Nitric oxide (NO) regulates the expression of multiple genes but in most cases its precise mechanism of action is unclear. We used baby hamster kidney (BHK) cells, which have very low soluble guanylate cyclase and cGMP-dependent protein kinase (G-kinase) activity, and C5-74 arterial smooth muscle cells, which express these two enzymes, to study NO regulation of the human fos promoter. The NO-releasing agent Deta-NONOate (ethanamine-2,2′-(hydroxynitrosohydrazone)bis-) had no effect on a chloramphenicol acetyltransferase (CAT) reporter gene under control of the fos promoter in BHK cells transfected with an empty vector or in cells transfected with a G-kinase Iβ expression vector. In BHK cells transfected with expression vectors for guanylate cyclase, Deta-NONOate markedly increased the intracellular cGMP concentration and caused a small (2-fold) increase in CAT activity; the increased CAT activity appeared to be from cGMP activation of cAMP-dependent protein kinase. In BHK cells co-transfected with guanylate cyclase and G-kinase expression vectors, CAT activity was increased 5-fold in the absence of Deta-NONOate and 7-fold in the presence of Deta-NONOate. Stimulation of CAT activity in the absence of Deta-NONOate appeared to be largely from endogenous NO since we found that: (i) BHK cells produced high amounts of NO; (ii) CAT activity was partially inhibited by a NO synthase inhibitor; and (iii) the inhibition by the NO synthase inhibitor was reversed by exogenous NO. In C5-74 cells, we found that NO increased fos promoter activity and that the increase was prevented by a guanylate cyclase inhibitor. In summary, we found that NO activates the fos promoter by a guanylate cyclase- and G-kinase-dependent mechanism.

Nitric oxide (NO) is a pluripotential molecule involved in regulating blood pressure, neurotransmission, and immune function. One of its major intracellular targets is the heme group of soluble guanylate cyclase with NO markedly stimulating enzymatic activity and thereby increasing the intracellular cGMP concentration (2, 3). Other NO targets are thiol-containing proteins, iron sulfur proteins and non-heme iron; in addition, NO can react with oxygen to produce peroxynitrite and hydroxyl radical, both of which can have physiological effects (4).

NO regulates the expression of multiple genes including c-fos, junB, heme oxygenase, smooth muscle α-actin, vascular endothelial growth factor, vascular cell adhesion molecule-1 (VCAM-1), and mitogen-activated kinase phosphatase-1 (5–10). In regulation of VCAM-1, NO appears to act independently of cGMP because its effect is not mimicked by cGMP analogs, while in regulation of c-fos and junB, NO and cGMP analogs induce similar changes (5, 6, 10). In cases where NO and cGMP analogs function similarly, it is likely that NO works through activation of soluble guanylate cyclase, but this has not been shown definitively. Moreover, it is not clear which of several cGMP target proteins, e.g., cGMP-dependent protein kinases (G-kinase), cGMP-gated ion channels, cGMP-activated phosphodiesterases, or cGMP-inhibited phosphodiesterases, mediates the effects of NO on gene expression (11).

In this study we used baby hamster kidney (BHK) cells, which have very low soluble guanylate cyclase and G-kinase activity, to determine whether NO regulates the fos promoter via a cGMP/G-kinase signal transduction pathway. We found that maximal stimulation of the fos promoter by NO in BHK cells required expression of both guanylate cyclase and G-kinase. In cells transfected with guanylate cyclase alone, NO caused a small activation of the fos promoter, apparently by cGMP activation of cAMP-dependent protein kinase (protein kinase A), either directly by cross-activation or indirectly by inhibition of phosphodiesterase activity (12). In cells containing endogenous guanylate cyclase and G-kinase, NO stimulation of the fos promoter was inhibited by the guanylate cyclase inhibitor ODQ. This, to our knowledge, is the first demonstration that NO regulates gene expression via cGMP activation of G-kinase.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transient Transfection Experiments—**BHK cells were cultured and transfected as described previously (13). All cells received 50 ng of pFos-CAT, which contains the chloramphenicol acetyltransferase (CAT) gene under control of the human fos promoter, and 50 ng of the luciferase expression vector pRSV luciferase to serve as an internal control for transfection efficiency (5, 13). As indicated, cells also received 300 ng of a human G-kinase Iβ expression vector (14) and/or 100 ng each of expression vectors encoding the α1 and β1 subunits of rat soluble guanylate cyclase (15); variable amounts of the empty vector pcDNA were used to keep the total amount of DNA constant. Where indicated, N5′-monoethyl-L-arginine mononacete (NMEA, Alexis Corp., San Diego, CA) was added 16 h prior to cell harvest and 8-Br-cGMP (Biolog Life Sciences, Bremen, Federal Republic of Germany), Deta-NONOate (ethanamine-2,2′-(hydroxynitrosohydrazone)bis-, Cayman

---

*This work was supported in part by National Institutes of Health Grant R01GM055586 (to R. B. P.) and American Heart Association Grant 9650582N (to G. R. B.). 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.

**To whom correspondence should be addressed. Tel.: 619-534-8805; Fax: 619-534-1421; E-mail: gboss@ucsd.edu.

The abbreviations used are: NO, nitric oxide; BHK, baby hamster kidney; G-kinase, cGMP-dependent protein kinase; protein kinase A, cAMP-dependent protein kinase; CAT, chloramphenicol acetyltransferase; SNAP, S-nitroso-Penicillamine; 8-Br-cGMP, 8-bromo-cGMP; PKI, protein kinase inhibitor; Deta-NONOate, ethanamine-2,2′-(hydroxynitrosohydrazone)bis; Deta, NONOate-releasing parent compound of Deta-NONOate; NMEA, N5′-monoethyl-L-arginine mononacete; ODQ, [1H-(1,2,4)oxadiazolo(4,3-a)quinolinal-1-one]; TRE, phorbol ester response element.**
Guanylate cyclase and G-kinase activity in transfected BHK cells

BHK cells were transfected with vectors encoding soluble guanylate cyclase (GC), G-kinase (GK), or the combination of the two, and 36 h later cells were harvested. Cytoplasmic and soluble guanylate cyclase activities were measured as described under “Experimental Procedures.” Guanylate cyclase activity was measured in the absence and presence of 50 μM SNAP. The data are the mean ± S.D. of at least three independent measurements performed in duplicate. Empty vector refers to the pCB6 parental vector without guanylate cyclase or G-kinase DNA, and ND is not determined.

Intracellular cGMP concentration in BHK cells

BHK cells were transfected as described in the legend to Table I, and 36 h later cells were harvested, and cGMP concentrations were measured as described under “Experimental Procedures”; 250 μM Deta-NONOate and/or 20 mM NMEA were added 8 h prior to cell extraction. The data are the mean ± S.D. of three independent experiments performed in duplicate. GC, soluble guanylate cyclase; GK, G-kinase; ND, not determined.

| DNA transfected | Guanylate cyclase activity | G-kinase activity |
|-----------------|----------------------------|------------------|
| No addition     | 50 μM SNAP                  |
| Empty vector    | 3.2 ± 1.3                  | 20 ± 15          |
| GC              | 35 ± 2                     | 242 ± 70         |
| GK              | ND                        | ND               |
| GC/GK           | 65 ± 16                    | 280 ± 60         |

| DNA transfected | No addition | Deta-NONOate | NMEA | NMEA + Deta-NONOate |
|-----------------|-------------|--------------|------|---------------------|
| Empty vector    | 0.2 ± 0.06  | 0.5 ± 0.2    | ND   | ND                  |
| GC              | 1.6 ± 0.8   | 50 ± 12      | ND   | ND                  |
| GK              | 0.5 ± 0.02  | 1.1 ± 0.4    | ND   | ND                  |
| GC/GK           | 12 ± 3      | 131 ± 15     | 5.5 ± 3 | 105 ± 15      |

Intracellular cGMP in Guanylate Cyclase- and G-kinase-transfected BHK Cells—In BHK cells transfected with empty vector, intracellular cGMP was low but measureable, consistent with the cells’ low guanylate cyclase activity, and Deta-NONOate caused a small increase in cGMP, compatible with the low endogenous guanylate cyclase being stimulated by NO (Table II). In cells transfected with guanylate cyclase, intracellular cGMP increased about 8-fold and Deta-NONOate increased cGMP ~250-fold (Table II; throughout the text, -fold increase is relative to cells transfected with empty vector receiving no drug treatment).

In cells transfected with both guanylate cyclase and G-kinase expression vectors, intracellular cGMP increased ~60-fold and Deta-NONOate increased cGMP >600-fold (Table II). The higher cGMP in cells co-transfected with guanylate cyclase and G-kinase compared with cells transfected with guanylate cyclase alone is probably because the cGMP assay measures total intracellular cGMP, including that which is protein-bound: the intracellular G-kinase concentration in cells transfected with this enzyme is 3–6 pmol/mg of protein corresponding to a cGMP binding capacity of 12–24 pmol/mg of protein (13). In addition, as shown below, BHK cells produce relatively high amounts of NO. Thus, high cGMP in the guanylate cyclase- and G-kinase-transfected cells in the absence of Deta-NONOate probably represents the combination of high endogenous NO stimulating the transfected guanylate cyclase with the cGMP produced binding to the transfected G-kinase. These results suggest that in cells co-transfected with guanylate cyclase and G-kinase, G-kinase is likely to be activated, even in the absence of exogenous NO.

Transactivation of the fos Promoter by NO in BHK Cells—We showed previously that NO-releasing agents activate synthetic TRE-containing promoters in REF52 fibroblasts and rat thyroid cells, and we and others (5, 6, 22–26) have shown that NO increases c-fos mRNA in many cell types. We have also shown that cGMP regulates the fos promoter in BHK cells via G-kinase activation and nuclear translocation (13, 27).

To test whether NO’s effect on the fos promoter in BHK cells was mediated by cGMP activation of G-kinase, we transfected cells with pFos-CAT in the absence or presence of guanylate cyclase and G-kinase expression vectors. We found that the NO-generating agent Deta-NONOate had no effect on CAT expression in cells transfected with empty vector (Fig. 1). When the cells were transfected singly with guanylate cyclase or G-kinase expression vectors, CAT activity increased a minimal 1.3-fold in the absence of drug treatment which was not statistically significant (Fig. 1). Deta-NONOate increased CAT activity a small, but statistically significant, 2.1-fold in the guanylate cyclase-transfected cells but had no effect in the G-kinase-transfected cells (Fig. 1). The small increase in CAT activity by Deta-NONOate in guanylate cyclase-expressing
cells points to a cGMP-mediated mechanism, and as described above, we found that under these conditions Deta-NONOate increased the intracellular cGMP concentration by 250-fold (Table II). This large increase in cGMP could activate the cells low endogenous G-kinase or, as shown later, cross-activate protein kinase A. Deta-NONOate increased CAT activity more than 8-Br-cGMP in the guanylate cyclase-transfected cells (Fig. 1); as discussed later, this difference could be because endogenously produced cGMP is more available than exogenously provided 8-Br-cGMP. As we showed previously (13), 8-Br-cGMP stimulated CAT expression about 4-fold in cells transfected with the G-kinase expression vector and was without effect in cells transfected with empty vector (Fig. 1), indicating that at the concentration used, 8-Br-cGMP did not cross-activate protein kinase A (Fig. 1).

When the cells were co-transfected with guanylate cyclase and G-kinase expression vectors, CAT expression increased 5.5-fold in the absence of drug treatment (Fig. 1). This substantial increase in CAT activity can be attributed to the large increase in intracellular cGMP under these conditions (Table II) activating the transfected G-kinase (discussed above). Deta-NONOate further increased CAT activity to about 7-fold in cells co-transfected with guanylate cyclase and G-kinase expression vectors (Fig. 1) and, as shown, further increased intracellular cGMP (Table II). The increase in CAT activity by Deta-NONOate in the guanylate cyclase- and G-kinase-transfected cells was similar to that observed when these cells were treated with 8-Br-cGMP (Fig. 1; the further increase in CAT activity by Deta-NONOate and 8-Br-cGMP was not statistically significant compared with the untreated cells). Deta, the parent compound of Deta-NONOate, which does not release NO, had no effect on CAT expression in guanylate cyclase- and/or G-kinase-expressing cells.

To examine whether high endogenous NO production in the guanylate cyclase- and G-kinase-expressing cells could be the cause of increased CAT expression in the absence of Deta-NONOate or 8-Br-cGMP, we treated cells with the competitive NO synthase inhibitor NMEA. We found that NMEA reduced CAT expression by 40% in cells transfected with guanylate cyclase and G-kinase expression vectors (Fig. 1). To eliminate possible effects of altered culture conditions, all cells were grown in medium containing 400 μM arginine; this relatively high arginine concentration may have prevented more complete inhibition of CAT expression by NMEA and other workers have also found that NO synthase inhibitors are only partially effective at high micromolar arginine concentrations (28). Corresponding to the 40% reduction in CAT expression, NMEA reduced intracellular cGMP by 50% in the guanylate cyclase- and G-kinase-transfected cells (Table II). When Deta-NONOate was added to the NMEA-treated cells, CAT expression returned to the levels observed in the absence of NMEA, providing evidence that NMEA was acting by inhibiting endogenous NO synthase (Fig. 1). Consistent with recovery of CAT activity, Deta-NONOate almost fully returned the intracellular cGMP concentration to the level observed in the absence of NMEA (Table II).

**NO Production by BHK Cells**—NO produced under physiological conditions is converted stoichiometrically to nitrite and nitrate (29). We found the sum of nitrite and nitrate in the medium of serum-starved BHK cells to be 1 μM after 24 h of culture corresponding to an NO production rate of 40 pmol/h/10⁶ cells. This NO production rate is similar to what vascular endothelial cells and glial cells, which have high NO synthase activity, produce under nonstimulated conditions (30, 31).

**Protein Kinase A Activation by Deta-NONOate in Guanylate Cyclase-transfected BHK Cells**—At high intracellular concentrations, cGMP can cross-activate protein kinase A and protein kinase A activation can lead to transactivation of the fos promoter (12, 13). The small increase in CAT expression on treating guanylate cyclase-transfected cells with Deta-NONOate (Fig. 1) could, therefore, be secondary to protein kinase A activation by high intracellular cGMP concentrations (Table II). To address this question, we measured the activity of the free catalytic subunit of protein kinase A in Deta-NONOate-treated, guanylate cyclase-transfected cells and found a 2.7-fold increase in enzyme activity (Table III). This increase in free catalytic subunit activity by Deta-NONOate was about half that observed in cells treated with 8-Br-cAMP (Table III); in control experiments we showed that free catalytic subunit release by 8-Br-cAMP was not secondary to drug carryover from the culture medium because adding 8-Br-cAMP immediately prior to cell washing had no effect on free catalytic subunit activity. In cells transfected with empty vector, Deta-NONOate had no effect, while 8-Br-cAMP yielded the same increase in enzyme activity as in cells transfected with guanylate cyclase expression vectors (Table III). Thus, in cells containing soluble guanylate cyclase, NO can increase the cGMP content sufficiently to cross-activate protein kinase A.

**Transactivation of the fos Promoter by NO in CS-54 Cells**—Having shown that NO activation of the fos promoter in BHK cells required expression of both soluble guanylate cyclase and G-kinase, we assessed NO's effect on the fos promoter in cells expressing both of these enzymes endogenously. We chose CS-54 cells, derived from the smooth muscle of rat pulmonary arteries (16), because primary vascular smooth muscle cells contain both soluble guanylate cyclase and G-kinase (11). We found that CS-54 cells contained 21 ± 3.4 and 335 ± 39 pmol/min/mg of protein of guanylate cyclase and G-kinase activity, respectively (mean ± S.D. of three independent experiments

---

**FIG. 1. Transactivation of the fos promoter by NO in guanylate cyclase- and G-kinase-transfected BHK cells.** BHK cells were transfected as described under “Experimental Procedures” with pFosCAT and pRSV-luciferase and, where indicated, with the parent vector pCB6 (empty vector) or with expression vectors for guanylate cyclase (GC) and/or G-kinase (GK). Cells were either left untreated (Control, open bars) or were treated with 1 mM 8-Br-cGMP (wide diagonal-striped bars), 250 μM Deta-NONOate (Deta-NO, filled bars), 20 μM NMEA (narrow diagonal-striped bars), or the combination of 20 μM NMEA with 250 μM Deta-NONOate (filled bars with narrow diagonal stripes). CAT activity was normalized to the luciferase activity in each sample and the CAT/luciferase activity of untreated cells transfected with empty vector was assigned a value of 1. The data are the mean ± S.D. of at least three independent experiments performed in duplicate; error bars are too small to be observed in drug-treated cells transfected with empty vector.

**DNA Transfected**

- Control
- 8-Br-cGMP
- Deta-NO
- NMEA
- NMEA + Deta-NO

**Relative CAT Activity**

- Empty Vector
- GC
- GK
- GC/GK

**NO Regulation of Gene Transcription**

9491
performed in duplicate; guanylate cyclase activity was simulated 7-fold by \( S\)-nitrosoypenicillamine). The activities of both of these enzymes are considerably more than found in untransformed BHK cells, and although less than in BHK cells transfected with guanylate cyclase or G-kinase expression vectors (Table I), they are within the range reported in primary smooth muscle cells and other mammalian cells (3, 21).

In CS-54 cells transfected with pFos-CAT, the guanylate cyclase inhibitor ODQ reduced CAT activity by >60% compared with untreated cells (Fig. 2). This suggests that CAT expression in untreated cells was determined in large part by endogenously produced cGMP stimulating cellular G-kinase; ODQ was clearly acting via guanylate cyclase inhibition because the membrane-permeable cGMP analog \( 8\)-para-chlorophenylthio-cGMP reversed ODQ’s inhibitory effect (data not shown). When the cells were treated with Deta-NONOate, CAT expression increased more than 2-fold compared with untreated cells and more than 5-fold compared with cells treated with ODQ (Fig. 2). Deta-NONOate added to ODQ-treated cells was without effect indicating that Deta-NONOate was acting via a GMP-dependent mechanism (Fig. 2).

**DISCUSSION**

We have shown that NO activation of the \( fos \) promoter is dependent on both guanylate cyclase and G-kinase activity and have, therefore, defined a specific signal transduction pathway which mediates NO’s effect on \( fos \) expression. We showed previously that G-kinase \( \beta \), the G-kinase isoform used in the present studies, activates the \( fos \) promoter via translocation to the nucleus and that this effect of G-kinase is mediated by several sequence elements, including the serum response element, the AP-1 binding site, and the cAMP response element (13, 27).

In the absence of drugs, \( fos \) promoter activity was not significantly affected when BHK cells were transfected singly with either guanylate cyclase or G-kinase expression vectors. However, we found a substantial increase in \( fos \) promoter activity when the cells were co-transfected with expression vectors for both enzymes, even in the absence of drug treatment. These data suggest that the transfected guanylate cyclase generated sufficient cGMP to activate the transfected G-kinase, and indeed, we found a 60-fold increase in the intracellular cGMP concentration compared with untransfected cells. For guanylate cyclase to produce such high amounts of cGMP, it seemed likely that the enzyme was stimulated by NO and we found high constitutive NO production by BHK cells. Moreover, NMEA, a NO synthase inhibitor, decreased CAT expression in the guanylate cyclase and G-kinase transfected cells significantly and adding Deta-NONOate to the NMEA-treated cells caused full recovery of CAT expression to maximal levels. Although CAT activity was high in the guanylate cyclase- and G-kinase-transfected cells in the absence of drugs, both Deta-NONOate and 8-Br-cGMP further increased CAT activity, suggesting that the amount of endogenously produced cGMP was not quite sufficient to maximally stimulate G-kinase. Together, these data provide evidence that NO activates the \( fos \) promoter through a guanylate cyclase- and G-kinase-dependent mechanism.

In BHK cells transfected with guanylate cyclase, Deta-NONOate increased the activity of the free catalytic subunit of protein kinase A, although less effectively than 8-Br-cAMP. In vascular smooth muscle cells, Cornwell et al. (32) also found that NO donors activate protein kinase A, although they did not compare the effect of the drugs to that of CAMP analogs. Protein kinase A activation by 8-Br-cAMP stimulates \( fos \) promoter activity about 6-fold in BHK cells (13). Thus, protein kinase A activation by Deta-NONOate in the guanylate cyclase-transfected cells likely caused the small increase in \( fos \) promoter activity under these conditions; the high amounts of cGMP produced by the cells could also have activated the low endogenous G-kinase present in BHK cells.

The increase in CAT activity in cells transfected with guanylate cyclase and G-kinase expression vectors was significantly more than the increase in CAT activity in cells transfected with the G-kinase expression vector only and treated with 8-Br-cGMP. This may be because 8-Br-cGMP, although more membrane-permeable than cGMP, still does not diffuse readily across cell membranes and intracellular concentrations.

**TABLE III**

*Activity of A-kinase free catalytic subunit or holoenzyme in guanylate cyclase-transfected BHK cells treated with 8-Br-cAMP or Deta-NONOate*

BHK cells were transfected as described in the legend to Table I with pCB6 (empty vector) or with expression vectors for soluble guanylate cyclase. Twenty-four hours later, the cells were either left untreated (no drug) or were treated for 1 h with 1 mM 8-Br-cAMP or 250 \( \mu \)M Deta-NONOate (Deta-NO). The activities of the protein kinase A free catalytic subunit and holoenzyme were measured as described under “Experimental Procedures.” The data are the mean ± S.D. of three independent experiments performed in duplicate.

| Protein kinase A species | Guanylate cyclase vectors | Empty vector |
|-------------------------|--------------------------|--------------|
|                         | No drug                  | 8-Br-cAMP    | Deta-NO       |
|                         | \( \text{nmol min}^{-1} \text{mg protein}^{-1} \) | \( \text{nmol min}^{-1} \text{mg protein}^{-1} \) | \( \text{nmol min}^{-1} \text{mg protein}^{-1} \) |
| Catalytic subunit       | 0.15 ± 0.02              | 0.81 ± 0.07  | 0.40 ± 0.03   |
| Holoenzyme              | 1.7 ± 0.3                | 0.95 ± 0.08  | 1.5 ± 0.16    |
|                         | 0.16 ± 0.03              | 0.71 ± 0.05  | 0.17 ± 0.03   |
|                         | 1.8 ± 0.3                | 0.95 ± 0.09  | 1.7 ± 0.25    |

**FIG. 2. Transactivation of the \( fos \) Promoter by NO in CS-54 Cells.** CS-54 cells were transfected as described under “Experimental Procedures” with pFos-CAT and pSVL-luciferase and were either left untreated (no additions) or were treated with 10 \( \mu \)M ODQ (ODQ), 250 \( \mu \)M Deta-NONOate (Deta-NO), or the combination of 10 \( \mu \)M ODQ plus 250 \( \mu \)M Deta-NONOate (ODQ + Deta-NO). CAT activity was normalized to the luciferase activity in each sample, and the CAT/luciferase activity of untreated cells was assigned a value of 1. The data are the mean ± S.D. of at least three independent experiments performed in duplicate.

- **Additions:** None, ODQ, Deta-NO, ODQ + Deta-NO
- **Relative CAT Activity**
- **BHK cells:** guanylate cyclase and G-kinase expression vectors
- **Data:** mean ± S.D. of at least three independent experiments performed in duplicate.
achieved by the drug are relatively low.\(^2\)\(^,\)\(^3\) In addition, it may be that cGMP produced endogenously from guanylate cyclase is a more effective G-kinase activator than exogenous 8-Br-cGMP because of the intracellular localization of the cyclase and kinase. We found similar results in our previous work with REF52 and rat thyroid cells where NO-releasing agents were more potent activators of TRE-containing reporter genes than was 8-Br-cGMP (5). In the guanylate cyclase- and G-kinase-transfected cells there is also likely some degree of protein kinase A activation that we showed previously does not occur in G-kinase-transfected cells treated with 8-Br-cGMP (13).

NO plays an important role in regulating pulmonary vascular resistance and growth of pulmonary arterial cells via activation of soluble guanylate cyclase and G-kinase (33). In CS-54 cells, CAT expression from the forsk promoting activity in these cells and that CAT activity observed in the presence of ODQ may be the more correct “basal” level than that observed in untreated cells. Although we did not define the type of G-kinase expressed in more potent activators of TRE-containing reporter genes than was 8-Br-cGMP (5). In the guanylate cyclase- and G-kinase-transfected cells there is also likely some degree of protein kinase A activation that we showed previously does not occur in G-kinase-transfected cells treated with 8-Br-cGMP (13).

The findings in these studies are likely of physiological significance for the following reasons. First, the amounts of guanylate cyclase and G-kinase in the transfected BHK cells are comparable with the amounts of these enzymes expressed endogenously in several cell types (3, 21). Second, forsk promoter activity was markedly stimulated by endogenously produced NO in BHK cells expressing both guanylate cyclase and G-kinase, and third, NO increased forsk promoter activity in CS-54 cells. Thus, we conclude that one mechanism whereby NO regulates gene expression in vivo is via a guanylate cyclase/cGMP/G-kinase transduction pathway.

Acknowledgments—We thank S. Lohmann for the G-kinase cDNA, F. Murad and M. Nakane for the guanylate cyclase cDNAs, and S. Taylor for providing purified recombinant PKI.

REFERENCES

1. Lowenstein, C. J., and Snyder, S. H. (1992) Cell 70, 705–707
2. Ignarro, L. J., Degnan, J. N., Baricos, W. H., Kadowitz, P. J., and Wolin, M. S. (1982) Biochim. Biophys. Acta 718, 49–59
3. Arnold, W. F., Mittal, C. K., Katsuki, S., and Murad, F. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3203–3207
4. Stamlser, J. S. (1994) Cell 78, 931–936
5. Pilz, R. B., Suhasini, M., Idriss, S., Meinkoth, J. L., and Boss, G. R. (1995) FASEB J. 9, 552–558
6. Haby, C., Lievenski, F., Aunis, D., and Zwiller, J. (1994) J. Neurochem. 62, 496–501
7. Durante, W., Kroll, M. H., Christodoulides, N., Peyton, K. J., and Schafer, A. I. (1997) Circ. Res. 80, 557–564
8. Eigenenthaler, M., Lohmann, S. M., Walter, U., and Pilz, R. B. (1998) Rev. Physiol. Biochem. Pharmacol. 135, 173–209
9. Tuder, R. M., Flook, B. E., and Voelkel, N. F. (1995) J. Clin. Invest. 95, 1798–1807
10. De Caterina, R., Libby, P., Peng, H. B., Thannickal, V. J., Rajavashisth, T. B., Gimbrone, M. A., Jr., Shin, W. S., and Liao, J. K. (1995) J. Clin. Invest. 96, 1730–1738
11. Schmidt, H. H. W., Lohmann, S. M., and Walter, U. (1993) Biochim. Biophys. Acta 1178, 153–175
12. Forte, L. R., Thorne, P. K., Eber, S. L., Krause, W. J., Freeman, R. H., Francis, S. H., and Corbin, J. D. (1992) Am. J. Physiol. 263, C607–C635
13. Gudi, T., Huvai, I., Meinecke, M., Lohmann, S. M., Boss, G. R., and Pilz, R. B. (1996) J. Biol. Chem. 271, 4597–4600
14. Meinecke, M., Geiger, J., Butt, E., Sandberg, M., Jahnson, T., Chakraborty, T., Walter, U., Jarchau, T., and Lohmann, S. (1994) Mol. Pharmacol. 46, 283–290
15. Nakane, M., Arak, K., Saheki, S., Kuno, T., Buechler, W., and Murad, F. (1990) J. Biol. Chem. 265, 16941–16945
16. Rothman, A., Kulik, T. J., Taubman, M. B., Berk, B. C., Smith, W. J., and Nadal-Ginard, B. (1992) Circulation 86, 1987–1986
17. Idriss, S. D., Pilz, R. B., Sharma, V. S., and Boss, G. R. (1992) Biochem. Biophys. Res. Commun. 183, 312–320
18. Pilz, R. B., Berjis, M., Idriss, S. D., Scheele, J. S., Suhasini, M., Gao, L., Scheffler, I. E., and Boss, G. R. (1994) J. Biol. Chem. 269, 32155–32161
19. Pilz, R. B., Eigenenthaler, M., and Boss, G. R. (1992) J. Biol. Chem. 267, 16161–16167
20. Schmidt, H. H. W., Warner, T. D., Nakane, M., Forstermann, U., and Murad, F. (1992) Mol. Pharmacol. 41, 615–624
21. Butt, E., Geiger, J., Jarchau, T., Lohmann, S. M., and Walter, U. (1993) Neurochem. Res. 18, 27–42
22. Peunova, N., and Einkolopov, G. (1993) Nature 364, 450–453
23. Morris, B. J. (1995) Am. J. Physiol. 268, C1405–C1413
24. Nakazawa, K., Karachot, L., Nakabeppu, Y., and Yamamori, T. (1993) J. Biol. Chem. 268, 16161–16167
25. El-Dada, M. D., and Quik, M. (1997) FASEB J. 11, 349–360
26. Wharton, A. R., Simonds, D. B., and Plantadosi, C. A. (1997) Am. J. Physiol. 272, L161–L166
27. Feinstein, D. L., Galea, E., and Reis, D. J. (1997) Nitric Oxide 1, 167–176
28. Cornwell, T. L., Arnold, E., Boerth, N. J., and Lincoln, T. M. (1994) Am. J. Physiol. C1405–C1413
29. Munceda, S., and Higgs, E. A. (1995) FASEB J. 9, 1319–1330
30. Wolfe, L., Corbin, J. D., and Francis, S. H. (1992) Mol. Pharmacol. 41, 552–5254
31. El-Dada, M. D., and Quik, M. (1997) Pharmcol. Exp. Ther. 281, 1463–1470
32. Archer, S. (1993) FASEB J. 7, 349–360
33. Wharton, A. R., Simonds, D. B., and Plantadosi, C. A. (1997) Am. J. Physiol. 272, L161–L166
34. Haby, C., Lisovoski, F., Aunis, D., and Zwiller, J. (1994) Circulation 89, 153–175
35. Matsuda, H., and Imaki, J. (1995) Am. J. Physiol. 269, 5244–5254
36. El-Dada, M. D., and Quik, M. (1997) FASEB J. 7, 349–360
37. Wharton, A. R., Simonds, D. B., and Plantadosi, C. A. (1997) Am. J. Physiol. 272, L161–L166
38. Feinstein, D. L., Galea, E., and Reis, D. J. (1997) Nitric Oxide 1, 167–176
39. Cornwell, T. L., Arnold, E., Boerth, N. J., and Lincoln, T. M. (1994) Am. J. Physiol. C1405–C1413
40. Munceda, S., and Higgs, E. A. (1995) FASEB J. 9, 1319–1330
41. Wolfe, L., Corbin, J. D., and Francis, S. H. (1992) J. Clin. Invest. 90, 2580–2587
42. Soff, G. A., Cornwell, T. L., Cundiff, D. L., Gately, S., and Lincoln, T. M. (1997) J. Clin. Invest. 100, 2580–2587

\(^2\) G. R. Boss and R. B. Pilz, unpublished observations.

\(^3\) M. Bartesch, J. Kruppa, C. Schultz, and B. Jastorff, 11th Protein Kinase Seminar: Cyclic Nucleotide-dependent Signaling Mechanisms, June 4–7, 1998, Bergen, Norway.