A New Pathway of Nitric Oxide/Cyclic GMP Signaling Involving S-Nitrosothioline*

(Received for publication, August 20, 1997, and in revised form, November 19, 1997)

Bernd Mayer‡§, Silvia Pfeiffer‡, Astrid Schrammel‡, Doris Koeslings§, Kurt Schmidt‡, and Friedrich Brunner‡

From the ‡Institut für Pharmakologie und Toxikologie, Karl-Franzens-Universität Graz, Universitätsplatz 2, A-8010 Graz, Austria and the §Institut für Pharmakologie, Freie Universität Berlin, Thielallee 69–73, D-14195 Berlin, Germany

Nitric oxide (NO), a physiologically important activator of soluble guanylyl cyclase (sGC), is synthesized from L-arginine and O2 in a reaction catalyzed by NO synthases (NOS). Previous studies with purified NOS failed to detect formation of free NO, presumably due to a fast inactivation of NO by simultaneously produced superoxide (O2). To characterize the products involved in NOS-induced sGC activation, we measured the formation of cyclic 3′,5′-guanosine monophosphate (cGMP) by purified sGC incubated in the absence and presence of GSH (1 mM) with drugs releasing different NO-related species or with purified neuronal NOS basal. sGC activity was 0.04 ± 0.01 and 0.19 ± 0.06 μmol of cGMP × mg−1 × min−1 without and with 1 mM GSH, respectively. The NO donor DEA/NO activated sGC in a GSH-independent manner. Peroxynitrite had no effect in the absence of GSH but significantly stimulated sGC activity 1.22 ± 0.06 μmol of cGMP × mg−1 × min−1 with 1 mM GSH, respectively. The NO donor DEA/NO and S-nitrosothioline (GSNO), which activated sGC through Cu+-catalyzed release of free NO. In contrast to NO/nitrosothioline, the novel NO/O2-triggered pathway was very efficient (25–45% GSNO) and insensitive to CO2.

The NO/cGMP pathway involving NO-mediated activation of soluble guanylyl cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2.; sGC1) is essential to signal transduction in several biological systems (1). In the vasculature, NO/cGMP signaling is important for the regulation of blood pressure and platelet function (2); in the brain, this pathway controls the release of neurotransmitters such as glutamate and acetylcholine (3). Biosynthesis of NO is triggered by autacoids increasing the intracellular concentration of free Ca2+, resulting in activation of Ca2+-calmodulin-dependent NOS (EC 1.14.13.39), complex homodimeric enzymes that catalyze the synthesis of NO from the guanidine moiety of the amino acid L-arginine (4–7). The oxidation of L-arginine is catalyzed by a cytochrome P450 type heme iron in the oxygenase domain of NOS with O2 serving as a cosubstrate. The electrons required for reduction of O2 are shunted from the cofactor NADPH to the heme via a flavin-containing cytochrome P450 reductase that forms the C-terminal half of the NOS protein. This electron transport chain only operates when Ca2+-calmodulin is bound to the enzyme, which then effects the Ca2+ regulation of endothelial and neuronal NOS synthesis.

At low concentrations of L-arginine or in its absence, the enzymatic reduction of O2 uncouples from substrate oxidation and results in the generation of superoxide anions and H2O2 (8–12). The effective coupling of the reaction requires not only saturation with L-arginine but also the pteridine cofactor H4biopterin (13). Since the two subunits of neuronal NOS bind H4biopterin in a highly anticooperative manner, the purified enzyme always contains ≥ 1 molecule of H4biopterin/dimer, i.e. it consists of a H4biopterin-containing and a H4biopterin-free subunit (14). In this state, the enzyme can form l-citrulline and is stimulated about 2-fold upon binding of H4biopterin to the low affinity site of the pteridine-free subunit. Together with our recent findings that the two NOS subunits function independently (15), this very unusual binding behavior of H4biopterin appears to have interesting functional consequences; at low concentrations of free H4biopterin, the H4biopterin-containing subunit of NOS is expected to form NO, whereas the uncoupled NADPH oxidation catalyzed by the pteridine-free subunit should generate O2*, which reacts at a nearly diffusion-controlled rate (k = 4.3–6.7 × 109 M−1 s−1) with NO to form peroxynitrite, a powerful oxidant and cytotoxic species (16). In keeping with the hypothesis that simultaneous production of NO and O2* is an intrinsic activity of NOS, the enzyme does not catalyze formation of free NO unless high concentrations of SOD are present to outcompete the peroxynitrite reaction (17, 18). The simultaneous production of NO and O2* could be important to prevent feedback inhibition of the enzyme by free NO (19). In the presence of saturating concentrations of H4biopterin, which prevent enzymatic O2* formation due to coupling of NADPH and L-arginine oxidation, NO becomes

NO, spermine/NO; SOD, superoxide dismutase; HPLC, high performance liquid chromatography.

* This work was supported by grants from the Fonds zur Förderung der Wissenschaftlichen Forschung in Österreich (to B. M.) and the der Wissenschaftlichen Forschung in Oesterreich (to B. M.) and the

§ To whom correspondence should be addressed. Tel.: 43-316-380-5567; Fax: 43-316-380-9880; E-mail: mayer@kfunigraz.ac.at.

¶ The abbreviations used are: sGC, soluble guanylyl cyclase; BCS, bathocuproine sulfonic acid; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio-1-propanesulfonate; DEA/NO, 2,2-diethyl-1-nitroso-oxypyridazine; GSNO, S-nitrosothioline; H4biopterin, (6R)-5,6,7,8-tetrahydro-L-bioterin; L-NNa, L-3-nitro-L-arginine; NO−, nitroxy anion; NOS, nitric oxide synthase; SIN-1, 3-(4-morpholinyl)-sydnonimine; SPER
inactivated by $O_2^·$ formed non-enzymatically by autoxidation of $H_2$biopterin (17, 20). As an alternative explanation of these puzzling results, it has been suggested that the true product of the presence of GSH and SOD. Our study yielded several un-purified sGC. This system allowed us to compare the action of NOS/sGC signaling, we have designed an in vitro study using a reconstituted system consisting of purified neuronal NOS and purified sGC. This system allowed us to compare the action profile of NOS with that of donor compounds of several nitrogen oxides and to study the activation of sGC by active NOS in the presence of GSH and SOD. Our study yielded several unexpected results. Most importantly, the data uncover a novel NO/O$_2^·$-triggered nitrosative pathway that outcompetes the reaction of $O_2^·$ with both NO and SOD.

EXPERIMENTAL PROCEDURES

Materials—Purified recombinant rat neuronal NOS was obtained from baculovirus-infected insect cells as described (25). sGC was purified from bovine lung by immunoaffinity chromatography as described previously (26). Alkaline stock solutions of peroxynitrite (50–100 mM) were prepared and quantified as described (27). 1-[3,4,5-$^3$H]Arginine hydrochloride (57 Ci/mmol) and $[^32P]GTP$ (400 Ci/mmol) were from Amersham, purchased through MedPro (Vienna, Austria). DEANO, SPER/NO, Angeli’s salt, and GSNO were obtained from Alexis (Läufelfingen, Switzerland). SIN-1 was a generous gift from Dr. K. Schönafinger (Hochst Marion Rousell Inc., Frankfurt, Germany). $H_2$Biopterin was from Dr. B. Schircks Laboratories (Jona, Switzerland) and NADPH from Boehringer Mannheim (Vienna, Austria). Other chemicals including Cu,Zn-SOD (specific activity 4,200 units/mg) were prepared and quantified as described (27). $[^3H]citrulline$ in the same samples, 20

Enzyme Assays—Stock solutions of purified bovine lung sGC (t$_{max}$ = ~ 16 μmol of cGMP × mg$^{-1}$ × min$^{-1}$) were diluted to 250-fold final concentrations (0.125 mg/ml) with chilled 50 mM potassium phosphate buffer (pH 7.4) containing 0.5 mg/ml bovine serum albumin. 0.4 μl of the diluted enzyme (50 ng) was added to 89.6 μl of 50 mM potassium phosphate buffer (pH 7.4), containing 0.55 mM [α-$^32P$]GTP (~200,000 cpm), 3.3 mM MgCl$_2$, 1.1 mM cGMP, 0.11 mM L-arginine, 55 μM NADPH, 11 μM CaCl$_2$, and 1.1 μg of calmodulin. GSH, SOD, drugs, and NaHCO$_3$ were present as indicated. Reactions were started by addition of 0.5–500 ng of purified GSNO (t$_{max}$ = 0.7–0.9 μmol of L-citrulline × mg$^{-1}$ × min$^{-1}$) in 10 μl of a chilled 50 mM potassium phosphate buffer (pH 7.4) containing 0.4 mM CHAPS, followed by incubation of the samples at 37 °C for 10 min, and isolation of $[^32P]$cGMP as described (31). Where indicated, sGC was incubated with donor compounds instead of NOS under identical conditions.

Specific NOS activity measured as formation of $[^3H]$citrulline from $[^3H]$arginine (32) was 0.08–0.1 μmol of L-citrulline × mg$^{-1}$ × min$^{-1}$ when determined under identical conditions but with $[^3H]$arginine (~50,000 counts per min) being present instead of [α-$^32P$]GTP. For electrochemical, photomultiplier and HPLC determination of GSNO together with $[^3H]$citrulline in the same samples, 20 μg of NOS were incubated in a total volume of 1 ml in open glass vials, followed by the removal of aliquots required for the various analyses and addition of Cu(NO$_3$)$_2$ for the electrochemical quantification of GSNO.

RESULTS

Effect of GSH on the Activation of sGC by NO Donors and Neuronal NOS—To allow a reliable comparison of the effects of NO donors with that of the enzymatic NOS product(s), determination of sGC activity was performed in 50 mM phosphate buffer (pH 7.4) containing 0.5 mM GTP, 3 mM MgCl$_2$, 1 mM cGMP, 0.1 mM L-arginine, 50 μM NADPH, 10 μM CaCl$_2$, and 10 μg/ml calmodulin. Under these conditions, NOS activity was 0.08–0.1 μmol of L-citrulline × mg$^{-1}$ × min$^{-1}$, whether or not GSH (1 mM) was present.

In the absence of added NO, purified sGC exhibited basal activities of 0.04 ± 0.01 and 0.19 ± 0.06 μmol of cGMP × mg$^{-1}$ × min$^{-1}$ without and with 1 mM GSH, respectively. As shown in Fig. 1, cGMP formation was increased about 300-fold to 12.92 ± 0.55 μmol of cGMP × mg$^{-1}$ × min$^{-1}$ by the NO donor DEA/NO (1 μM), the identical maximal enzyme activity was obtained from the presence of GSH. Peroxynitrite (0.1 mM) had no effect in the absence of the thiol but led to a moderate enzyme stimulation (about 20-fold) when incubated with 1 mM GSH. The effect of the NO/O$_2^·$ donor SIN-1 (33) differed considerably from that of peroxynitrite. At a concentration of 10 μM, SIN-1 produced only a slight enzyme stimulation in the absence of
GSNO as Intermediate in NO/cGMP Signaling

GSH (1.01 ± 0.19 μmol of cGMP × mg⁻¹ × min⁻¹) but was nearly as effective as DEA/NO in the presence of the thiol (9.50 ± 1.01 μmol of cGMP × mg⁻¹ × min⁻¹). The nitroxy donor Angeli’s salt decomposes with a half-life close to that of DEA/NO (τ₁/₂ at 37 °C and pH 7.4 = 2.1 and 2.3 min, respectively) (34). At 10 μM, i.e. a concentration that yields 10-fold higher rates of product release than the maximally active concentration of DEAN/O (1 μM), Angeli’s salt produced only little effects in the absence and presence of GSH (0.64 ± 0.09 and 1.46 ± 0.29 μmol of cGMP × mg⁻¹ × min⁻¹, respectively). The effects of the nitrogen oxide donors were compared with that of Ca²⁺/calmodulin-activated neuronal NOS (containing ~0.4 eq of H₂biopterin/monomer) coincubated with sGC at saturating concentrations of L-arginine but in the absence of exogenous H₂biopterin. At a maximally effective concentration of 200 ng/0.1 ml, NOS stimulated sGC to 1.22 ± 0.12 μmol of cGMP × mg⁻¹ × min⁻¹ in the absence of GSH but led to a pronounced accumulation of cGMP in the presence of the thiol (8.51 ± 0.88 μmol of cGMP × mg⁻¹ × min⁻¹). GSH triggered sGC activation by NOS with an EC₅₀ value of 0.21 ± 0.06 μM (data not shown).

Effects of SOD on sGC Activation by NOS—As shown in Fig. 2A, presence of SOD led to an apparently GSH-independent activation of sGC by NOS (200 ng/0.1 ml). The effective concentrations of SOD (EC₅₀ = 0.66 ± 0.13 unit/ml) were about 200-fold lower than those required to detect free NO under the same conditions (EC₅₀ = 135 ± 7 units/ml; data not shown). Identical data were obtained with MnSOD from Escherichia coli (data not shown). In the presence of GSH, SOD increased NOS-stimulated cGMP formation to 13.74 ± 1.28 μmol of cGMP × mg⁻¹ × min⁻¹, a value close to that obtained with DEAN/O (cf. Fig. 1). As evident from the concentration-response curves shown in

Fig. 2B, the system was highly sensitive to NOS. In the presence of GSH, the Ca²⁺/calmodulin-activated enzyme stimulated sGC with an EC₅₀ value of 22.5 ± 7.5 ng/0.1 ml (0.7 nM) (filled circles). SOD (unfilled squares) decreased the EC₅₀ of NOS to 2.2 ± 0.6 ng/0.1 ml. The slight increase of basal sGC activity (2.56 ± 0.74 μmol of cGMP × mg⁻¹ × min⁻¹) was inhibited by hemoglobin and the heme-site inhibitor ODQ (35) (data not shown), indicating that it was due to stabilization of ambient NO (36). If SOD and GSH were present together (filled squares), the resulting EC₅₀ (8.2 ± 1.2 ng of NOS/0.1 ml) was between the values obtained with either SOD or GSH alone.

Characterization of NOS-induced sGC Activation in the Presence of GSH—The surprising difference between sGC activation by NO/NO₂ (generated by NOS or SIN-1) and peroxynitrite (cf. Fig. 1) prompted us to perform further experiments to better define their respective modes of action and to characterize possible intermediates of these pathways. Very similar data to those described below were obtained when sGC was activated with the NO/NO₂ donor SIN-1 instead of NOS.³

Under physiological conditions, the reactivity of peroxynitrite is dramatically modified by its rapid reaction with CO₂ (37). As shown in Fig. 3, CO₂ (delivered as NaHCO₃) almost completely blocked GSH-dependent activation of sGC by peroxynitrite (A) but only slightly reduced cGMP accumulation triggered by NOS (B). Since latter effect was also observed when DEA/NO was used to activate sGC (data not shown), NaHCO₃ and/or CO₂ apparently slightly interfere with NO stimulation of sGC.

These data showed that the GSH-dependent activation of sGC by the NO products NO/NO₂ is not mediated by peroxynitrite, indicating that peroxynitrite formation is at least partially outcompeted by a rapid reaction of NO/NO₂ with GSH to an

³ A. Schrammel, S. Pfeiffer, D. Koesling, K. Schmidt, and B. Mayer, submitted for publication.
intermediate with NO-like biological activity. The thionitrite GSNO appeared to be a likely candidate. This compound is not a direct activator of sGC but releases free NO in the presence of trace amounts of Cu\(^{2+}\) and reducing agents such as thiols or ascorbate (24, 29). We analyzed the product of NOS and GSH by HPLC and obtained a peak with an absorbance maximum at 338 nm, which co-eluted with authentic GSNO (Fig. 4). GSNO formation triggered by NO/O\(_2\)\(_{3}\)-nitrosothiol was further confirmed by incubation of NOS and GSH with Cu(NO\(_3\))\(_2\) (Fig. 5A). The same GSNO was obtained when Cu(NO\(_3\))\(_2\) was added to the incubation mixtures 6 min later. The shape of the signal was identical to that obtained by addition of Cu(NO\(_3\))\(_2\) to authentic GSNO, and Cu(NO\(_3\))\(_2\) was without effect when NO was incubated in the absence of a thiol (data not shown). Together with the HPLC data, these results strongly suggested that incubation of NOS with GSH led to formation of GSNO, which released NO in a copper-dependent reaction. The enzymatic formation of GSNO was insensitive to NaHCO\(_3\) (Fig. 5A), in marked contrast to the nitrosation reaction triggered by ONOO\(^-\) (Fig. 5B). The same differential effects of NaHCO\(_3\) were observed when GSNO was analyzed by HPLC (data not shown).

The electrochemical method was used to quantify GSNO formation by NOS incubated in the presence of 1 mM GSH. To compare the values with NOS activity, formation of L-citrulline was determined in the same samples. As shown in Fig. 6, formation of GSNO depended on the enzyme concentration and accounted for 25–45% of the L-citrulline production. For instance, at a NOS concentration of 100 ng/0.1 ml, which produced ~80% of maximal sGC stimulation (cf. Fig. 2B), NOS generated 0.61 ± 0.12 \(\mu\)M L-citrulline and 0.23 ± 0.03 \(\mu\)M GSNO. Since authentic GSNO stimulated sGC under identical conditions with an \(EC_{50}\) of 0.35 ± 0.06 \(\mu\)M (data not shown), these data suggest that sGC activation by NOS is explained by enzymatic formation of GSNO.

Incubation of NOS at a high concentration (2,000 ng/0.1 ml) but under otherwise identical conditions allowed the quantitative determination of GSNO with additional, less sensitive
analitical techniques and a comparison of these values with the formation of l-citrulline. Within 10 min, the enzyme converted 0.1 m\text{m}\ [3\text{H}]l-arginine to 14 m\text{m}\ [3\text{H}]l-citrulline; the corresponding concentrations of GSNO were 2.52 \pm 0.24 \text{m}\text{m} (NO electrode), 2.68 \pm 0.30 \text{m}\text{m} (HPLC), and 2.53 \pm 0.70 \text{m}\text{m} (photometric determination of thionitrites).

Physiological Significance of NO/O$_2$-triggered S-Nitrosation—S-Nitrosation triggered by peroxynitrite occurs at low yields (23, 24) and is blocked by CO$_2$ (cf. Figs. 3 and 5), suggesting that it may not represent an important pathway of NO/cGMP signaling in vivo. However, the present data show that S-nitrosation by NOS is efficient at physiologically low enzyme concentrations and not inhibited by CO$_2$. Thus, the novel NO/O$_2$-triggered nitrosative pathway described here could be physiologically relevant. To study the possible contribution of GSNO as an intermediate in the NO/cGMP signal transduction cascade, we decided to test whether inhibition of non-enzymatic GSNO decomposition by chelators of copper ions affects agonist-induced cGMP formation in a perfused isolated rat heart system. As shown in Fig. 7A, the endothelium-dependent vasodilator bradykinin stimulated a $N^\alpha$-nitro-l-arginine (l-NNA)-sensitive release of cGMP into the coronary effluent, which was strongly inhibited by the Cu$^{2+}$-selective chelators neocuproine and BCS but not by the Cu$^{2+}$ chelator cuprizone (38). To exclude that these effects were due to an interference of the drugs with sGC stimulation or cGMP outward transport, similar experiments were performed with the NONOate SPER/NO, which is expected to activate cardiac sGC directly. Release of cGMP triggered by the NO donor was not affected by neocuproine or by cuprizone (Fig. 7B), demonstrating that Cu$^{2+}$-specific chelators are specific inhibitors of agonist-induced cGMP release from rat heart.

Discussion

The present study was designed to elucidate the molecular mechanisms involved in cGMP accumulation triggered by Cu$^{2+}$-activated NOS. Purified sGC was functionally reconstituted with purified neuronal NOS or incubated with donors of nitrogen oxides to learn about the conditions required for the effective transduction of the NOS signal and the identity of the intermediates involved. Special emphasis was given to the role of the sulfhydryl compound GSH, which occurs in concentrations of 1–10 m\text{m} in mammalian cells (39). The NO donor DEA/NO stimulated sGC in a GSH-independent manner, demonstrating that the direct activation of the enzyme by NO does not require the presence of a thiol. However, earlier reports appear to show the opposite (40), but sodium nitroprusside or organic nitrates, which were used as NO donors before better drugs became available in the early 1990s, do not release free NO in well defined first order reactions but require chemical transformations to become biologically active.

NOS has never been observed to produce NO unless fairly high amounts of SOD were present (17, 18). Based on the findings that (i) NOS generates O$_2^-$ if not saturated with both l-arginine and H$_2$bioperin (9, 10), (ii) the enzyme is only half-saturated with H$_2$bioperin over a wide range of exogenous H$_2$bioperin concentrations due to anticooperative pteridine binding (14), (iii) the two subunits of NOS dimers function independently (15), and (iv) autoxidation of exogenous H$_2$bioperin results in O$_2^-$-mediated inactivation of NOS (17), it appears safe to conclude that NOS generates NO and O$_2^-$ simultaneously under most circumstances and that SOD acts via scavenging O$_2^-$. This view is further supported by the present results showing that the effect of NOS resembled that of the NO/O$_2^-$ donor SIN-1 (33), with a pronounced GSH dependence of sGC activation that was overcome by addition of SOD. According to an alternative proposal, NOS produces nitroxyl (NO$^-$), which is converted to NO by stoichiometric amounts of SOD (18).

However, we observed only minor activation of sGC with Angeli’s salt at a concentration yielding a 10-fold higher rate of product release than achieved with a maximally active concentration of DEA/NO (1 m\text{m}). The small effect that we observed with Angeli’s salt may be due to release of minor amounts of NO and/or aerobic conversion of NO$^-$ to NO (41). Together with the present knowledge about NOS enzymology, our results argue against NO$^-$ as the biologically active species produced by NOS, although we cannot exclude that the enzyme produces an NO$^-$ species with different chemical properties than the NO$^-$ released from Angeli’s salt.

Activation of sGC by NOS was rendered GSH-independent by remarkably low concentrations of SOD, which are clearly below the average concentration of SOD in tissues (10 m\text{m} corresponding to about 1,000 units/ml) (42). A maximal effect was obtained with 5 units of SOD/ml, conditions under which enzymatic NO formation is below the detection limit of the NO electrode. These surprising results are difficult to explain but may be of utmost physiological importance because they suggest that low levels of SOD, which are far below the levels required to outcompete the peroxynitrite reaction, may be sufficient for the effective functional coupling of NOS activity to cGMP accumulation. At a first glance, the data also seem to suggest that the reaction of NO/O$_2^-$ with GSH is less important because of the ubiquitous occurrence of SOD in tissues. However, GSH largely antagonized the pronounced leftward shift of the NOS concentration-response curve caused by SOD (cf. Fig. 2B), indicating that NO/O$_2^-$ preferentially reacts with the thiol when both GSH and SOD are present. It is interesting that

4 S. Pfeiffer and B. Mayer, unpublished data.
of NO/O_2 decomposition may be more relevant than the nonenzymatic Cu^+ mechanism. Several enzymes including GSH peroxidase (70, 71), thioredoxin reductase (72), and γ-glutamyl transpeptidase (73) appear to catalyze reactions leading to NO release from GSNO. Of note, a Cu^+-dependent enzymatic activity was reported to catalyze GSNO decomposition in platelets (74). In the present study, we found that Cu^+-selective chelators led to a pronounced inhibition of bradykinin-induced release of cGMP into the coronary effluent of isolated perfused rat hearts, whereas cGMP release upon direct activation of cardiac sGC was not affected by the chelators. Although these data are good circumstantial evidence that Cu^+-dependent release of NO from endogenous thionitrites may be essentially involved in cardiac NO/cGMP signaling, further studies are needed to unequivocally demonstrate the role of GSNO as a product of the NOS pathway in mammalian tissues.

Acknowledgments—We thank Eva Pitters, Gerald Wölkart, Margit Rehn, and Jürgen Malkewitz for excellent technical assistance; Dr. Kim Q. Do (Institute for Brain Research, Zürich, Switzerland) for helpful suggestions on the HPLC analysis of GSNO; and Dr. Benjamin Hemmens for critical reading of the manuscript.

REFERENCES

1. Ignarro, L. J. (1991) Biochem. Pharmacol. 41, 485–490
2. Moncada, S., Palmer, R. M. J., and Higgs, E. A. (1991) Pharmacol. Rev. 43, 109–142
3. Garthwaite, J., and Boulton, C. L. (1995) Annu. Rev. Physiol. 57, 683–706
4. Marletta, M. A. (1994) Cell 78, 927–930
5. Mayer, B. (1995) in Nitric Oxide in the Nervous System (Ventric, S. R., ed) pp. 21–42, Academic Press, New York
6. Masters, B. S., McMillan, K., Sheta, E. A., Nishimura, J. S., Roman, L. J., and Martasek, P. (1996) FASEB J. 10, 552–558
7. Stuehr, D. J. (1997) Annu. Rev. Pharmacol. Toxicol. 37, 339–359
8. Mayer, B.; John, M., Heinzle, B., Werner, E. R., Wachter, H., Schultz, G., and Böhm, E. (1991) FEMS Lett. 288, 157–191
9. Heinzle, B., John, M., Klatt, P., Böhm, E., and Mayer, B. (1992) Biochem. J. 281, 627–630
10. Pau, S., Pou, W. S., Breidt, D. S., Snyder, S. H., and Rosen, G. M. (1992) J. Biol. Chem. 267, 24173–24176
11. Klatt, P., Schmidt, K., Uray, G., and Mayer, B. (1993) J. Biol. Chem. 268, 14781–14787
12. Cucea, M., Lafon-Cazal, M., Pietri, S., and Boekaert, J. (1994) J. Biol. Chem. 269, 12558–12563
13. Mayer, B., and Werner, E. R. (1995) in Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 453–463
14. Gorren, A. C. F., List, B. M., Schrammel, A., Pitters, E., Hemmens, B., Werner, E. R., Schmidt, K., and Mayer, B. (1996) Biochemistry 35, 16735–16745
15. Gorren, A. C. F., Schrammel, A., Schmidt, K., and Mayer, B. (1997) Biochemistry 36, 4360–4366
16. Beckman, J. S., and Koppenol, W. H. (1996) Am. J. Physiol. 40, C1242–C1437
17. Mayer, B., Klatt, P., Werner, E. R., and Schmidt, K. (1995) J. Biol. Chem. 270, 655–659
18. Schmidt, H. H. W., Hofmann, H., Schindler, U., Shutenko, Z. S., Cunningham, D. D., and Feilisch, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14492–14497
A New Pathway of Nitric Oxide/Cyclic GMP Signaling Involving
-Nitrosoglutathione
Bernd Mayer, Silvia Pfeiffer, Astrid Schrammel, Doris Koesling, Kurt Schmidt and
Friedrich Brunner

_J. Biol. Chem._ 1998, 273:3264-3270.
doi: 10.1074/jbc.273.6.3264

Access the most updated version of this article at [http://www.jbc.org/content/273/6/3264](http://www.jbc.org/content/273/6/3264)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click [here](http://www.jbc.org/content/273/6/3264.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 71 references, 28 of which can be accessed free at [http://www.jbc.org/content/273/6/3264.full.html#ref-list-1](http://www.jbc.org/content/273/6/3264.full.html#ref-list-1)