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Biliverdin IX is an endogenous inhibitor of soluble guanylyl cyclase

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Abstract

Heme oxygenase (HO) converts heme to carbon monoxide (CO) and biliverdin IX. CO is a weak activator of soluble guanylyl cyclase (SGC), the enzyme that catalyzes the conversion of GTP to the second messenger cGMP. HO overexpression has recently been shown to inhibit production of cGMP by SGC *in vivo*. The aim of the present study was to investigate a possible influence of biliverdin IX on SGC activity. Using recombinant α_1/β_1 isoform of SGC, we show an inhibitory effect of biliverdin IX in the micromolar range both on basal and NO stimulated guanylyl cyclase activity. Bilirubin IX which differs from biliverdin IX in two hydrogen atoms had no effect. Biliverdin IX reduced maximal guanylyl cyclase activity (V_{max} values) while it had no effect on the K_M values indicating unchanged affinity towards the substrate GTP. Concentration response experiments using the NO donor, 2,2-diethyl-1-nitroso-oxyhydrazine (DEA/NO), showed that enzyme activities at maximal DEA/NO concentration were reduced by biliverdin IX. The affinity of the NO-donor, DEA/NO, towards SGC was significantly reduced in the presence of biliverdin IX. Biliverdin IX lowered enzyme activity at maximal activator concentrations of YC-1 and protoporphyrin IX (PPIX) while it had no significant effect on the EC_{50} values of these two NO independent activators. The inhibitory effect of biliverdin IX on PPIX activated enzyme activity is not shared by ODQ, which indicates that the inhibitory mechanism of biliverdin IX is different from ODQ.

Keywords: Bliverdin IX; Soluble guanylyl cyclase; Heme oxygenase

1. Introduction

Soluble guanylyl cyclase (SGC) in smooth muscle cells of blood vessels catalyzes the conversion of GTP to cGMP [EC 4.6.1.2] and is activated by nitric oxide (NO) released from the endothelial lining of the vessels (for review [1]). The SGC has been purified as a heterodimeric, heme containing enzyme consisting of an α_1 and β_1 subunit. After cloning of the α_1 and β_1 cDNAs two other subunit cDNAs have been cloned by homology screening: The β_2 subunit from rat kidney and the α_2 subunit from human fetal brain [2,3]. Coexpression of the α_1/β_1 and α_2/β_1 cDNAs yielded NO-sensitive enzymes in expression systems [3] and the α_2/β_1 heterodimeric enzyme has been demonstrated on the protein level in human placenta by coprecipitation experiments [4]. We have recently shown that the β_2 subunit shows NO sensitive guanylyl cyclase activity in the absence of an additional α subunit and most likely forms a homomeric enzyme complex [5]. While activation of the different SGC isoforms by NO is firmly established another gaseous molecule, carbon monoxide (CO), has been put forward as a putative activator of SGC [6,7]. The CO generating enzyme heme oxygenase (HO) colocalizes with SGC in numerous brain regions [8]. Its substrate is ferric protoporphyrin IX (PPIX) and the enzyme catalyzes the reaction to biliverdin IX besides CO. The gaseous molecule CO activates SGC only modestly and only at very high concentrations [9]. Very recently it has been shown that overexpression of HO in vascular smooth muscle cells attenuates NO-induced vasodilation in these transgenic mice [10]. This paradox raised our interest in the HO product biliverdin IX as a possible novel SGC inhibitor. In the current study, we show that biliverdin IX inhibits SGC in the micromolar range and represents a novel SGC inhibitor.

2. Materials and methods

2.1. Materials

3-(5′-Hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ)

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Abbreviations: CO, carbon monoxide; HO, heme oxygenase; NO, nitric oxide; PPIX, protoporphyrin IX; SGC, soluble guanylyl cyclase.

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was from Alexis Biochemicals. Biliverdin IX was from Porphyrin Products Inc. and bilirubin IX was from Fluka. 2,2-Diethyl-1-nitroso-oxyhydrazine (DEA/NO) and all other chemicals were obtained from Sigma in the highest grade of purity. Products for Sf9 cell culture were from Life Technologies.

2.2. Generation of recombinant baculovirus, expression of guanylyl cyclase subunits in Sf9 cell culture and cytosol preparation

Cloning of the α_1 subunit (a kind gift of Dr. Georges Guellaën, Créteil [11]) and the β_1 subunit have been described earlier [5,12]. Recombinant baculoviruses of respective subunits were generated according to the BAC-TO-BAC™ System. Sf9 cells were cultured in Sf-900 II serum-free medium supplemented with 1% penicillin/streptomycin and 10% fetal calf serum. Spinner cultures were grown to a cell density of 3.0×10^6 cells/mL and then diluted to 1.2×10^6 cells/mL for infection. About 30 mL cell solution were infected with multiplicities of infection of 1. Cell harvesting and cytosol preparation is described earlier [5].

2.3. Purification of SGC

All purification steps were performed at 4°. The cell pellet from 1400 mL cell solution were homogenized with a cell disruption bomb (Parr®) at 60 bar for 1 hr in 150 mL 50 mM TEA/HCl pH 8.0 containing 10 mM DTT, 1 mM benzamidine and 900 μL protease inhibitor mix (Sigma). The homogenate was centrifuged at 40,000 *g* for 30 min, and 140 mL supernatant was collected. All chromatographic steps were performed on a FPLC system. The protease inhibitor benzamidine (1 mM) and DTT (10 mM) were used in all chromatographic steps. The supernatant was immediately applied to a Q-Sepharose column (20 mL volume) at 1.5 mL/min. Ion exchange buffer A contained 50 mM TEA/HCl pH 8.0. Ion exchange buffer B was prepared by adding 1 M NaCl to buffer A. The column was washed with 10% buffer B until the absorption measured at 280 nm was stable. A linear gradient from 10% to 100% B for 60 mL was used to elute SGC. The SGC containing fractions were pooled by determining SGC activity. The pooled fractions (40 mL) were applied immediately to a ceramic hydroxyapatite column (Bio-Rad, 5 mL volume) at 1 mL/min. Hydroxyapatite buffer A containing 10 mM potassium phosphate (pH 6.8) and hydroxyapatite buffer B containing 400 mM potassium phosphate (pH 6.8). The column was then washed with 9% buffer B until the absorption measured at 280 nm was stable. The enzyme was eluted with a linear gradient running from 9% to 100% buffer B for 40 mL. The SGC containing fractions (15 mL) were again pooled as described above and concentrated in centrifugal devices with a 30 kDa cut-off (Millipore) to 2 mL. The enzyme was then loaded on a Superdex 200 column (Pharmacia 60 cm × 2.6 cm) and eluted with 50 mM TEA/HCl pH 8.0 containing 250 mM NaCl. Fractions with the highest SGC activity were pooled and concentrated as described above to a final volume of approximately 200 μL. The partially purified enzyme was stored with 10% (v/v) glycerol at −80°. For spectroscopic measurements 100 μL of partially purified enzyme were used. For measurements of SGC activity purified enzyme was diluted 1:30 with 50 mM TEA/HCl pH 8.0 containing DTT and benzamidine.

2.4. Determination of protein concentration and guanylyl cyclase activity assay

Protein concentrations were determined by the method of Bradford using bovine plasma gamma globulin (Bio-Rad Protein Assay Standard I) as standard. Guanylyl cyclase activity was measured as described previously [13]. YC-1 was dissolved in 25% (v/v) DMSO so that the final DMSO concentration in the enzyme assay did not exceed 2.5% (v/v). At this concentration no effects of DMSO on enzyme activity were observed. DEA/NO, PPIX, biliverdin IX and bilirubin IX were dissolved in 10 mM NaOH, which also did not effect the enzyme activity.

2.5. Generation of the α_1-1200 and the β_1-89 antibodies, SDS–PAGE and immunoblotting

The α_1-1200 antibody was raised against two peptides (EP012493: H_2N-FTPRSREELPPNFP-COOH and EP012494: H_2N-CFQKKDVEDGNANFLGKASGID-COOH) of the C-terminal domain of the human α_1-subunit and the β_1-89 antibody was raised against the C-terminal peptide (EP990255: H_2N-CSRKNTGTEETKQDDD-COOH). Antibodies were coupled by an additional cysteine to keyhole-limpet hemocyanin. Rabbits were immunized on days 0, 14, 28, 56 and were finally bled on day 80. Successful antigen response was estimated by ELISA. For monitoring the purity of enzyme preparations and for the determination of apparent molecular masses of the purified enzyme, SDS–PAGE was performed in 10% slab gels and were stained with Coomassie blue G-250. For immunoblotting, protein fractions were subjected to 10% SDS–PAGE and then transferred electrophoretically to a nitrocellulose membrane. 16 μg protein was loaded per lane. The membrane was reversibly stained with Ponceau S and unspecific binding sites were saturated by immersing the membrane for 1 hr in TBST-buffer (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) containing 5% nonfat dry milk. The membranes were incubated for 1.5 hr in TBST-buffer containing α_1-1200 and β_1-89 in a 1:1000 dilution and 0.5% dry milk. Negative control reactions were run in additional presence of synthetic peptides used for immunization in different combinations (5 μg/mL). The membranes were washed three times for 10 min with

TBST and subsequently incubated for 1 hr with horseradish peroxidase labeled anti-rabbit-IgG antibodies (diluted 1:4000, Sigma). After three washes with TBST the membranes were processed with the ECL western blotting detection system according to the recommendations of the manufacturer (Amersham).

2.6. Statistical analysis

A student t test was used to examine significance of difference in two groups. For comparison of multiple groups one-way ANOVA analysis followed by a Newman–Keuls post test was used. A value of $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

Previous studies have indicated that overexpression of HO inhibits NO/cGMP dependent signaling, although the

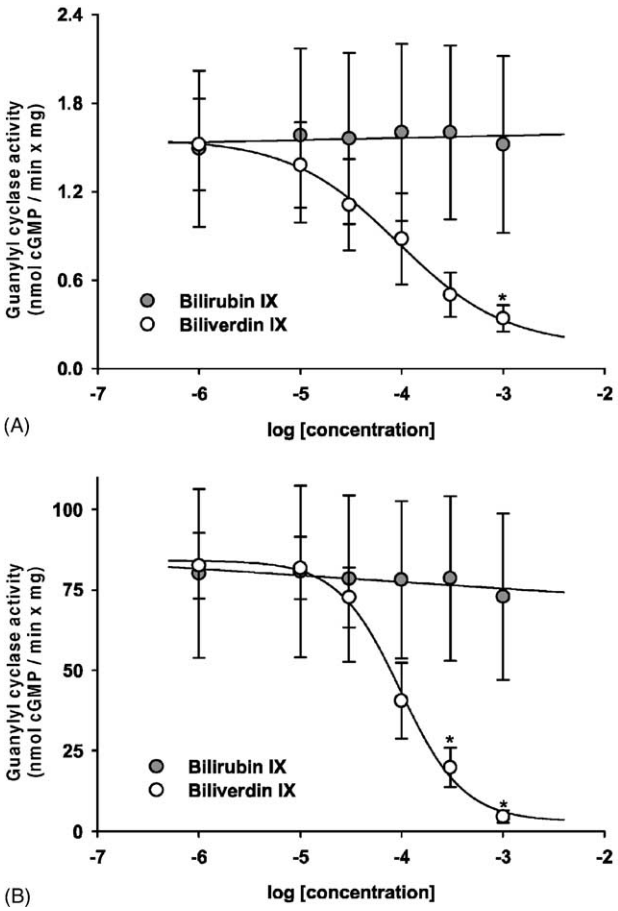

Fig. 1. Concentration-dependent effect of biliverdin IX or bilirubin IX on guanylyl cyclase activity in Sf9 cell cytosol after infection with α_1/β_1. Guanylyl cyclase activity was measured under basal conditions (A) or NO-stimulated conditions (100 μM DEA/NO; B) in the presence of increasing concentrations of biliverdin IX (white circles) or bilirubin IX (gray circles). Data represent the means (±SE) of at least four independent experiments performed in duplicate.

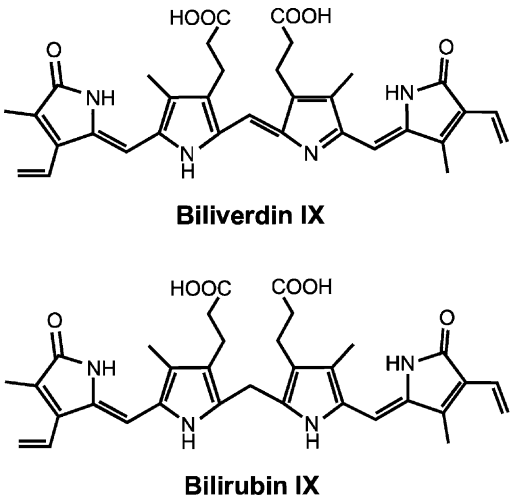

Fig. 2. Chemical structure of biliverdin IX and bilirubin IX.

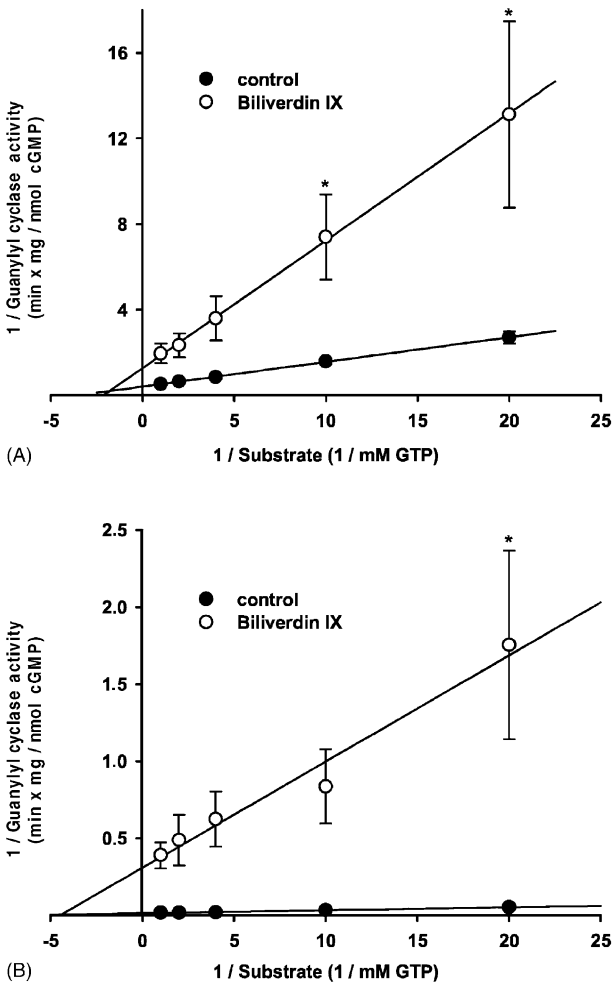

Fig. 3. Guanylyl cyclase activity as a function of MgGTP concentration. Guanylyl cyclase activity is plotted in double-reciprocal form. The experiments were performed in the absence (closed circles) or presence of 100 μM biliverdin IX (open circles) with GTP concentrations between 0.050 and 1 mM under basal conditions (A) or NO-stimulated conditions (100 μM DEA/NO; B). Data represent the means (±SE) of three independent experiments performed in duplicate. For closed circles symbols are larger than error bars.

enzyme produces CO which is considered a weak activator of NO-sensitive guanylyl cyclase [10]. We hypothesized that another product of the HO reaction could account for the effects of HO overexpression. Conversion of heme from the HO leads to production of biliverdin IX in addition to CO. In the present study, we investigate the influence of biliverdin IX and its reduction product bilirubin IX on NO-sensitive guanylyl cyclase activity. Fig. 1 shows the influence of both bile pigments on cytosolic enzyme activity of α_1/β_1 expressed in the baculovirus/Sf9 cell system. While biliverdin IX led to a significant reduction in enzyme activity under basal and NO-stimulated conditions (100 μM DEA/NO), its reduction product bilirubin IX showed no effect, although both bile pigments differ only in two hydrogen atoms (Fig. 2). At basal conditions, biliverdin IX inhibited guanylyl cyclase activity in a dose-dependent fashion with an IC_{50} value of 110 ± 47 μM (N = 5; Fig. 1A). In experiments performed in parallel, no inhibitory effect of bilirubin IX was observed. A similar result was obtained when the experiments were done in presence of 100 μM DEA/NO (Fig. 1B). The dose-dependent inhibition of NO-stimulated guanylyl cyclase resulted in an IC_{50} value of 143 ± 61 μM for biliverdin IX (N = 4). Again, there was no significant effect of bilirubin IX at equal concentrations. To test whether biliverdin IX binds to the substrate binding site of SGC or induces conformational changes altering this site, we determined the kinetic parameters of SGC in the presence and absence of biliverdin IX (Fig. 3). The slight difference for the K_M values in the presence (450 ± 113 μM GTP) or absence (287 ± 50 μM GTP) of biliverdin IX was not significant ($P = 0.26$; N = 3). In contrast, maximal enzyme activities (V_{max} values) were significantly lower in the presence of biliverdin IX (2.48 ± 0.20 nmol cGMP/min mg vs. 0.82 ± 0.11 nmol cGMP/min mg; $P < 0.01$; N = 3). The same set of experiments was performed in presence of 100 μM DEA/NO (Fig. 3B). There was no significant difference for the K_M values in the presence (212 ± 41 μM GTP) or absence (148 ± 14 μM GTP) of biliverdin IX ($P = 0.21$; N = 3). In contrast V_{max} values were significantly lower in the presence of biliverdin IX (82.2 ± 17.8 nmol cGMP/min mg vs. 3.8 ± 1.0 nmol cGMP/min mg; $P < 0.05$; N = 3). These findings indicate, that the inhibitory effect of biliverdin IX on SGC does not affect the substrate binding site.

Since biliverdin IX is a heme-degradation product and bears chemical similarities to heme or other porphyrins, we speculated that it might interfere with NO-activation by binding to the heme pocket of SGC to displace endogenous heme (Fig. 4). Concentration response experiments using the NO donor DEA/NO showed that enzyme activities at maximal DEA/NO concentration were significantly reduced in the presence of biliverdin IX (89.9 ± 33.3 nmol cGMP/min mg vs. 19.0 ± 9.3 nmol cGMP/min mg; $P < 0.05$; N = 4). This raises the possibility that biliverdin IX displaces endogenous heme, since heme content of SGC closely correlates with fold-stimulation by NO-donors [14]. The affinity of the NO-donor, DEA/NO, towards SGC was significantly reduced in the presence of biliverdin IX, as suggested by the respective EC_{50} values (1.61 ± 0.34 μM vs. 0.32 ± 0.13 μM; $P < 0.01$; N = 4). This finding prompted us to test whether NO-independent activation of SGC can also be inhibited by biliverdin IX. Fig. 5 shows the respective concentration response curves for YC-1 in the presence or absence of biliverdin IX. Biliverdin IX had no significant effect on the EC_{50} for YC-1, but significantly lowered enzyme activity at maximal YC-1 concentrations (23.4 ± 5.2 nmol cGMP/min mg vs. 6.9 ± 1.2 nmol cGMP/min mg; $P < 0.05$; N = 3). Likewise, biliverdin IX had no effect on the EC_{50} of PPIX, another NO-independent

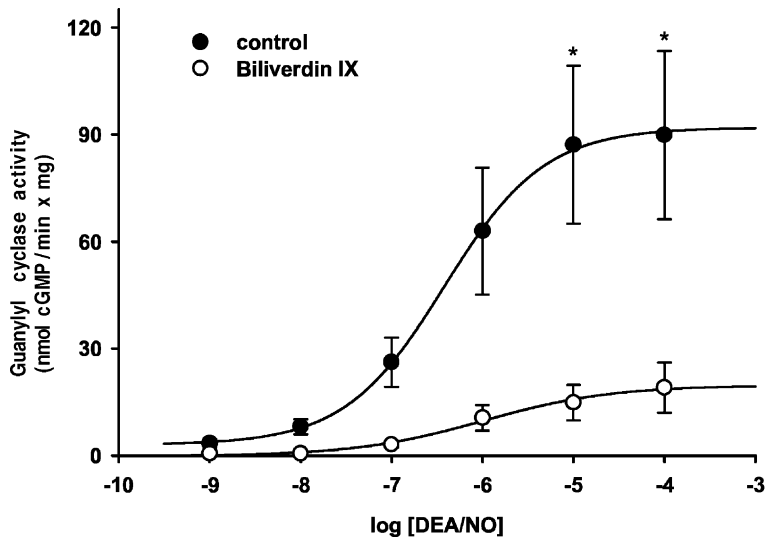

Fig. 4. Effect of biliverdin IX on α_1/β_1 heterodimeric guanylyl cyclase stimulated with increasing concentrations of DEA/NO. Guanylyl cyclase was stimulated with DEA/NO in a concentration range between 1 nM and 100 μM. Measurements were performed in the absence (closed circles) or presence of 100 μM biliverdin IX (open circles). Data represent means (±SE) of four independent experiments performed in duplicate.

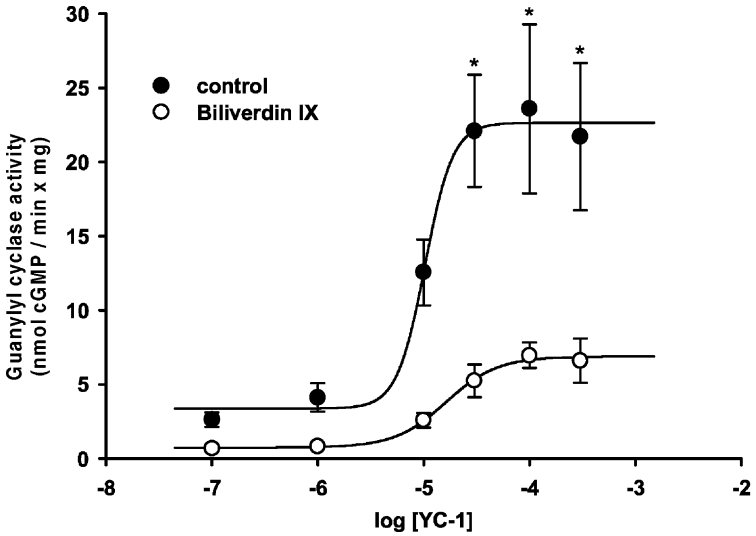

Fig. 5. Effect of biliverdin IX on α_1/β_1 heterodimeric guanylyl cyclase stimulated with increasing concentrations of YC-1. Guanylyl cyclase was stimulated with increasing concentrations of YC-1 (0.1–300 μM) in the absence (closed circles) or presence (open circles) of biliverdin IX (100 μM). Data represent the means (±SE) of three independent experiments performed in duplicate.

activator of SGC, but significantly lowered enzyme activity in the presence of maximal PPIX concentrations (Fig. 6; 40.5 ± 3.8 nmol cGMP/min mg vs. 14.3 ± 1.6 nmol cGMP/min mg, $P < 0.001$; $N = 4$). Again, bilirubin IX was used as additional control and did not show any difference from the control curve (see Fig. 6). To test for the possibility that the inhibitory effect of biliverdin IX is not due to a direct interaction with SGC, we partially purified α_1/β_1 as described in Section 2. Fig. 7 shows a Coomassie blue stained SDS–PAGE. Two bands with molecular masses consistent with the α_1 and β_1 SGC subunits were apparent (left panels, arrows). Immunoblotting using specific antibodies and blocking with the respective peptides identified these bands as α_1 and β_1 SGC subunits (Fig. 7; right panel). Spectroscopic analysis of this enzyme preparation showed a Soret peak with a maximal absorption at 431 nm under basal conditions and a shift to 398 nm in the presence of DEA/NO typical of SGC (Fig. 8). Similar to the previous findings, biliverdin IX inhibited guanylyl cyclase activity in a dose-dependent fashion in this enzyme preparation with an IC_{50} value of 55 ± 19 μM ($N = 5$; Fig. 9A). In experiments performed in parallel, no inhibitory effect of bilirubin IX was observed. A similar result was obtained when the experiments were done in presence of 100 μM DEA/NO (Fig. 9B). The dose-dependent inhibition of NO-stimulated

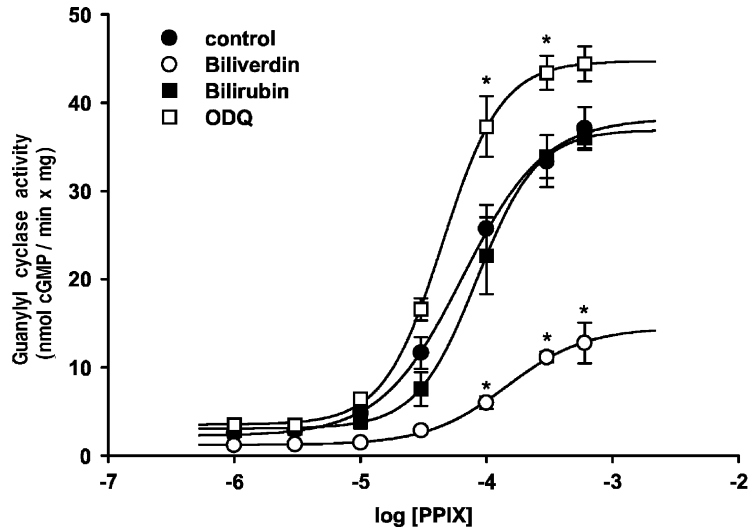

Fig. 6. Effect of biliverdin IX, bilirubin IX and ODQ on α_1/β_1 heterodimeric guanylyl cyclase stimulated with increasing concentrations of PPIX. Guanylyl cyclase was stimulated with increasing concentrations of PPIX in the absence of test substance (closed circles) or presence of biliverdin IX (100 μM, open circles), bilirubin IX (100 μM, closed squares) or ODQ (10 μM, open squares). Data represent the means (±SE) of at least three independent experiments performed in duplicate.

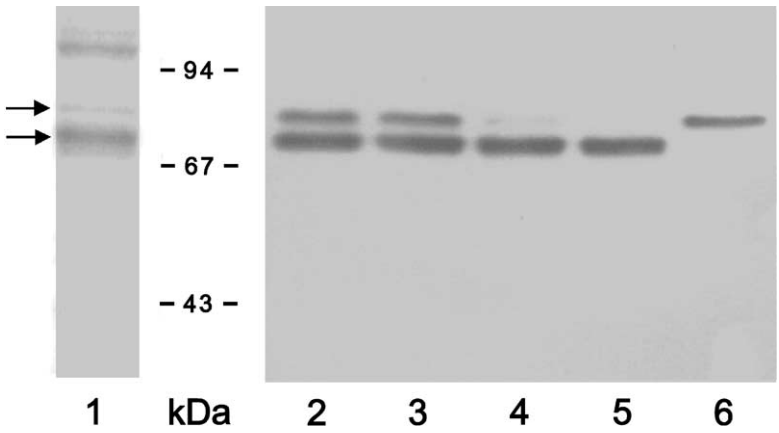

Fig. 7. SDS–PAGE and immunoblotting analysis of partially purified SGC α_1/β_1 isoform. 16 μg partially purified enzyme were electrophoresed by 10% SDS–PAGE and stained with Coomassie blue (lane 1). The electrophoresed proteins were transferred to nitrocellulose membranes and incubated with α_1-1200 and β_1-89 in a 1:1000 dilution (lane 2). The α_1 and β_1 subunit was determined by blocking antibody signals with 5 μg/mL of the respective peptides (lane 3: EP012493; lane 4: EP012494; lane 5: EP012493 + EP012494; lane 6: EP990255).

guanylyl cyclase resulted in an IC_{50} value of 128 ± 19 μM for biliverdin IX (N = 5). Again, there was no significant effect of bilirubin IX at equal concentrations. The lack of effect of bilirubin IX vs. biliverdin IX could either be explained by conformational differences: bilirubin IX adopts a folded conformation shaped like the ridge-tile of a roof and stabilized by intramolecular hydrogen bonding between its carboxy groups and the pyrromethenone lactam and –NH groups, while biliverdin IX prefers to adopt helical porphyrin-like conformations [15,16]. On the other hand it is reasonable to assume that differences in the redox status of both molecules could explain the inhibitory effect, since ODQ has been shown to inhibit SGC by oxidizing the ferrous to a ferric heme group [17]. If biliverdin IX acts as heme-oxidizing agent similar to ODQ, this would also explain the lack of effect of bilirubin IX, a known antioxidant [18]. As shown in Fig. 6 ODQ did not inhibit PPIX activated SGC activity. The significant effect of biliverdin IX under the same experimental conditions strongly argues for a mode of inhibitory action different from ODQ.

We found in the current study that halfmaximal inhibition of NO-stimulated guanylyl cyclase occurs at a concentration of 143 μM biliverdin IX. While there are only scarce data on the concentrations of biliverdin IX throughout the body, plasma concentrations of biliverdin IX between 30 and 40 μM have been measured in patients with obstructive jaundice secondary to malignant diseases [19]. It is likely that in pathological states local biliverdin IX concentrations in the liver or other organs will be sufficient to inhibit endogenous NO-cGMP-signaling. In a rat model it has been shown that oxidation products of bilirubin like biliverdin produced after bleeding in the cerebrospinal fluid in states of oxidative stress can cause prolonged vasospasm of rat cerebral vessels which is consistent with an inhibitory effect on NO-cGMP mediated

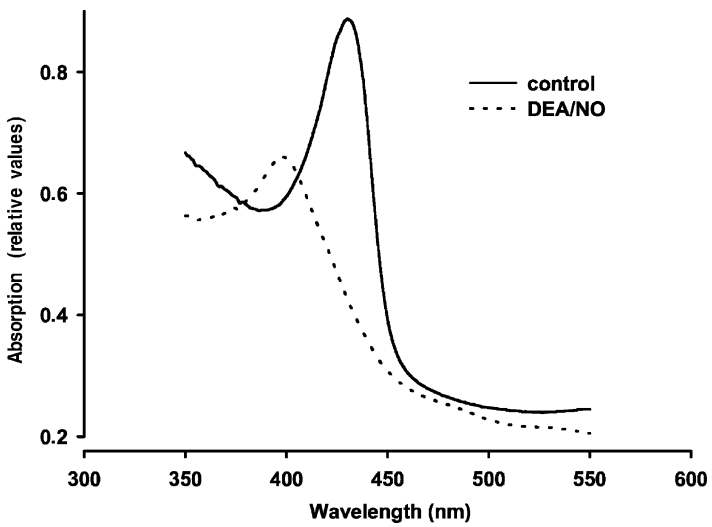

Fig. 8. Heme spectra of partially purified SGC. The spectra were performed under basal conditions (solid line) and in the presence of 100 μM DEA/NO (dotted line).

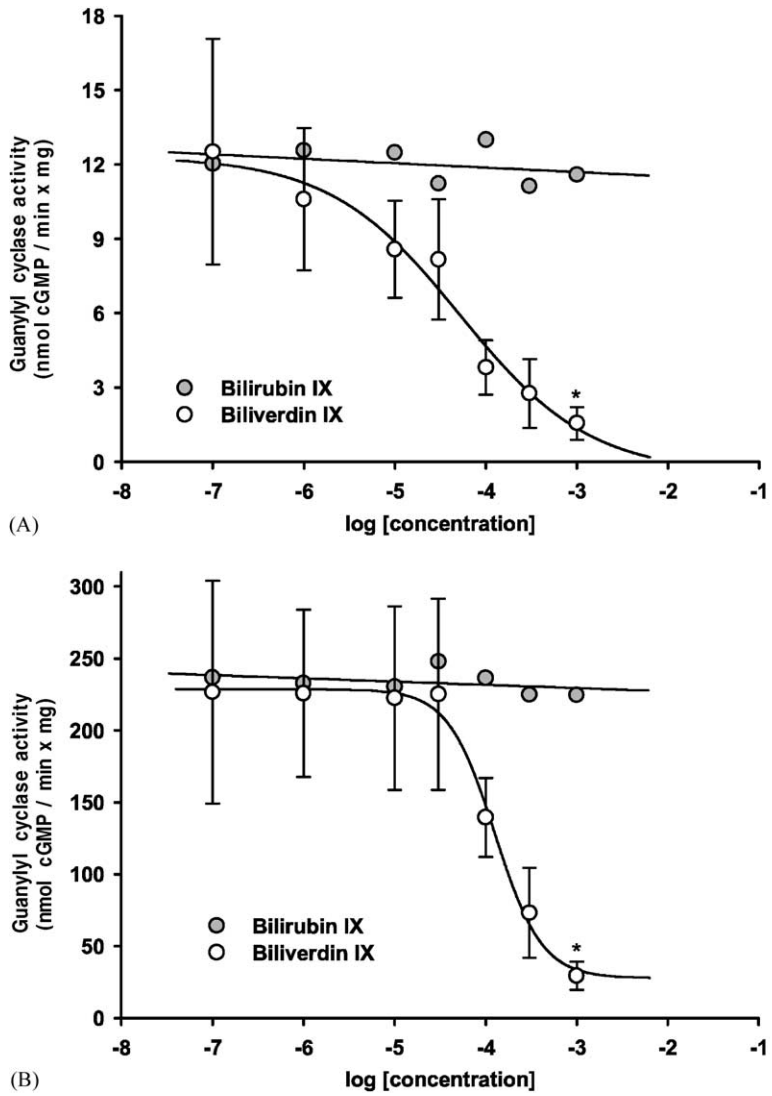

Fig. 9. Concentration-dependent effect of biliverdin IX or bilirubin IX on guanylyl cyclase activity of partially purified α_1/β_1. Guanylyl cyclase activity was measured under basal conditions (A) or NO-stimulated conditions (100 μM DEA/NO; B) in the presence of increasing concentrations of biliverdin IX (white circles) or bilirubin IX (gray circles). Data for biliverdin IX represent the means (±SE) of five independent experiments performed in duplicate. Data for bilirubin IX represent only one control experiment.

vasorelaxation [20]. The further study of the mechanism of action of biliverdin IX might be useful for the development of a class of more selective and potent inhibitors of SGC, which could be useful as drugs in septic shock or migraine [21,22].

References

[1] Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, Waldman SA. Guanylyl cyclases and signaling by cyclic GMP. Pharmacol Rev 2000;52:375–414.

[2] Yuen PST, Potter LR, Garbers DL. A new form of guanylyl cyclase is preferentially expressed in rat kidney. Biochemistry 1990;29:10872–8.

[3] Harteneck C, Wedel B, Koesling D, Malkewitz J, Böhme E, Schultz G. Molecular cloning and expression of a new α subunit of soluble guanylyl cyclase. Interchangeability of the α subunits of the enzyme. FEBS Lett 1991;292:217–22.

[4] Russwurm M, Behrends S, Harteneck C, Koesling D. Functional properties of a naturally occurring isoform of soluble guanylyl cyclase. Biochem J 1998;335:125–30.

[5] Koglin M, Vehse K, Budaeus L, Scholz H, Behrends S. Nitric oxide activates the β_2 subunit of soluble guanylyl cyclase in the absence of a second subunit. J Biol Chem 2001;276:30737–43.

[6] Brüne B, Ullrich V. Inhibition of platelet aggregation by carbon monoxide is mediated by activation of guanylate cyclase. Mol Pharmacol 1987;32:497–504.

[7] Stone JR, Marletta MA. Soluble guanylate cyclase from bovine lung: activation with nitric oxide and carbon monoxide and spectral characterization of the ferrous and ferric states. Biochemistry 1994;33:5636–40.

[8] Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: a putative neural messenger. Science 1993;259:381–4.

[9] Cary SP, Marletta MA. The case of CO signaling: why the jury is still out. J Clin Invest 2001;107:1071–3.

[10] Imai T, Morita T, Shindo T, Nagai R, Yazaki Y, Kurihara H, Suematsu M, Katayama S. Vascular smooth muscle cell-directed overexpression of heme oxygenase-1 elevates blood pressure through attenuation of nitric oxide-induced vasodilation in mice. Circ Res 2001;89:55–62.

[11] Giuili G, Scholl U, Bulle F, Guellaën G. Molecular cloning of the cDNAs coding for the two subunits of soluble guanylyl cyclase from human brain. FEBS Lett 1992;304:83–8.
[12] Koglin M, Behrends S. Cloning and functional expression of the rat α_2 subunit of soluble guanylyl cyclase. Biochim Biophys Acta 2000;1494:286–9.
[13] Behrends S, Harteneck C, Schultz G, Koesling D. A variant of soluble guanylyl cyclase contains an insert homologous to adenylyl cyclase and functions as a dominant negative protein. J Biol Chem 1995;270:21109–13.
[14] Foerster J, Harteneck C, Malkewitz J, Schultz G, Koesling D. A functional heme-binding site of soluble guanylyl cyclase requires intact N-termini of α_1 and β_1 subunits. Eur J Biochem 1996;240:380–6.
[15] Falk H. The chemistry of linear oligopyrroles and bile pigments. Springer: New York, 1989.
[16] Person RV, Peterson BR, Lightner DA. Bilirubin conformational analysis and circular dichroism. J Am Chem Soc 1994;116:42–59.
[17] Schrammel A, Behrends S, Schmidt K, Koesling D, Mayer B. Characterization of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one as a heme-site inhibitor of nitric oxide-sensitive guanylyl cyclase. Mol Pharmacol 1996;50:1–5.
[18] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–6.
[19] Tickner TR, Gutteridge JMC. A simple colorimetric method for the estimation of plasma biliverdin. Clin Chim Acta 1978;85:125–9.
[20] Clark JF, Reilly M, Sharp FR. Oxidation of bilirubin produces compounds that cause prolonged vasospasm of rat cerebral vessels: a contributor to subarachnoid hemorrhage-induced vasospasm. J Cereb Blood Flow Metab 2002;22:472–8.
[21] Zingarelli B, Hasko G, Salzman AL, Szabo C. Effects of a novel guanylyl cyclase inhibitor on the vascular actions of nitric oxide and peroxynitrite in immunostimulated smooth muscle cells and in endotoxic shock. Crit Care Med 1999;27:1701–7.
[22] Hobbs AJ, Higgs A, Moncada S. Inhibition of nitric oxide synthase as a potential therapeutic target. Annu Rev Pharmacol Toxicol 1999;39:191–220.