Volume 307, number 1, 102–107 FEBS 11176 July 1992

Minireview

NO•, CO and •OH
Endogenous soluble guanylyl cyclase-activating factors

Harald H.H.W. Schmidt

Northwestern University Medical School, Department of Pharmacology, Chicago, IL 60611, USA

27 April 1992

Several low molecular weight compounds are capable of activating soluble guanylyl cyclase. Recent evidence suggests that some of these are formed under physiological conditions: the nitric oxide radical, carbon monoxide and the hydroxyl radical. Thus, multiple signal transduction pathways appear to exist that form a family of guanylyl cyclase activating factors and thereby regulate the intracellular cyclic guanosine 3′,5′-monophosphate level.

L-Arginine; Heme oxygenase; Carbon monoxide; Lipid peroxidation; Xanthine oxidase; Hydroxyl radical; Cyclic GMP

1. INTRODUCTION

The second messenger molecule guanosine cyclic 3′,5′-monophosphate (cGMP) regulates various protein kinases, nucleosid 3′,5′-monophosphate phosphodiesterases and ion channels [1]. Its intracellular concentration is regulated by cGMP-forming enzymes, i.e. guanylyl cyclases (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2), and cGMP-degrading enzymes, i.e. cGMP phosphodiesterases [2].

2. SOLUBLE GUANYLYL CYCLASES

Guanylyl cyclases can be classified into soluble (GC-S) and particulate isoforms. In all particulate guanylyl cyclases (detergent-extractable or cytoskeletal) a single transmembrane domain separates the intracellular catalytic portion from an extracellular receptor portion for different peptides, e.g. natriuretic peptides [3] or guanylin [4], which stimulate enzyme activity.

GC-S are not activated by these peptides and have a different structure. They are heterodimeric proteins which exhibit the typical visual absorption spectrum of hemoproteins, a pyridine hemochrome spectrum corresponding to that of ferroprotoporphyrine IX [5] and, in addition to iron, contain copper as another transition metal [5]. Unlike the particulate isozymes, the regulation of GC-S activity is much less understood. It has been suggested that GC-S activity is regulated by Ca^{2+}-dependent [6] and redox [3, 7] mechanisms. Recent data suggest that this regulation is mediated by a family of endogenously formed guanylyl cyclase-activating-factors (GAFs) [8], i.e. low molecular weight monoxides of nitrogen (NO), carbon (CO) or hydrogen (OH). Their formation [9,10] is catalyzed by different GAF synthases (Fig. 1), some of which can be transcriptionally induced.

Abbreviations: biopterin, 6-(L-*erythro*-1′,2′-dihydroxyprop yl)-pterin; cGMP, cyclic guanosine 3′,5′-monophosphate; CPR, NADPH-cytochrome P_{450} reductase; GAF, soluble guanylyl cyclase-activating-factor; GC-S, soluble guanylyl cyclase; H_2biopterin, (6*R*)-7,8-dihydrobiopterin; H_4biopterin, (6*R*)-5,6,7,8-tetrahydrobiopterin; LPO, lipid peroxidation; NO, nitric oxide; NOS, NO synthase; OH, hydroxyl radical; q-H_2biopterin, (6*R*)-6,7-dihydrobiopterin; XOD, xanthine oxidase.

Correspondence and present address: H.H.H.W. Schmidt, Medizinische Universitätsklinik, Klinische Forschergruppe, Josef-Schneider Str. 2, W-8700 Würzburg, Germany.

3. NITRIC OXIDE

Nitric oxide (NO) is formed enzymatically from a terminal guanidino-nitrogen of L-arginine [11–13] by so-called NO synthases (NOSs) that yield L-citrulline as a co-product. The mechanism, an unusual five-electron oxidation, has not been elucidated but two intermediates N^G-hydroxy-L-arginine [14] and a N^G-hydroxy-cation radical [15] have been postulated. Alternatively, the latter compound may represent the endproduct of enzymatic conversion of L-arginine and may spontaneously, i.e. non-enzymatically, decompose to release NO. After its formation, NO may also be stabilized by thiols [16,17] procluding its detection as a free radical [18,19]. So far, three families of NOS exist [8] (Table I). The basis for their classification is subcellular location and regulation by the free Ca^{2+} (usually mediated by cal-

Published by Elsevier Science Publishers B.V.

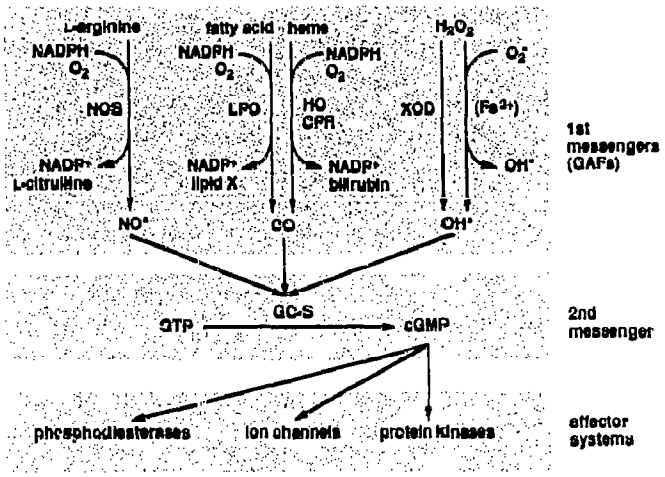

Fig. 1. Regulation of soluble guanylyl cyclase (GC-S) by different guanylyl cyclase-activating factors (GAFs). The 1st messenger pathways summarize (from left to right) the following GAF synthesizing enzyme systems: nitric oxide synthase (NOS), lipid peroxidation (LPO), heme oxygenase (HO), xanthine oxidase (XOD) and the non-enzymatic iron-catalyzed Haber–Weiss reaction.

modulin). Types I and III are constitutively expressed whereas type II is expressed only after immunological activation of cells with different cytokines or endotoxin.

NOSs require L-arginine, molecular oxygen, NADPH, and/or contain tetrahydrobiopterin (H_4biopterin) [20,21], FAD [22], FMN [23–25], and iron [24], respectively, classifying NOSs as biopteroflavo proteins [25]. The homodimer represents the native form of NOS [8]; the monomeric protein is inactive, and dimerization requires H_4biopterin [26]. From the primary structure of NOS, one mole of each flavin and NADPH would be predicted per monomer [23]. Upon dimerization, binding sites for some cofactors appear to dissociate into high and low affinity sites resulting in a recovery or incorporation of less than one mol cofactor per monomer [25]. Non-stoichometric amounts of exogenous FAD [22] and H_4biopterin [8,27] stimulate the activity of purified NOS, possibly by occupying the respective low-affinity binding site in the dimeric complex. The degree of activation of NOS that can be achieved by exogenous H_4biopterin inversely correlates to the amount of enzyme-bound cofactor, which explains apparently contradictory initial reports on the role of H_4biopterin in NOS catalysis [8,27–30]. In all known H_4biopterin-utilizing enzymes, the quinoid form of H_2biopterin (q-H_2biopterin) is the product of normal catalysis and is regenerated to H_4biopterin by a q-H_2biopterin reductase (dihydropteridine reductase). Purified NOS contains biopterin only in its fully reduced form (H_4biopterin) which may indicate a q-H_2biopterin reductase activity [25] by which NOS NADPH-dependently recycles H_4biopterin. Alternatively, it was suggested that H_4biopterin acts as an allosteric activator of NOS and is not oxidized during normal catalysis [30]. The biopterin binding site of NOS may, however, be different from the one in any known H_4biopterin-metabolizing enzyme as indicated by unique K_m values [31,32], substrate specificity [20,21,30] and resistance to inhibition by methotrexate [30].

The putative q-H_2biopterin reductase activity of NOS may in part or completely be related to its NADPH-diaphorase activity [33], i.e. the ability of NOS to reduce nitroblue tetrazolium to a blue diformazan. This activity is remarkably stable enabling the convenient localization of NOS in fixed tissue sections [33,34]. NOS also reduces the cytochromes *c* [11] and P_{450} [35]. Notably, the turnover number for cytochrome *c* is almost two orders of magnitude higher than that for L-arginine [35]; in conjunction with the reported sequence homologies [23], the latter activity would qualify NOS also as an isoform of microsomal NADPH-cytochrome P450 reductase (CPR). In the case of NOS type I, O_2 uptake displays the same Ca^{2+}-dependency, as does L-arginine turnover [24]. Non-heme iron is possibly involved in the electron transfer from NADPH to oxygen [24,25] (Fig. 2) and, in the absence of L-arginine, NOS becomes an NADPH oxidase, i.e. generates H_2O_2 [24].

The Ca^{2+}-independent NOS type II is not constitutively expressed. Its induction by cytokines is transcriptionally based and can be suppressed by glucocorticoids [36]. A similar effect on cytokine-mediated induction was recently described for certain serine protease inhibitors [37–39]; however, their mechanisms is unrelated to protease inhibition but involves interference with protein synthesis [40] and intracellular thiol pools [41]. Furthermore, cytokines can also down-regulate the expression of constitutive NOS (Ca^{2+}-dependent type I) by mechanisms which are yet unclear [39]. Once expressed, phosphorylation may be a common regulatory mechanism for all known isoforms of NOS. The predicted amino acid sequence of the NOS type I cDNA contains consensus sites for phosphorylation by cAMP-dependent protein kinases [23]. Although forskolin-induced increases in intracellular cAMP levels [39] and cAMP-dependent protein kinases [42] do not regulate NOS activity, protein kinase C and Ca^{2+}/calmodulin-dependent kinase [43] and the phosphatase inhibitor okadaic acid [39] clearly modulate NOS activity.

The subcellular localization of the various NOS is different, and also the same isoform may be distributed between the soluble and particulate subcellular fraction [39]. Since none of the described NOS appear to have a transmembrane domain ([23,44] and T. Michel, personal communication), co- or posttranslational modifications, e.g. myristilation, palmitation, isoprenylation and/or glycosylation, may regulate the subcellular localization of the enzyme and represent an additional mechanism of regulation of its activity.

Long before its endogenous biosynthesis was discovered, NO [45] and NO-containing compounds such as sodium nitroprusside [46,47] were known to potently

activate GC-S. These early studies partially elucidated the mechanism of action of NO, which binds to the heme moiety of GC-S, dislocates the heme-iron and thereby induces a conformational change of the protein which in turn activates the catalytic site [48]. Heme-free GC-S no longer responds to NO [5,48]. Some aspects of this proposed mechanism, however, still need experimental support.

4. CARBON MONOXIDE

Carbon monoxide (CO) is detected in the exhaled air of mammals [49] (recently also shown for NO [50]). It can be generated endogenously from at least two biological sources, fatty acids and heme, and both processes appear to be enzymatic.

NADPH-dependent enzymatic peroxidation of microsomal membrane lipids, e.g. methyl linolenate [49] and other polyunsaturated fatty acids, can produce a carbon chain cleavage [51] and eventually CO [52,53]. In microsomal preparations, the formation of CO is quantitatively sufficient to cause the characteristic P_{450}-CO absorbance spectrum [53,54]. Lipid peroxidation and CO formation can be further enhanced by iron and chelators such as adenosine diphosphate ($ADP^{\cdot}Fe^{3+}$) and parallels that of malondialdehyde formation [54].

Table I

Classification and properties of NO and CO synthesizing enzymes

	NO synthase			NADPH cytochrome P_{450} reductase	Heme oxygenase	
E.C.	1.14.23			1.6.2.4	1.14.99.3	
Reaction	L-arginine → NO + L-citrulline			heme → CO + propentdyopent	heme → CO + biliverdin IXa + iron	
Isoforms	Type I	Type II	Type III		Type I	Type II
Subcellular Location	soluble	soluble> particulate	particulate> soluble	microsomal	microsomal	microsomal
Mass, Denatured	160 kD	130 kD	135 kD	74 kD	30 kD	36 kD
Native State	dimer	dimer		dimer	monomer	monomer
Induction	[c]	cytokines endotoxin		phenobarbital	cytokines heat shock hematin metal ions bromobenzene	
Cofactors	NADPH FAD, FMN H_4biopterin iron	NADPH FAD, FMN H_4biopterin	NADPH FAD, FMN H_4biopterin	NADPH FAD, FMN	NADPH iron heme (also substrate)	
Regulation	free Ca^{2+} [a] phosphor.		free Ca^{2+} [a]			
Remarks	homologous to Cytochrome P_{450} reductase [b]			homologous to NO Synthase [b]	requires Cytochrome P_{450} reductase	

[a] in most cases mediated by calmodulin. [b] based on this homology and the shared characteristic of cytochrome P_{450} reduction [35], NO synthase may be considered an isoform of NADPH-cytochrome P_{450} reductase. [c] down regulated by cytokines, endotoxin

Moreover, cytochrome b_5 or another microsomal component closely related to cytochrome P_{450} can function as a peroxidase [55] (see below) and may, therefore, also play a role in microsomal enzymatic CO formation.

At least three enzymatic[1] systems for oxidative heme destruction have been identified and all have destinct mechanisms. However, all have in common to generate CO, utilize NADPH and O_2, and to depend on CPR. First, the bulk of in vivo heme metabolism is believed to be provided by a mixed function oxidase which consists of CPR and the heme-binding and heat-shock [58] protein heme, hydrogen donor:oxygen oxidoreductase (α-methene-oxidizing, hydroxylating) termed heme oxygenase. This two-enzyme system converts iron protoporphyrine IX (FePPIX) and several other hemoproteins yielding biliverdin IXα and CO. Second, P_{450} is destroyed during microsomal lipid peroxidation[2], which process will yield CO [53]. Third, P_{450} and FePPIX are destroyed in the presence of CPR [59, 62] by a mechanism that appears to be distinct from that in which heme oxygenase converts heme to biliverdin [63]. Because of the numerous similarities between NOS and CPR, some isoforms of NOS may also share this heme degrading activity of CPR. Moreover, under certain conditions the heme moiety of GC-S may represent an alternate substrate for heme oxygenase and the other described heme degrading mechanisms. It is intriguing that intracellular GC-S activity might be downregulated by such mechanisms.

The activation of GC-S by CO in vitro [64] and in isolated cells [65,66] has effects similar to NO, i.e. inhibition of platelet aggregation [65] and vascular smooth muscle relaxation [67], and is accompanied by increases in intracellular cGMP. The characteristics and mechanism of GC-S activation by CO also seem to be identical with NO [65]. The fact that hemin [68], heme [69] and tin [70], all potent metabolic inducers of heme oxygenase, all lower blood pressure in spontaneously hypertensive rats suggests that endogenous CO formation can be sufficient in vivo to stimulate vascular smooth muscle GC-S and induce vasodilatation.

5. HYDROXYL RADICAL

The hydroxyl radical (OH) has been shown to activate GC-S, whereas other oxygen radicals are either inhibitory (superoxide anion) or have no effect (peroxide). OH was, therefore, suggested to function as a physiological GAF [71] and, in mouse cerebral arterioles [72], as the mediator of endothelium-dependent relaxation[3]. The mechanism of activation of GC-S by OH is unclear but appears to be different from that of NO and CO. Instead of the heme-iron, OH might interact with regulatory thiol groups of GC-S [7].

In intact mammalian cells, the formation of OH has been most extensively studied in human neutrophils where it was clearly identified by electron paramagnetic resonance (EPR) spectroscopy in conjunction with spin-trap compounds [73]. Three non-enzymatic and enzymatic mechanisms, respectively, are capable of generating OH. Non-enzymatic OH formation can take place either by the iron-catalyzed Haber–Weiss reaction [74,75] or the iron-independent peroxinitrite pathway [76]. Both pathways require superoxide anions, which interact directly either with Fe^{3+} or NO at second order rate constants of 10^6 $M^{-1}\cdot s^{-1}$ [77] or 3.4×10^7 $M^{-1}\cdot s^{-1}$, respectively. In the latter reaction superoxide and NO form peroxinitrite [76], which then homolytically decomposes to yield OH and NO_2 [76]. OH is also formed enzymatically, namely by xanthine oxidase [78], a posttranslational modification of xanthine dehydrogenase in postischemic tissues [79]. This was first observed by the production of ethylene from methional [80] and later confirmed by EPR spectroscopy [78]. Peroxide,

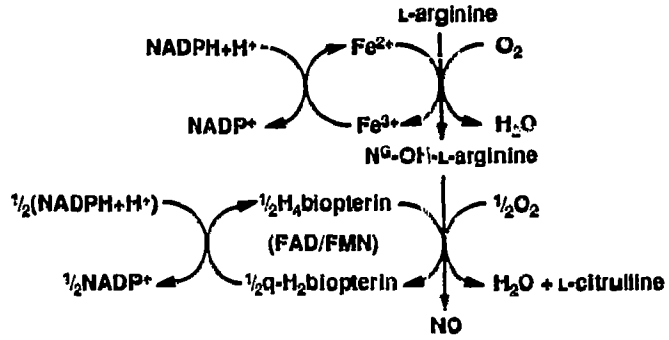

Fig. 2. Three activities of NOS: L-arginine dioxygenase, NADPH-diaphorase and q-H_2biopterin reductase (modified from ref. [25]). Molecular oxygen (dioxygenase) is incorporated into both NO and L-citrulline [82]. In the case of NOS type I, oxygen consumption is Ca^{2+}/CaM-dependent [24]. Non-heme iron may be involved in the electron transfer to oxygen [24]. The primary reaction is the hydroxylation of L-arginine which requires 1 mol of the electron donor NADPH. The oxidation of the intermediate N^G-hydroxy-L-arginine to L-citrulline requires an additional 0.5 mol NADPH [14] and is dependent on H_4biopterin. NOS has bound stoichometric amounts of its cofactors H_4biopterin, FAD and FMN. It was suggested [25] that iron and flavins are part of different domains and that H_4biopterin is recycled in a Ca^{2+}/CaM-independent fashion. This yet putative q-H_2biopterin reductase activity may be closely related to the NADPH-diaphorase activity of NOS (reduction of Nitroblue tetrazolium, NBT). NBT also non-competitively inhibits the conversion of L-arginine [25].

[1]Degradation of heme and hemoproteins to bile pigments has also been described in coupled systems using ascorbate or mild reducing agents [56]. This mechanism is thought to account for the artifactual 'heme α-methenyl oxygenase' activity reported by Nakajima [57].

[2]P_{450} is also destroyed by products of its mixed function oxidative metabolism of certain olefins, such as vinyl chloride [59], secobarbital [60], and allylisopropylacetamide [61].

[3]In the majority of all other blood vessels, this endothelium-derived relaxing factor appears to be identical with NO [9,10].

but not superoxide, is an intermediate in this reaction. Which of the mentioned sources for OH, enzymatic or non-enzymatic, is quantitatively more relevant for GC-S activation remains to be determined. The relative importance of each of these pathways may also vary between different tissues and oxygen tensions. The peroxynitrite pathway, for example, is likely to operate when in a given biological system sufficient concomitant NO and superoxide formation take place [76] as is the case in reperfused tissue [81]. However, OH (and NO) is also highly cytotoxic. Depending on its concentration, activation of GC-S or tissue damage can, therefore, be expected.

6. CONCLUSION

Several potent and physiological GAFs have been identified, most of which are synthesized enzymatically. The role of endogenous NO as a signal transduction molecule regulating soluble guanylyl cyclase activity is clearly established. Evidence for a similar role for endogenous CO and OH is strong but yet mostly indirect. The mechanisms of GAF formation imply that cGMP levels function as a sensor for intracellular events such as Ca^{2+} movements, heat shock, and redox status.

Acknowledgements: I thank J.S. Beckman, W.R. Bidlack and V. Walter for helpful discussions.

REFERENCES

[1] Walter, U. (1989) Rev. Physiol. Biochem. Pharmacol. 113, 41–88.
[2] Beavo, J.A. (1988) Adv. Second Messenger Phosphoprotein Res. 22, 1–38.
[3] Waldman, S.A. and Murad, F. (1987) Pharmacol. Rev. 39, 163–196.
[4] Currie, M.G., Fok, K.F., Kato, J., Moore, R. J., Hamra, F.K., Duffin, K.L. and Smith, C.E. (1992) Proc. Natl. Acad. Sci. USA 89, 947–951.
[5] Gerzer, R., Böhme, E., Hofmann, F. and Schultz, G. (1981) FEBS Lett. 132, 71–74.
[6] Schultz, G., Hardman, J.G., Schultz, K., Baird, C.E. and Sutherland, E.W. (1973) Proc. Natl. Acad. Sci. USA 70, 3889–3893.
[7] Wu, X.-B., Brüne, B., von Appen, F. and Ullrich, V. (1992) Arch. Biochem. Biophys. 294, 75–82.
[8] Schmidt, H.H.H.W., Pollock, J.S.P., Nakane, M., Gorsky, L.D., Förstermann, U. and Murad, F. (1991) Proc. Natl. Acad. Sci. USA 88, 365–369.
[9] Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E. and Chaudhuri, G. (1987) Proc. Natl. Acad. Sci. USA 84, 9265–9269.
[10] Palmer, R.M.J., Ferrige, A.G. and Moncada, S. (1987) Nature 327, 524–526.
[11] Palmer, R.M.J., Ashton, D.S. and Moncada, S. (1988) Nature 333, 664–666.
[12] Schmidt, H.H.H.W., Klein, M.M., Niroomand, F. and Böhme, E. (1988) Eur. J. Pharmacol. 148, 293–295.
[13] Schmidt, H.H.H.W., Nau, H., Wittfoht, W., Gerlach, J., Prescher, K.-E., Klein, M.M., Niroomand, F. and Böhme, E. (1988) Eur. J. Pharmacol. 154, 213–216.
[14] Stuehr, D.J., Kwon, N.S., Nathan, C.F., Griffith, O.W., Feldman, P.L. and Wiseman, J. (1991) J. Biol. Chem. 266, 6259–6263.
[15] Prónai, L., Ichimori, K., Nozaki, H., Nakazawa, H., Okino, H., Carmichael, A.J. and Arroyo, C.M. (1991) Eur. J. Biochem. 202, 923–930.
[16] Myers, P.R., Minor Jr., R.L., Guerra Jr., R., Bates, J.N. and Harrison, D.G. (1990) Nature 345, 161–163.
[17] Mordvintsev, P.I., Vedernikov, Y.P., Malenkova, I.V. and Vanin, A.F. (1990) Dokl. A.N. USSR. 312, 1006–1010.
[18] Greenberg, S.S., Wilcox, D.E. and Rubanyi, G.M. (1990) Circ. Res. 67, 1446–1452.
[19] Forray, C., Arroyo, C.M., El-Fakahany, E.E. and Rosen, G.M. (1990) FASEB J. 4, A1121.
[20] Kwon, N.S., Nathan, C.F. and Stuehr, D.J. (1989) J. Biol. Chem. 264, 20496–20501.
[21] Tayeh, M.A. and Marletta, M.A. (1989) J. Biol. Chem. 264, 19654–19658.
[22] Stuehr, D.J., Kwon, N.S. and Nathan, C.F. (1990) Biochem. Biophys. Res. Commun. 168, 558–565.
[23] Bredt, D.S., Hwang, P.M., Glatt, C.E., Lowenstein, C., Reed, R.R. and Snyder, S.H. (1991) Nature 351, {11}714–718.
[24] Mayer, B., John, M., Heinzel, B., Werner, E.R., Wachter, H., Schultz, G. and Böhme, E. (1991) FEBS Lett. 288, 187–191.
[25] Schmidt, H.H.H.W., Smith, R.M., Nakane, M. and Murad, F. (1992) Biochemistry. 31, 3243–3249.
[26] Stuehr, D.J., Cho, H.J., Kwon, N.S. and Nathan, C.F. (1991) Proc. Natl. Acad. Sci. USA 88, 7773–7777.
[27] Mayer, B., John, M. and Böhme, E. (1990) FEBS Lett. 277, 215–219.
[28] Snyder, S.H. and Bredt, D.S. (1991) Trends Pharmacol. Sci. 12, 125–128.
[29] Knowles, R.G., Palacios, M., Palmer, R.M.J. and Moncada, S. (1989) Proc. Natl. Acad. Sci. USA 86, 5159–5162.
[30] Giovanelli, J., Campos, K.L. and Kaufman, S. (1991) Proc. Natl. Acad. Sci. USA 88, 7091–7095.
[31] Craine, J.E., Hall, E.S. and Kaufman, S. (1972) J. Biol. Chem. 247, 6082–6091.
[32] Firgaira, F.A., Cotton, R.G.H. and Danks, D.M. (1981) Biochem. J. 197, 31–43.
[33] Hope, B.T., Michael, G.J., Knigge, K.M. and Vincent, S.R. (1991) Proc. Natl. Acad. Sci. USA 88, 2811–2814.
[34] Schmidt, H.H.H.W., Warner, T.D., Ishii, K., Sheng, H. and Murad, F. (1992) Science. 255, 721–723.
[35] Mayer, B., John, M., Heinzel, B., Klatt, P., Werner, E.R. and Böhme, E. (1992) in: Biology of Nitric Oxide (S. Moncada, M.A. Marletta, J.B. Hibbs and E.A. Higgs, Eds.), Portland Press, Colchester, in press.
[36] Radomski, M.W., Palmer, R.M.J. and Moncada, S. (1990) Proc. Natl. Acad. Sci. USA 87, 10043–10047.
[37] Welsh, N., Eizirik, D.L., Bendtzen, K. and Sandler, S. (1991) Endocrinology. 129, 3167–3173.
[38] Dijkmans, R. and Billiau, A. (1991) Eur. J. Biochem. 202, 151–159.
[39] Schmidt, H.H.H.W., Warner, T.D., Nakane, M., Förstermann, U. and Murad, F. (1992) Mol. Pharmacol. 41, 615–624.
[40] Noonan, N.E. and Noonan, K.D. (1977) J. Cell. Physiol. 92, 137–144.
[41] Rossman, T., Norris, C. and Troll, W. (1974) J. Biol. Chem. 249, 3412–3417.
[42] Brüne, B. and Lapetina, E.G. (1991) Biochem. Biophys. Res. Commun. 181, 921–926.
[43] Nakane, M., Mitchell, J., Förstermann, U. and Murad, F. (1991) Biochem. Biophys. Res. Commun. 180, 1396–1402.
[44] Lyons, C.R., Orloff, G.J. and Cunningham, J.M. (1992) J. Biol. Chem. 267, 6370–6374.
[45] Arnold, W.P., Mittal, C.K., Katsuki, S. and Murad, F. (1977) Proc. Natl. Acad. Sci. USA 74, 3203–3207.
[46] Böhme, E., Graf, H. and Schultz, G. (1978) Adv. Cyclic Nucleotide Res. 9, 131–143.
[47] Schultz, K.D., Schultz, K. and Schultz, G. (1977) Nature 256, 750–751.

[48] Ignarro, L.J., Wood, K.S. and Wolin, M.S. (1984) Adv. Cyclic Nucleotide Prot. Phosph. Res. 17, 267–274.
[49] Lindstrom, T.D. and Anders, M.W. (1978) Toxicol. Lett. 1, 307–314.
[50] Gustafsson, L.E., Leone, A.M., Persson, M.G., Wiklund, N.P. and Moncada, S. (1991) Biochem. Biophys. Res. Commun. 181, 852–857.
[51] May, H.E. and McCay, P.B. (1968) J. Biol. Chem. 243, 2288–2295.
[52] De Matteis, F., Gibbs, A.H. and Unseld, A. (1977) Biochem. J. 168, 417–422.
[53] Nishibayashi, H., Omura, T., Sato, R. and Estabrook, R.W. (1968) in: Structure and Function of Cytochromes (K. Okunuki, M.D. Kamen and I. Sekuzu, Eds.), University Park Press, Baltimore, pp. 658–665.
[54] Wolff, D.G. and Bidlack, W.R. (1976) Biochem. Biophys. Res. Commun. 73, 850–857.
[55] Bidlack, W.R. and Hochstein, P. (1974) Life Sci. 14, 2003–2010.
[56] Lemberg, R. (1956) Rev. Pure Appl. Chem. 6, 1.
[57] Nakajima, H. (1963) J. Biol. Chem. 238, 3797.
[58] Shibahara, S., Müller, R.M. and Taguchi, H. (1987) J. Biol. Chem. 262, 12889–12892.
[59] Guengerich, F.P. and Strickland, T.W. (1977) Mol. Pharmacol. 13, 993.
[60] Levin, W., Lu, A.Y.H., Jacobson, M., Kuntzman, R., Poyer, J.L. and McCay, P.B. (1973) Arch. Biochem. Biophys. 158, 842.
[61] DeMatteis, F. (1971) Biochem. J. 124, 767.
[62] Masters, B.S.S. and Schacter, B.A. (1976) Ann. Clin. Res. 8, 18.
[63] Guengrich, F.P. (1978) Biochemistry 17, 3633–3639.
[64] Brüne, B., Schmidt, K.-U. and Ullrich, V. (1990) Eur. J. Biochem. 192, 683–688.
[65] Brüne, B. and Ullrich, V. (1987) Mol. Pharmacol. 32, 497–504.
[66] Ramos, K.S., Lin, H. and McGrath, J.J. (1989) Biochem. Pharmacol. 38, 1368–1370.
[67] McFaul, S.J. and McGrath, J.J. (1987) Toxicol. Appl. Pharmacol. 87, 464–473.
[68] Martasek, P., Schwartzman, M.L., Goodman, A.I., Solangi, K.B., Levere, R.D. and Abraham, N.G. (1991) J. Am. Soc. Nephrol. 2, 1078–1084.
[69] Levere, R.D., Martasek, P., Escalante, B., Schwartzman, M.L. and Abraham, N.G. (1990) J. Clin. Invest. 86, 213–219.
[70] Sacerdoti, D., Escalante, B., Abraham, N.G., McGiff, J. C., Levere, R.D. and Schwartzman, M.L. (1989) Science 243, 388–390.
[71] Mittal, C.K. and Murad, F. (1977) Proc. Natl. Acad. Sci. USA 74, 4360–4364.
[72] Rosenblum, W.I. (1987) Circulation Res. 61, 601–603.
[73] Green, M.R., Hill, A.O., Okolow-Zubkowska, M.J. and Segal, A.W. (1979) FEBS Lett. 100, 23–26.
[74] Haber, F. and Weiss, J. (1934) Proc. Roy. Soc. Ser. A. 147, 332–351.
[75] Cohen, G. (1977) in: Superoxide and superoxide dismutase (A.M. Michelson, J.M. McCord and I. Fridovich, Eds.), Academic Press, London, pp. 3177ndash;321.
[76] Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. (1990) Proc. Natl. Acad. Sci. USA 87, 1620–1624.
[77] Winterbourn, C.C. (1991) Biochem. J. 182, 625–628.
[78] Kuppusamy, P. and Zweier, J.L. (1989) J. Biol. Chem. 264, 9880–9884.
[79] McCord, J.M. (1985) N. Engl. J. Med. 312, 159–163.
[80] Beauchamp, C. and Fridovich, I. (1970) J. Biol. Chem. 245, 4641–4646.
[81] Matheis, G., Sherman, M.P., Buckberg, G.D., Haybron, D.M., Young, H.H. and Ignarro, L.J. (1992) Am. J. Physiol. 262, H616-H620.
[82] Leone, A.M., Palmer, R.M.J., Knowles, R.G., Francis, P.L., Ashton, D.S. and Moncada, S. (1991) J. Biol. Chem. 266, 23790–23795.