for a 10-cm dish or 0.15 ml for a well of a six-well plate, respectively) was added to the cells and incubated for 4 h. After changing the medium cells were incubated for an additional 20–24 h before experimentation. The expression of sT516a driven by the MT-1 promoter was induced by changing the infection of the transfected cells for an additional 16 h in RPMI 1640 containing 2.5% fetal bovine serum and 100 \( \mu \text{M} \) ZnCl\(_2\). In some studies, including the luciferase reporter experiments, cells were serum-starved for 12–16 h before use.

Cloning and Analysis of Wild-type and Mutant PKG1a—Two fragments of PKG1a were cloned by PCR using the PCR-Script vector according to manufacturer instructions (Stratagene, La Jolla, CA). The template cDNA was prepared by reverse transcriptase using human lung RNA (CLONTECH, Palo Alto, CA). The two fragments were joined using a unique Pst site restriction site, and the full-length construct was subcloned into BamHI and EcoRI sites of the pCDNA3.1 expression vector. The Pkg1a mutant T516A was made with the 3 \( \text{Pst} \) fragment containing the catalytic region by PCR, and the mutant fragment was joined via the Pst site into PCR-Script vector as mentioned above. Verification of both wild-type and mutant forms of PKG1a was assessed by sequencing, which indicated no mutations from published sequence except for the T516A replacement. Expression of the PKG1a constructs in pCDNA3.1 was assessed by transfecting the 293T cells followed by Western blotting with a commercially available COOH-terminal specific antiserum PKG antibody (Upstate Biotechnology Inc., Lake Placid, NY). The kinase activity of the expressed enzymes was measured in cell homogenates using BPDEtide as substrate as detailed previously (31) except that 0.5 \( \mu \text{g} \) of the PKA inhibitor H89 was incorporated in the reaction mixture instead of inhibitory peptide. A truncated version of type 1 PKG corresponding to the PKA-C1 catalytic subunit gene was generated by PCR using the full-length cDNA sequence described above and the fragment subcloned into the pCDNA3.1 expression vector.

Measuring p38 MAPK Phosphorylation—Assessment of the phosphorylation state of p38 MAPK in 293T cells was accomplished by Western blotting using a polyclonal anti-phospho-p38 MAPK antibody (New England Biolabs, Beverly, MA) and visualized with enhanced chemiluminescence (Pierce). Briefly, the medium was removed from cells grown on 10-cm dishes and replaced by serum-free RPMI 1640. After treatment in this medium the plates were immediately placed on ice and the RPMI replaced by 10 ml of ice-cold phosphate-buffered saline. The cells were removed from the plates by repeated pipetting and harvested by centrifugation at 1000 \( \times g \) at 4 \( ^\circ \text{C} \). For some experiments cell extracts were made by resuspending the cells in denaturing sample buffer and analyzing these proteins on Western blots directly. In other experiments the protein loading in each lane (approximately 20 \( \mu \text{g} \)) was assessed by reprobing the blots with antibodies against unphosphorylated p38.

In other experiments involving transfection of FLAG epitope-tagged p38 (a generous gift from Dr. J. Han, Scripps Research Institute), the cell pellet was resuspended in lysis buffer (50 nm Tris-HCl, pH 8.0, 1 mm EDTA, 1% Nonidet P-40, 150 mm NaCl, 1 mm sodium vanadate, 5 mm sodium fluoride, and 1 \( \times \) protease inhibitor mixture) and agitated at 4 \( ^\circ \text{C} \) for 20 min. Following clarification by centrifugation, the exogenously expressed p38 was immunoprecipitated from the extracts using anti-FLAG M2 coupled to agarose (Eastman Kodak Co.). The p38 in the washed precipitates was then eluted from the beads by incubating 10 min on ice in 400 \( \mu \text{g} \) ml FLAG peptide, and after removing the beads, fractions of the supernatant were analyzed by Western blotting with the anti-phospho-p38 antibody. Reprobing with monoclonal anti-FLAG M2 did standardization of the p38 loading on the blots. In some instances quantitation of p38 MAPK phosphorylation was accomplished by scanning autoradiograms followed by densitometric analysis with ImageQuant software (V3.2, Molecular Dynamics). The intensity of the phosphorylated p38 was standardized with the intensity of the total p38 MAPK and expressed as a ratio of the basal level.

Assay for ATF-2-dependent Gene Expression—Transcriptional activity stimulated by p38 activation was measured using the Path-Detect ATF-2-dependent luciferase reporter assay (Stratagene, La Jolla, CA). In these experiments the 293T cells were cultured in six-well dishes and transfected with 150 \( \mu \text{g} \) of DNA precipitate containing 75 ng of luciferase reporter and 0.4 ng of ATF-2 fusion vector as detailed above. After approximately 36 h the transfected cells were stimulated for 6 additional hours. Luciferase activity in cell lysates was determined using luminal substrate according to the manufacturer’s recommendations.

## RESULTS

The MAPKs are activated by phosphorylation of specific tyrosine and threonine residues by dual specificity MEKs. Thus the phosphorylation state of any MAPK is a good measure of the activation of the enzyme. The ability of the nitric oxide-releasing compound sodium nitroprusside (SNP-1) to activate p38 MAPK in 293T cells was assessed by Western blotting with a polyclonal antiserum specific for the phosphorylated version of p38 MAPK. There was some background phosphorylation of p38 in untreated cells, but as early as 5 min after challenge with 100 \( \mu \text{M} \) SNP-1, this level increased and was maximal after 15 min (Fig. 1A). A major effector function of NO is guanylyl cyclase, which leads to an increase in cellular cGMP levels. Treatment of 293T cells with 100 \( \mu \text{M} \) SNP-1 resulted in a rapid increase in cellular cGMP and was maximal 15–20 min following stimulation (Fig. 1B). The kinetics of cGMP formation
Activation of p38 by cGMP-dependent Protein Kinase

2813

**Fig. 2.** Phosphorylation of p38 MAPK by nitric oxide in transfectected cells leads to ATF-2-dependent gene expression. The activation of p38 MAPK in 293T cells transfected with p38 tagged with the FLAG epitope was assessed by stimulating the transfected cells with 8-Br-cGMP for various times followed by analysis of phospho-p38 in immunoprecipitates prepared using anti-FLAG-agarose. A comparison of the effectiveness of TNFα (400 ng/ml, 30 min), 8-Br-cGMP (250 μM, 5 min), and SNP-1 (100 μM, 30 min) to cause phospho-p38 accumulation was assessed in transfected 293T cells by immunoprecipitation of FLAG-tagged p38 followed by Western blotting. The lower panel shows the loading of total p38 in the immunoprecipitates as parallel blots were probed with anti-FLAG M2 antibody (A). Blot shown is representative of results from at least three experiments. The activity of phospho-p38 to cause ATF-2 dependent gene expression was assessed by transfecting 293T cells with ATF-2/luciferase reporter followed by stimulation with TNFα (400 ng/ml), 8-Br-cGMP (250 μM), and SNP-1 (100 μM). After 6 h the cells were harvested, and the expression of luciferase activity in homogenates was measured in a luminometer (B). Error bars represent the S.E. from at least three independent experiments.

Although there are several cGMP-binding proteins present in cells, PKG is believed to function in a signaling capacity and has been demonstrated to be downstream of NO (12). Because of the ability of cyclic nucleotides to cross-activate both PKA and PKG, it was important to determine whether the exogenously added 8-Br-cGMP was able to activate PKA. To test this, a cyclic nucleotide unresponsive regulatory subunit for PKA (δRIα), which behaves in a dominant negative fashion, was transfected into 293T cells. To measure the activation of PKA and PKG we took advantage of the fact that VASP is a substrate for both kinases and expressed a FLAG epitope-tagged version, which could be immunoprecipitated from the cells and its phosphorylation measured as a mobility shift on Western blots (31). It was found that 250 μM 8-Br-cAMP or cGMP could phosphorylate VASP, but the cAMP was twice as effective (Fig. 3A). The greater efficacy of cAMP likely reflects differences in expression levels of the two kinases as PKG isoforms are generally less abundant. Expression of δRIα effectively blocked the ability of the cAMP to phosphorylate VASP, reducing the amount of phospho-VASP to close to basal levels. Overexpression of δRIα did not significantly reduce the phosphorylation of VASP by cGMP. Taken together these data demonstrate the effectiveness of the δRIα to shut down the PKA system and suggest that at 250 μM 8-Br-cAMP or cGMP there is no cross-activation of PKA and PKG in these cells. The importance of PKG downstream of cGMP in that activation of p38 MAPK was further supported by the ability of this nucleotide to cause p38 MAPK phosphorylation in cells transfected to express δRIα with almost identical kinetics to cells with a functional PKA system (Fig. 3B).

The type 1 isoforms of PKG are more widely distributed than type 2 and are the enzymes found in immune cells (32). To test the importance of PKG in the activation of p38 MAPK by NO, PKG1α was cloned, and a catalytically inactive mutant construct of this enzyme was generated for use in the 293T cell transfection system. The T516A mutation has been previously characterized and shown to prevent the phosphorylation of a conserved threonine residue located in the catalytic region of the enzyme, a process thought to be essential for the catalytic function of this enzyme (33). Low levels of PKG activity were measured in untransfected or mock-transfected 293T cells using a peptide substrate based assay (Fig. 4). Transfection of the cells with either wild-type or the T516A mutant construct resulted in overexpression of the protein which was detected as an intense band migrating at 80-kDa on Western blots probed with a polyclonal anti-PKG antibody (Fig. 4, inset). PKG activity in cell homogenates was not significantly altered by transfection of the T516A mutant confirming the absence of catalytic function. In homogenates transfected with the wild-type PKG1α, the activity was approximately 10-fold higher than in mock-transfected cells and the ratio of basal to cGMP stimulated activity was identical. The importance of PKG in cGMP-mediated stimulation of phospho-p38 was assessed by cotransflecting the wild-type and T516A mutant isoforms of PKG1α with the FLAG epitope-tagged p38 MAPK followed by immu-
noprecipitation and Western blotting. In these experiments overexpression of the catalytic mutant blocked the ability of 8-Br-cGMP to increase p38 phosphorylation (Fig. 5). Furthermore, the increased levels of phospho-p38 that were found in 8-Br-cGMP-stimulated cells was further elevated in cells transfected to overexpress PKG1α. The ability of the wild-type PKG construct to enhance cGMP-stimulated p38 MAPK activation was variable and probably depends on the abundance of endogenous PKG, which was also appeared to vary with culture age (not shown). Other than the cyclic nucleotide specificity, a major difference between PKA and PKG is that distinct PKA genes encode the regulatory and catalytic subunits whereas they are present as separate domains in a single polypeptide in PKG. Expression of the catalytic region of type 1 PKG produces a constitutive activity that is insensitive to cGMP (31). When overexpressed, the truncated PKG was able to stimulate p38

Fig. 3. Activation of p38 MAPK with 250 μM cGMP does not involve PKA. To determine whether the cGMP added to cells was able to activate PKA as well as PKG, the common substrate for both enzymes VASP was transfected into 293T cells either alone or in combination with a dominant negative regulatory subunit for PKA (cAMP-R, cGMP-R). Cells were either untreated (Basal) or stimulated for 10 min with 250 μM of either cAMP or cGMP (as indicated), and the levels of phosphorylated VASP in homogenates was measured by immunoprecipitation of the VASP (via a FLAG tag) followed by Western blotting. The relative levels of phosphorylated to unphosphorylated VASP in the extracts are shown graphically by densitometric analysis of the autoradiogram. The expression of the dominant negative R1α subunit in the transfected cells was assessed by Western blot analysis of the lysates (lower panel) (A). The effect of the dominant negative PKA-R1α on the ability of 8-Br-cGMP (250 μM) to stimulate phosphorylation of p38 MAPK was assessed by transfection of the cells to express either FLAG epitope-tagged p38 alone (open bars) or cotransfected with dominant negative PKA-R1α (filled bars). At different times following stimulation, the phospho-p38 MAPK was assessed by Western blotting and the phospho-p38 levels quantitated by densitometry and normalized with the total p38 MAPK loaded per lane. The levels of phospho-p38 shown are relative to the basal amount which was arbitrarily set as 1.0 (B).

Fig. 4. Overexpression of wild-type and a catalytic mutant of PKG1α in 293T cells. The amount of cGMP-dependent protein kinase activity found in 293T fibroblasts transfected with empty vector (Mock), wild-type PKG1α (WT), or mutant PKG1α (T516A) was determined using BPDEtide as detailed under “Experimental Procedures.” The transfer of phosphate to the PKG substrate peptide was measured in the absence (open bars) and the presence (hatched bars) of 20 μM cGMP. The relative expression of PKG protein in the cell homogenates was compared by Western blotting with an anti-PKG (COOH terminus) antibody (inset). The error bars show the S.E. for three independent experiments.

Fig. 5. Involvement of PKG in cGMP-induced phosphorylation of p38 MAPK. The effect of transfecting 293T fibroblasts to express wild-type or mutant PKG1α (T516A) on cGMP stimulated p38 phosphorylation was determined. Cells were cotransfected with 1 μg of FLAG-p38, and either 0.1 or 1 μg of PKG construct and 24 h later were stimulated with 250 μM 8-Br-cGMP for 5 min. The cell homogenates were subjected to immunoprecipitation with anti-FLAG M2 and the precipitates analyzed for phospho-p38 content by Western blotting (top panel). The level of phospho-p38 found in unstimulated cells is shown as “Basal.” Equal loading of the lanes is shown by probing the immunoprecipitates with anti-FLAG M2 antibody (middle panel). The expression of PKG1α in the transfected cells was determined by measuring PKG protein in the cell homogenates by Western blotting with anti-PKG serum (lower panel) (A). The phosphorylation of p38 MAPK detected by Western blot analysis was quantitated by densitometry and standardized with the intensity of FLAG-p38 in the precipitates (B). The data shown were reproduced in at least two independent experiments.
MAPK phosphorylation in the absence of cGMP stimulation (Fig. 6). Of interest, the constitutive PKG produced lower levels of p38 MAPK phosphorylation than overexpressed ASK-1, which is known to directly activate MEK3 in the p38 pathway.

Because of its pleiotropic effects, there exists a possibility that NO might activate p38 MAPK by more than one means. To address this issue the effect of overexpression of PKG1α (T516A) on p38 MAPK activation by NO treatment was measured by both Western blotting and by ATF-2-dependent luciferase activity. The 293T cells stimulated with 100 μM SNP-1 showed a high level of p38 MAPK phosphorylation without mutant PKG (T516A) (Fig. 7A). At 1 μg of DNA/plate the amount of phospho-p38 in the SNP-1-stimulated cells was reduced to basal levels. Similarly, the activation of ATF-2-dependent luciferase activity stimulated by SNP-1 was completely inhibited by expression of the catalytic mutant of PKG1α (Fig. 7B).

**DISCUSSION**

Nitric oxide synthesis is induced by stimulation with a variety of different ligands although the function of this in most systems is poorly defined. Synthesis of nitric oxide contributes to vascular-endothelial growth factor stimulation of cell proliferation by activating the ERK pathway (17, 28). In a recent study, we demonstrated a role for NO in LPS-stimulated p38 MAPK phosphorylation, a process that requires cGMP accumulation (15). Results shown here demonstrate that NO can activate p38 MAPK in 293T fibroblasts, suggesting that NO induced p38 MAPK activation is not confined to certain differentiated cell types but is a widespread phenomenon. The induction of ATF-2-dependent gene expression by either NO or 8-Br-cGMP lends further support to the physiological significance of this pathway.

As a first step toward understanding the mechanism underlying cGMP-induced activation of p38 MAPK, the current study evaluated the role of cGMP-dependent protein kinase (PKG). Whereas much attention has focused upon PKA as a cyclic nucleotide responsive protein kinase, the function of PKG and how this protein might mediate the effects of NO on cell behavior have not been fully elucidated. Results shown here reveal that blockade of the PKA system does not affect activation of p38 MAPK by cGMP and that the levels of cGMP causing phosphorylation of p38 MAPK do not lead to PKA activation. Together these data indicate that as in other systems, PKG is the likely downstream effector of NO in the pathway leading to activation of p38 MAPK. PKG has three isoforms: PKG1α, PKG1β (products of alternative splicing), and PKG2. These enzymes differ predominantly in the amino-terminal regulatory region and in the intracellular and tissue distribution patterns (2, 34). Overexpression of wild-type PKG1α potentiated the activation of p38 MAPK by cGMP, and expression of the constitutive catalytic domain of PKG1α conferred increased levels of phosphorylated p38 MAPK in the absence of exogenous cGMP. These data demonstrate that PKG is capable of activating p38 MAPK in these cells. Exogenous expression of catalytically inactive mutants of many kinases has been very useful in establishing specific functions for these proteins as the mutants often behave in a dominant negative fashion. The T516A mutant of PKG has been reported previously to be catalytically inactive in vitro when purified from baculovirus infected SF9 insect cells (33). Here we show that transfection of this mutant into 293T cells does not confer kinase activity confirming indeed that this mutant has lost kinase activity. Furthermore, our results provide direct evidence for a dominant negative function of this mutant PKG, as it possesses the ability to block cGMP and NO stimulated activation of p38 MAPK. In cells stimulated with exogenous NO, blockade of both p38 phosphorylation and ATF-2-dependent gene expression with the T516A mutant suggests that PKG activation is essential to this pathway and that other mechanisms such as protein nitrosylation are less important. Thus, this mutant PKG will complement the use of the inhibitor KT5823 in studies of PKG where inhibition of its function is required. The mechanism for the dominant negative action of this mutant PKG is currently not known, nor is its effect on other PKG isotypes. Many protein kinases localize to specific regions of the cell by interacting with specific anchoring proteins, and this interaction is essential for enzyme function (35). Because such proteins exist for PKG (36), it is possible that at higher levels of expression the mutant PKG might displace the endogenous enzyme from its anchoring proteins. Further characterization of its mode of action may provide useful information for future application of this dominant negative kinase. It is well established that p38 MAPK can be activated by a large number of environmental factors, and all of them do not activate the same signaling mechanism. Although significant compared with the basal level, the degree of p38 MAPK acti-
Activation of p38 by cGMP-dependent Protein Kinase

Michael Uhler, Jiahui Han, and Tatyana. Voyno-Yasenyetskaya for their generosity in providing the cDNA constructs used in this study.

REFERENCES

1. Moilanen, E., and Vapaatalo, H. (1995) Ann. Med. 27, 359–367
2. Pfeifer, A., Klett, P., Massberg, S., Ny, L., Saubier, M., Hinnebus, C., Wang, G. X., Korth, M., Assodi, A., Andersson, K. E., Krombach, F., Mayerhofer, A., Ruth, P., Fassler, R., and Hofmann, F. (1998) EMBO J. 17, 3045–3051
3. Wang, X., and Robinson, F. J. (1997) J. Neurochem. 68, 443–456
4. Zhou, M., Hu, Y., Schultz, C., Kandel, E. R., and Hawkins, R. D. (1994) Nature 368, 635–639
5. Archer, S. L., Huang, J. M., Hamp, V., Nelson, D. P., Shultz, P. J., and Weir, E. K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7583–7587
6. McDonald, L. J., and Moss, J. (1994) Mol. Cell. Biochem. 138, 201–206
7. Stamler, S. J. (1994) Cell 78, 931–936
8. Brune, B., Dimmel, S., Molina y Vedia, L., and Lapetina, E. G. (1994) Life Sci. 54, 61–70
9. Brune, B., Mohr, S., and Messmer, U. K. (1996) Rev. Physiol. Biochem. Pharmacol. 127, 1–30
10. Willnow, T. E., Seh, J. K., Waleth, T. F., Lee, H. C., White, A. M., and Gallione, A. (1996) J. Biol. Chem. 271, 3689–3705
11. Kim, Y. M., Talanian, R. V., and Billiar, T. R. (1997) J. Biol. Chem. 272, 31138–31148
12. Hohes, A. J. (1997) Trends Pharmacol. Sci. 18, 484–491
13. Callisen, D., Pleischlaffer, J., and Brune, B. (1999) J. Immunol. 161, 4582–4588
14. Callisen, D., and Brune, B. (1999) Biochemistry 38, 2279–2286
15. Browning, D. D., Windes, N. D., and Ye, R. D. (1999) J. Biol. Chem. 274, 537–542
16. Kanteriewicz, B. I., Knapp, L. T., and Klann, E. (1998) J. Neurochem. 70, 1099–1106
17. Parenti, A., Morbidelli, L., Cai, X. L., Douglas, J. G., Hood, J. D., Graner, H. J., Ledda, F., and Ziche, M. (1998) J. Biol. Chem. 273, 4220–4226
18. Schieke, S. M., Briviba, K., Klotz, L. O., and Sies, H. (1999) FEBS Lett. 448, 301–303
19. Sciorati, C., Nistico, G., Meldolesi, J., and Clementi, E. (1997) Br. J. Pharmacol. 122, 687–697
20. Lander, H. M., Jacobina, A. T., Davis, R. J., and Tauras, J. M. (1996) J. Biol. Chem. 271, 19755–19769
21. Paul, A., Wilson, S., Belham, C. M., Robinson, C. J., Scott, P. H., Gould, G. W., and Plevin, R. (1997) Cell. Signalling 9, 405–410
22. Robinson, M. J., and Cobb, M. H. (1997) Curr. Opin. Cell Biol. 9, 180–186
23. Seger, R., Ahn, N. G., Posada, J., Munar, E. S., Jensen, A. M., Cooper, J. A., Cobb, M. H., and Krebs, E. G. (1992) J. Biol. Chem. 267, 14373–14381
24. Su, B., and Karin, M. (1996) Curr. Opin. Immunol. 8, 402–411
25. Treisman, R. (1996) Curr. Opin. Cell Biol. 8, 205–215
26. Tamura, N., Itoh, H., Ogawa, Y., Nakagawa, O., Harada, M., Chun, T. H., Suga, S., Yoshimasa, T., and Nakao, K. (1996) Hypertension 27, 552–557
27. Yun, H. Y., Gonzalez-Zulueta, M., Dawson, V. L., and Dawson, T. M. (1998) J. Biol. Chem. 273, 687–697
28. Hood, J., and Granger, H. J. (1998) J. Biol. Chem. 273, 23504–23508
29. Suhasihi, M. K., Li, H., Lohmann, S. M., Boss, G. R., and Pilz, R. B. (1998) Mol. Cell. Biol. 18, 6985–6993
30. Lo, Y. C., Wong, J. M. S., and Cruz, T. F. (1996) J. Biol. Chem. 271, 15703–15707
31. Collins, S. R., and Uhler, M. D. (1999) J. Biol. Chem. 274, 8391–8404
32. Lohmann, S. M., Vaandrager, A. B., Smolenzaki, A., Walter, U., and De Jonge, H. R. (1997) Trends Biochem. Sci. 22, 307–312
33. Feil, R., Kellermann, J., and Hofmann, F. (1995) Biochemistry 34, 13152–13158
34. Lincoln, T. M., Komalavilas, P., Boerth, N. J., MacMillan-Crow, L. A., and Cornwell, T. L. (1995) Adv. Pharmacol. 34, 305–322
35. Mochly-Rosen, D. (1996) Science 268, 247–251
36. Vo, N. K., Gettemy, J. M., and Coghlan, V. M. (1998) J. Biol. Chem. 273, 3045–3051
37. Acknowledgments—We are grateful to Drs. Stanley McKnight,