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Inhibition of platelet aggregation by *S*-nitroso-cysteine via cGMP-independent mechanisms: evidence of inhibition of thromboxane A_2 synthesis in human blood platelets

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Abstract ***S*-Nitroso-cysteine (SNC), a putative endothelium-derived relaxing factor, potently inhibited collagen- and arachidonic acid-induced platelet aggregation (IC_{50} = 100 nM) and thromboxane A_2 (TxA_2) synthesis of human blood platelets. ODQ, a selective inhibitor of the soluble guanylyl cyclase, inhibited SNC-induced formation of cGMP but did not reverse inhibition by SNC of collagen- and arachidonic acid-induced platelet aggregation. Combination of ODQ with SQ-29548, a specific platelet TxA_2 receptor antagonist, did not modify the antiaggregatory action of SNC. Our study shows that SNC inhibits platelet aggregation by cGMP-independent mechanisms that may involve inhibition of TxA_2 synthesis in human platelets.**

Key words: Aggregation; *S*-Nitroso-L-cysteine; 1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one; SQ-29548; cGMP; *S*-Nitrosylation

1. Introduction

The mechanism by which endothelium-derived relaxing factor (EDRF), nitric oxide (NO), *S*-nitroso compounds, sodium nitroprusside (SNP) and other NO-containing compounds act as inhibitors of platelet aggregation and vasodilators is thought to be by activation of the soluble guanylyl cyclase (sGC) following binding of NO to the heme moiety of this enzyme and subsequent release of cGMP in platelets and in vascular tissue [1–5]. Recently, a potent and selective inhibitor of the NO-sensitive sGC was discovered [6]. This compound, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), has been reported to inhibit sGC but not to affect the activity of the particulate guanylyl cyclase, adenylyl cyclase and NO synthase in endothelial cells and platelets [6–8]. By means of ODQ it has recently been demonstrated that the NO-containing compounds SNP, 3-morpholinosydnonymine, *S*-nitrosoglutathione, *S*-nitroso-DL-penicillamine and *S*-nitroso-*N*-acetyl-DL-penicillamine exert their platelet inhibitory and vasodilatory actions via stimulation of the sGC [6–8].

NO and *S*-nitroso-cysteine (SNC) are the most favored chemical structures for L-arginine-derived EDRF [9–17]. EDRF has been reported to inhibit platelet aggregation [18–23]. Both vasodilatation and inhibition of platelet aggregation by EDRF have been shown to be associated with the activation of the sGC [22,23]. At present only a few reports exist on the effects of SNC on platelets. It has been shown that SNC-induced inhibition of human platelet secretion correlated with increases in platelet cGMP levels in vitro [24]. In vivo, SNC has been shown to be a potent vasodilator causing a reduction in blood pressure [25,26]. In humans, *S*-nitroso-glutathione has been shown to be more effective as an inhibitor of platelet activation than as a vasodilator [27]. Comparable data on SNC are currently not available. It has been suggested [8,28–34] that the platelet-inhibitory action of NO and NO-containing compounds may include cGMP-independent mechanisms in addition to the cGMP-dependent mechanism. In the present work, we investigated the effect of SNC on aggregation and cGMP formation in human platelets. Utilizing ODQ and the selective thromboxane A_2 (TxA_2) receptor antagonist SQ-29548 [35–37] we demonstrate that SNC inhibits platelet aggregation via cGMP-independent mechanisms.

2. Materials and methods

2.1. Materials

Sodium [^{15}N]nitrite (98%+ at ^{15}N) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). L- and D-cysteine were obtained from Aldrich (Steinheim, Germany). SNP and rabbit hemoglobin (type VI) were bought from Sigma (Deisenhofen, Germany). ODQ was purchased from ALEXIS Corporation (San Diego, CA, USA). SQ-29548 was bought from Biomol (Hamburg, Germany). Collagen was obtained from Hormonchemie (Munich, Germany). All other chemicals were bought from Merck (Darmstadt, Germany). SNC and ^{15}N-labeled SNC were synthesized from L- or D-cysteine and [^{15}N]nitrite as described [38]. The structure of SNC and ^{15}N-labeled SNC was elucidated by flow injection analysis electrospray ionization mass spectrometry as described previously [39]. Intense mass fragments were obtained at *m/z* 151 and *m/z* 152 corresponding to the protonated molecular cations of ^{14}N-labeled and ^{15}N-labeled SNC. Dilutions of stock solutions in isotonic saline were prepared immediately prior to the start of the experiments and stored on ice until use.

2.2. Preparation of platelet suspensions

Human blood (10 ml, EDTA as the anticoagulating agent) was drawn from antecubital veins of healthy volunteers who had not received aspirin or other non-steroidal antiphlogistic drugs for at least 10 days. Platelet-rich plasma (PRP) was prepared by centrifugation of blood (200×*g*, 15 min). Platelet-poor plasma (PPP) was prepared from the remaining volume of blood by centrifugation (400×*g*, 15 min). Suspensions of washed platelets (WP) were prepared as described [20]. The pellet obtained was washed once with Tris-buffered saline and the platelets were resuspended in Ca^{2+}-containing (0.7 mM) phosphate-buffered saline (PBS), pH 7.3. The platelet count was adjusted to approx. 4×10^8 cells/ml. The platelets were stored at room temperature under gentle shaking and used within 3 h.

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Abbreviations: EDRF, endothelium-derived relaxing factor; NO, nitric oxide; SNP, sodium nitroprusside; sGC, soluble guanylyl cyclase; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; SNC, *S*-nitroso-cysteine; Tx, thromboxane; PRP, platelet-rich plasma; PPP, platelet-poor plasma; WP, washed platelets; PBS, phosphate-buffered saline; DMSO, dimethylsulfoxide

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2.3. Measurement of platelet aggregation and platelet-derived cGMP and TxB_2

Platelet aggregation measurements were performed in duplicate with 250-μl aliquots of WP suspensions (both approx. 10^8 cells) which were kept in suspension in the aggregation cuvettes by constant stirring at 37°C. Platelet aggregation was induced by collagen (1.0 μg/ml) or arachidonic acid (1 mM). These agonists were added to the cuvettes 3 min after addition of SNC or SNP. In experiments with ODQ and SQ-29548, stock solutions were freshly prepared in dimethylsulfoxide (DMSO) and further diluted with isotonic saline solutions. 10-μl aliquots of the final solutions were added to the WP suspensions. The final concentration of DMSO was less than 1 vol%. In the concentration range tested DMSO did not influence platelet aggregation. When ODQ and SQ-29548 were used, WP were preincubated with either or both for 5 min. Aliquots of SNC, SNP or isotonic NaCl (serving as a control) solutions were added to the WP suspensions. Platelet aggregation was monitored for 5 min by the increase in light transmission according to the method of Born and Cross [40] with an Apact dual-channel aggregometer (LAbor, Hamburg, Germany). For measurement of intraplatelet cGMP and TxA_2, platelet aggregation was stopped by the addition of 0.75-ml aliquots of −20°C cool ethanol to each cuvette and suspensions were stored at −80°C until analysis. Immediately prior to measurement of cGMP in duplicate by radioimmunoassay using a [^{125}I]iodotyrosine cGMP derivative as tracer (DuPont de Nemours, Dreieich, Germany) as described [41], samples were thawed and centrifuged at 4°C (1800×*g*, 10 min). Platelet-derived TxA_2 was quantitated as its stable analog TxB_2 by gas chromatography-tandem mass spectrometry as described elsewhere [42].

2.4. Measurement of NO and intraplatelet [^{15}N]nitrite

NO was measured by an NO electrode equipped with ISO-NOP200, a 200 μm diameter shielded micro-sensor (World Precision Instruments, Sarasota, FL). For measurement of intraplatelet [^{15}N]nitrite, *S*-[^{15}N]nitroso compounds were incubated (0–200 μM) in 500-μl aliquots of PRP (about 10^8 platelets) or PPP (used as a control) in siliconized 1.3-ml conical glass vials for 1 min at room temperature. After incubation the samples were treated with 500-μl aliquots of PBS and centrifuged (200×*g*, 5 min). Supernatants were decanted, pellets were resuspended in 1-ml aliquots of PBS and centrifuged again (200×*g*, 5 min). This procedure was repeated three times. Pellets from the last centrifugation were resuspended in 100-μl aliquots of distilled water. These samples were treated with acetone (400 μl) and mixed by vortexing for 1 min in order to disrupt the platelets. After centrifugation (1800×*g*, 5 min) the supernatants were taken up and treated for quantitation of nitrite by GC-MS [38]. The ratio *m/z* 47 ([^{15}N]nitrite) to *m/z* 46 (nitrite) was used as a measure of [^{15}N]nitrite accumulation in platelets.

2.5. Statistical analysis

Values are reported as mean ± S.E.M from experiments on inhibition of platelet aggregation, cGMP and TxA_2 formation and as mean ± S.D. for results from experiments on accumulation of [^{15}N]nitrite in the platelets and NO release. The significance of differences was determined with the paired *t*-test. $P < 0.05$ was considered significant.

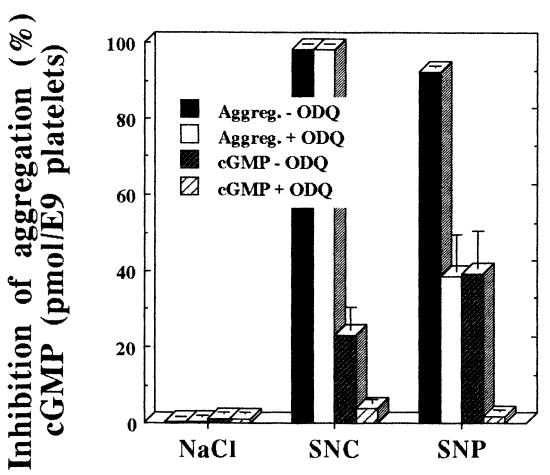

Fig. 1. Effect of ODQ (10 μM) on the extent of inhibition of collagen-induced (1 μg/ml) aggregation of washed platelets and intraplatelet cGMP formation by 0.9% NaCl, SNC and SNP each at 1 μM. Data are presented as mean ± S.E.M., $n = 4$–7.

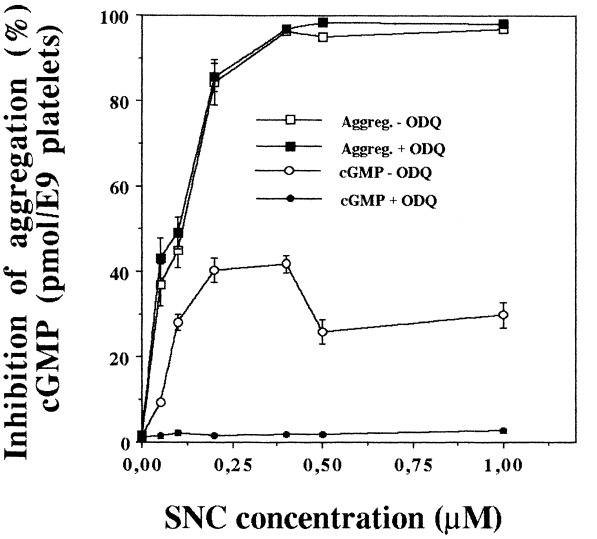

Fig. 2. Effect of various concentrations of SNC on the extent of inhibition of collagen-induced (1 μg/ml) aggregation of washed platelets and intraplatelet cGPM formation in the presence (10 μM) and in the absence of ODQ. Data are presented as mean ± S.E.M, $n = 3$.

3. Results

Fig. 1 shows that SNC (1.0 μM) almost completely inhibited collagen-induced platelet aggregation (extent of inhibition, 98 ± 0.2%) whereas ODQ did not affect the inhibitory action of SNC at all (extent of inhibition, 98 ± 0.2%). In the absence of ODQ, inhibition of platelet aggregation by SNC was accompanied by a significant increase in the cGMP levels (23.1 ± 5.9 pmol/10^9 platelets). Even though ODQ had no effect on the inhibition of aggregation by SNC, ODQ significantly ($P < 0.05$) inhibited SNC-induced formation of cGMP (3.8 ± 1.0 pmol/10^9 platelets). On the other hand, ODQ reversed the action of SNP (1.0 μM) partially with respect to the extent of inhibition (92 ± 0.4 vs. 38.6 ± 9.6%, $P < 0.05$) and completely with respect to the cGMP levels (39.4 ± 9.8 vs. 1.76 ± 0.4 pmol/10^9 platelets, $P < 0.05$). On a molar basis, SNP was less effective in inhibiting platelet aggregation than SNC, but caused a higher formation rate of cGMP, which was decreased almost to basal levels by ODQ. Fig. 1 shows that ODQ did not influence the extent of collagen-induced platelet aggregation at all (0.50 ± 0.01% vs. 0.50 ± 0.05%) nor cGMP levels (1.2 ± 0.2 pmol/10^9 platelets vs. 1.1 ± 0.2 pmol/10^9 platelets) suggesting that platelet function is not affected by ODQ. These results suggest that SNC inhibits platelet aggregation by cGMP-independent mechanisms, and that SNP inhibits platelet aggregation both by cGMP-dependent and cGMP-independent mechanisms.

Fig. 2 shows the dose-response relationship of SNC on platelet aggregation and cGMP formation. SNC emerges as a very potent inhibitor (IC_{50} approx. 100 nM) of collagen-induced aggregation in human WP. Fig. 2 also shows that ODQ effectively inhibited SNC-induced formation of cGMP

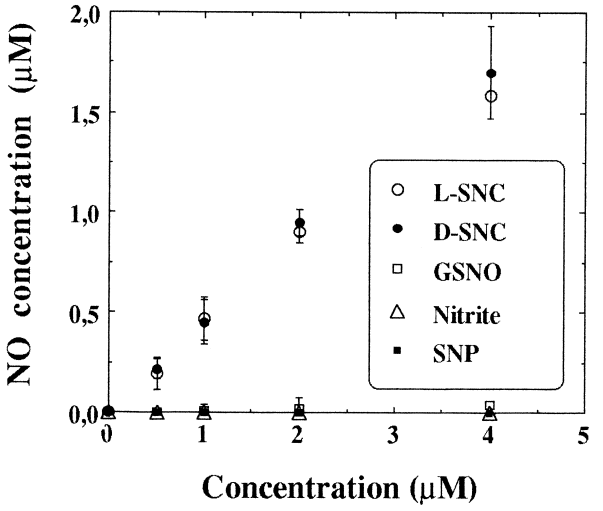

Fig. 3. L-SNC, D-SNC, GSNO, SNP and nitrite were separately incubated in 50 mM potassium phosphate buffer at pH 7.4 at the indicated concentrations. Each experiment was performed in triplicate.

but did not significantly affect the inhibition of aggregation by SNC. No significant differences were found between L-SNC and D-SNC (each at 1 μM) with respect to both the extent of platelet inhibition and cGMP levels in the presence and in the absence of ODQ (data not shown).

Fig. 3 shows that L-SNC and D-SNC are equally effective and much more potent NO donors than *S*-nitroso-glutathione (GSNO). SNP and nitrite did not release any NO under the experimental conditions used.

Intraplatelet formation of [^{15}N]nitrite – expressed as the ratio of *m/z* 47 to *m/z* 46 – was observed when platelets from PRP were incubated with ^{15}N-labeled L-SNC or ^{15}N-labeled D-SNC (Fig. 4) but not with ^{15}N-labeled GSNO or [^{15}N]nitrite (not shown). No accumulation of [^{15}N]nitrite was found when platelets from PPP used as control were incubated with ^{15}N-labeled L-SNC. Intraplatelet accumulation of [^{15}N]nitrite was found to depend on the concentration of ^{15}N-labeled L-SNC (Fig. 5) and to be inhibited by a molar excess

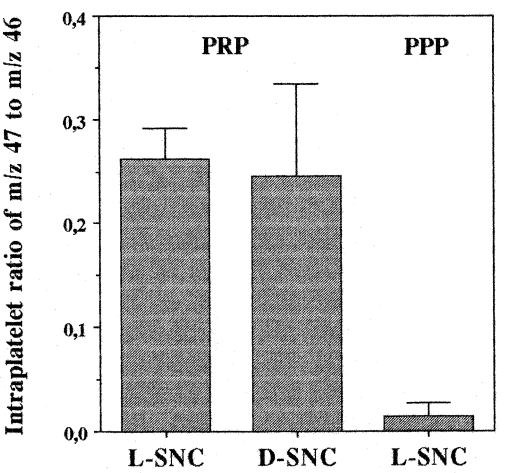

Fig. 4. Two sets of six 500-μl aliquots of PRP were incubated separately for 1 min at room temperature with 200 μM of ^{15}N-labeled L-SNC or ^{15}N-labeled D-SNC. Six 500-μl aliquots of PPP were similarly incubated with ^{15}N-labeled L-SNC and served as control.

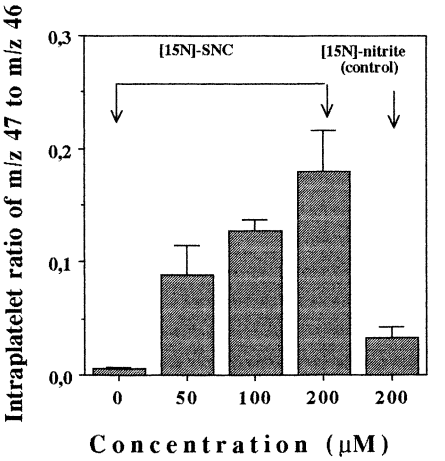

Fig. 5. Two sets of six 500-μl aliquots of PRP were incubated separately for 1 min at room temperature with the indicated concentrations of ^{15}N-labeled L-SNC and ^{15}N-labeled nitrite (at 200 μM) served as the control.

of hemoglobin over ^{15}N-labeled L-SNC and ^{15}N-labeled D-SNC (data not shown).

Arachidonic acid-induced platelet aggregation was completely inhibited by acetylsalicylic acid (ASA) and SNC (Fig. 6A). While ASA completely inhibited TxA_2 formation, SNC caused only partial inhibition of TxA_2 formation in platelets. ODQ did not reverse the effects of SNC on either platelet aggregation or TxA_2 formation (Fig. 6A). Fig. 6B shows that the combination of ODQ with SQ-29548 weakly inhibited collagen-induced platelet aggregation. In the presence of ODQ and SQ-29548, SNC completely inhibited collagen-induced aggregation (Fig. 6B). SQ-29548 (1 μM) completely inhibited arachidonic acid-induced platelet aggregation (not shown).

4. Discussion

The present study shows for the first time that SNC, a putative EDRF, inhibits potently collagen- and arachidonic acid-induced aggregation of platelets by cGMP-independent mechanisms. This work also shows for the first time that SNC inhibits TxA_2 synthesis in human platelets. Our results suggest that inhibition of TxA_2 synthesis and/or antagonization of the TxA_2 receptor of the platelets are novel cGMP-independent mechanisms by which SNC inhibits platelet aggregation. The mechanism involving inhibition of TxA_2 formation in the platelets is supported by the recent finding of our group that intravenous infusion of L-arginine in healthy volunteers resulted in NO-associated inhibition of platelet TxA_2 formation ex vivo [43]. This mechanism is also supported by the observation that SNC and other *S*-nitroso compounds including *S*-nitroso-glutathione inhibit TxA_2 synthesis by isolated cyclooxygenase (unpublished observations). The finding that SNC inhibits platelet aggregation despite complete inhibition of the sGC and complete antagonization of the TxA_2 receptor of the platelets suggests that additional cGMP-independent mechanisms for SNC may exist. These mechanisms could include inhibition of the low K_M cAMP phosphodiesterase, of glyceraldehyde 3-phosphate dehydrogenase, ADP ribosylation, and increases in intracellular Ca^{2+} concentrations independent of sGC [28–34].

Incubation of L- and D-SNC with human platelets did not

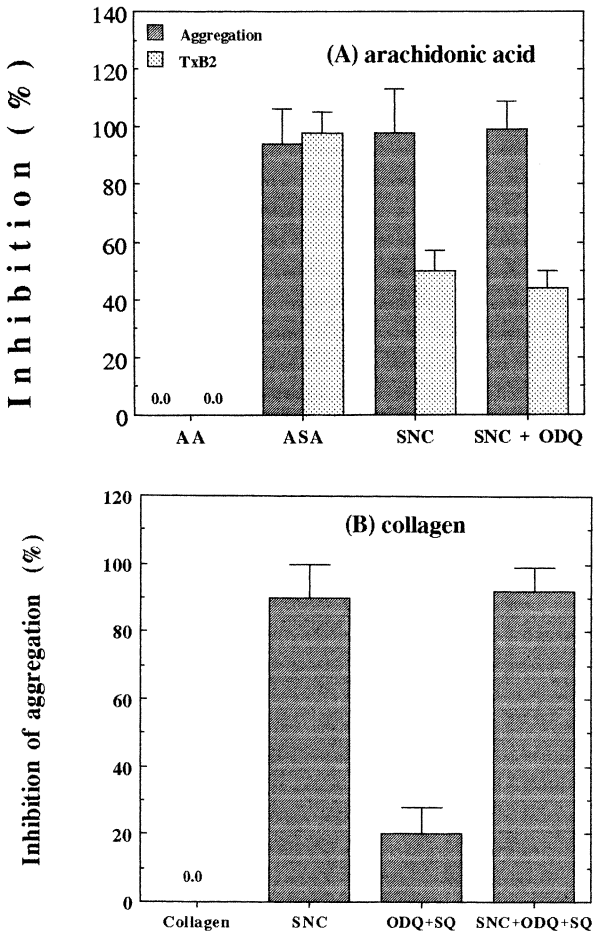

Fig. 6. A: Inhibition of aggregation and TxB_2 formation by SNC (10 μM) and ASA (200 μM) on arachidonic acid (AA, 1 mM)-induced aggregation in PRP. B: Inhibition of aggregation by SNC (10 μM) of collagen (1 μg/ml)-induced aggregation of washed human platelets. The concentrations of ODQ and SQ-29548 were 10 and 1 μM, respectively. All data are presented as mean ± S.E.M., $n = 3$.

result in significantly different inhibition of aggregation and intraplatelet levels of nitrite. A possible explanation for the apparent non-stereoselective antiaggregatory action of L- and D-SNC may be that activation of sGC, inhibition of TxA_2 synthesis in the platelets and possibly other cGMP-independent mechanisms were not caused by L- or D-SNC themselves but by NO which was released outside the platelets by these compounds that are equally potent NO donors. However, rapid free diffusion of SNC-derived NO and slower transmembranous transport of SNC into the platelets could take place simultaneously so that the differences in the effects caused by transmembranously transported L- or D-SNC could be abolished. Therefore, the cGMP-independent antiaggregatory actions of SNC could indeed be initiated by a specific transport system for SNC in human platelets. In contrast to SNC, SNP is a poor spontaneous NO donor. It seems likely that SNP activates the sGC by itself by directly binding to this enzyme rather by NO released from SNP. In the cGMP-independent mechanism, intraplatelet SNP could act by the mechanisms mentioned above for NO [28–34] but at a much weaker potency than SNC.

Our study shows that SNC potently stimulates the sGC. Although ODQ did not reverse the antiaggregatory action of SNC our study does not exclude that SNC inhibits platelet aggregation by a cGMP-dependent mechanism too, analogous to SNP and to other *S*-nitroso compounds such as *S*-nitrosopenicillamine and *S*-nitroso-*N*-acetyl-penicillamine [8]. Obviously, the cGMP-dependent mechanism of SNC is substantially less potent than the cGMP-independent mechanisms. Further, it is possible that the cGMP-independent mechanisms of SNC differ from those of other *S*-nitroso compounds.

An alternative cGMP-independent mechanism of the inhibition of platelet aggregation by SNC could involve *S*-nitrosylation of platelet membrane constituents such as the TxA_2 receptor. This mechanism is supported by the high nitrosylating potency of SNC against proteins and enzymes [44,45], the existence of membrane-bound *S*-nitroso compound receptors within the cardiovascular system [26], and the finding that *S*-nitroso compounds are targeted to red blood cells [46].

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References

[1] Craven, P.A. and DeRubertis, F.R. (1978) J. Biol. Chem. 253, 8433–8443.
[2] Feelisch, M. and Noack, E.A. (1987) Eur. J. Pharmacol. 139, 19–30.
[3] Murad, F. (1986) J. Clin. Invest. 78, 1–5.
[4] Mellion, B.T., Ignarro, L.J., Ohlstein, E.H., Pontecorvo, E.G., Hyman, A.L. and Kadowitz, P.J. (1981) Blood 57, 946–955.
[5] Radomski, M.W., Palmer, R.M.J. and Moncada, S. (1987) Br. J. Pharmacol. 92, 639–646.
[6] Garthwaite, J., Southam, E., Boulton, C.L., Nielsen, E.B., Schmidt, K. and Mayer, B. (1995) Mol. Pharmacol. 48, 184–188.
[7] Brunner, F., Schmidt, K., Nielsen, E.B. and Mayer, B. (1996) J. Pharmacol. Exp. Ther. 277, 48–53.
[8] Moro, M.A., Russel, R.J., Cellek, S., Lizasoain, I., Su, Y., Darley-Usmar, V.M., Radosmki, M.W. and Moncada, S. (1996) Proc. Natl. Acad. Sci. USA 93, 1480–1485.
[9] Furchgott, R.F. and Zawadski, J.V. (1990) Nature 288, 373–376.
[10] Palmer, R.M.J., Ferrige, A.G. and Moncada, S. (1987) Nature 327, 524–526.
[11] Ignarro, L.J., Buga, G.M., Wood, K.S., Byrus, R.E. and Chandhuri, G. (1987) Proc. Natl. Acad. Sci. USA 84, 9265–9269.
[12] Palmer, R.M.J., Ashton, D.S. and Moncada, S. (1988) Nature 333, 664–666.
[13] Schmidt, H.H.H.W., Nau, H., Wittfoht, W., Gerlach, J., Prescher, K.E., Klein, M.M. and Böhme, E. (1988) Eur. J. Pharmacol. 154, 213–216.
[14] Feelisch, M., te Poel, M., Zamora, R., Deussen, A. and Moncada, S. (1994) Nature 368, 62–65.
[15] Furchgott, R.F. and Vanhoutte, P.M. (1989) FASEB J. 3, 2007–2018.
[16] Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1992) Pharmacol. Rev. 43, 109–142.
[17] Myers, P.R., Minor, R.L., Guerra, R., Bates, J.N. and Harrison, D.G. (1990) Nature 345, 161–163.
[18] Azuma, H., Ishikawa, M. and Sekizaki, S. (1986) Br. J. Pharmacol. 88, 411–415.
[19] Furlong, B., Henderson, A.H., Lewis, M.J. and Smith, J.A. (1987) Br. J. Pharmacol. 90, 687–692.
[20] Alheid, U., Frölich, J.C. and Förstermann, U. (1987) Thromb. Res. 47, 561–571.
[21] Busse, R., Luckhoff, A. and Bassenge, E. (1987) Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 566–571.
[22] Hawkins, D.R., Meyrick, B.O. and Murray, J.J. (1988) Biochim. Biophys. Acta 969, 289–296.

[23] Ignarro, L.J. (1989) FASEB J. 3, 31–36.
[24] Lieberman, E.H., O'Neill, S. and Mendelsohn, M. (1991) Circ. Res. 68, 1722–1728.
[25] Keaney Jr., J.F., Simon, D.I., Stamler, J.S., Jaraki, O., Scharfstein, J., Vita, J.A. and Loscalzo, J. (1993) J. Clin. Invest. 91, 1582–1589.
[26] Davisson, R.L., Travis, M.D., Bates, J.N. and Lewis, S.J. (1996) Circ. Res. 79, 256–262.
[27] de Belder, A.J., MacAllister, R., Radomski, M.W., Moncada, S. and Vallance, P.J.T. (1994) Cardiovasc. Res. 28, 691–694.
[28] Maurice, D.H. and Haslam, R.J. (1990) Mol. Pharmacol. 37, 671–681.
[29] Dimmeler, S., Lottspeich, F. and Brune, B. (1992) J. Biol. Chem. 267, 16771–16774.
[30] Molina y Vedia, L., McDonald, B., Reep, B., Brune, B., Di Silvio, M., Billiar, T.R. and Lapetina, E.G. (1992) J. Biol. Chem. 267, 24929–24932.
[31] Zhang, J. and Snyder, S.H. (1992) Proc. Natl. Acad. Sci. USA 89, 9382–9385.
[32] Brune, B. and Lapetina, E.G. (1989) J. Biol. Chem. 264, 8455–8458.
[33] Ivanova, K., Schaefer, M., Drummer, C. and Gerzer, R. (1993) Eur. J. Pharmacol. 244, 37–47.
[34] Menschikov, M.Yu., Ivanova, K., Schaefer, M., Drummer, C. and Gerzer, R. (1993) Eur. J. Pharmacol. 245, 281–284.
[35] Ogletree, M.L., Harris, D.N., Greenberg, R., Haslanger, M.F. and Nakane, M. (1985) J. Pharmacol. Exp. Ther. 234, 435–441.
[36] Nalane, M., Reid, J.A., Haslanger, M.F., Garber, D.P., Harris, D.N., Ogletree, M.L. and Greenberg, R. (1985) Adv. Prostaglandins Thromboxane Res. 15, 291–293.
[37] Kattelman, E.J., Venton, D.L. and Breton, G.C. (1986) Thromb. Res. 41, 471–481.
[38] Tsikas, D., Gutzki, F.M., Rossa, S., Bauer, H., Neumann, Ch., Dockendorff, K., Sandmann, J. and Frölich, J.C. (1997) Anal. Biochem. 244, 208–220.
[39] Tsikas, D., Rossa, S., Stichtenoth, D.O., Gutzki, F.M., Raida, M. and Frölich, J.C. (1996) Biochem. Biophys. Res. Commun. 220, 939–944.
[40] Born, C.V.R. and Cross, M.J. (1963) J. Physiol. 168, 178–195.
[41] Kuhn, M., Otten, A., Frölich, J.C. and Förstermann, U. (1991) J. Pharmacol. Exp. Ther. 256, 677–682.
[42] Tewes, K.S., Tsikas, D., Gutzki, F.M. and Frölich, J.C. (1998) Anal. Biochem. 261, 121–124.
[43] Bode-Böger, S.M., Böger, R.H., Galland, A. and Frölich, J.C. (1998) Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 143–150.
[44] Meyer, D.J., Kramer, H., Özer, N., Coles, B. and Ketterer, B. (1994) FEBS Lett. 345, 177–180.
[45] Scharfstein, J.S., Keaney Jr., J.F., Slivka, A., Welch, G.N., Vita, J.A., Stamler, J.S. and Loscalzo, J. (1994) J. Clin. Invest. 94, 1432–1439.
[46] Pietraforte, D., Mallozzi, C., Scorza, G. and Minetti, M. (1995) Biochemistry 34, 7177–7185.