Functional properties of a naturally occurring isoform of soluble guanylyl cyclase

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Soluble guanylyl cyclase (sGC), the target enzyme of the signalling molecule NO, contains one prosthetic haem group and consists of an α and a β subunit. So far, only the $\alpha_1\beta_1$ heterodimer has been shown to exist in different cells and tissues, and most biochemical studies of sGC have been performed with the $\alpha_1\beta_1$ heterodimer. Here we demonstrate for the first time the natural occurrence of the α_2 subunit on the protein level. The α_2 subunit co-precipitated with the β_1 subunit from human placenta, showing the existence of the $\alpha_2\beta_1$ isoform *in vivo*. The new enzyme was expressed in and purified from cells from the *Spodoptera frugiperda* ovary cell line Sf 9. Spectral analysis showed that the $\alpha_2\beta_1$ heterodimer contains a prosthetic haem group revealing the same characteristics as the haem in the $\alpha_1\beta_1$ form. The kinetic

properties of both isoforms and sensitivity towards NO were indistinguishable. ³H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a selective inhibitor of sGC, abolished NO-stimulated activity of both heterodimers. The new NO-independent activator, 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1), increased the maximal NO-stimulated activity of the new isoform, caused a leftward-shift in the NO concentration–response curve and turned CO into an effective activator, as it did for the $\alpha_1\beta_1$ heterodimer (200-fold activation). In summary, the differences in primary structure of both α subunits are contrasted by their functional similarity. Further studies will be needed to elucidate the physiological purpose of the new isoform.

INTRODUCTION

Guanylyl cyclases [GTP pyrophosphate-lyase (cyclizing); EC 4.6.1.2], the enzymes that catalyse the conversion of GTP to cGMP, exist in membrane-bound and soluble forms [1,2]. The membrane-bound forms, which belong to the group of receptor-linked enzymes, are stimulated by different peptide hormones. In contrast to the membrane-bound enzymes, which form homodimers or higher ordered structures, soluble guanylyl cyclase (sGC) consists of two different subunits, designated α and β , both required for catalytic activity [3,4]. The enzyme is stimulated by the intra- and inter-cellular signalling molecule nitric oxide (NO), which, by binding to the enzyme's prosthetic haem group induces conformational changes leading to an up to 400-fold increase in catalytic activity [5,6]. Recently, new modulators of sGC activity have been identified. ³H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) has been shown to act as a specific inhibitor of NO-stimulated activity of sGC, thus representing an important tool in the study the cGMP-dependent mediation of NO effects [7]. We have been able to show that the substance 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1), a benzyl indazole derivative, stimulates sGC independently of NO, increases the responsiveness of the enzyme towards NO, and turns CO into an effective activator [8]. Most functional studies of sGC described so far have been performed with the enzyme purified from bovine lung, i.e. the $\alpha_1\beta_1$ heterodimer.

By homology screening, two other sGC subunits, α_2 and β_2 , have been identified [9,10]. As heterodimer formation between an α and a β subunit is required for enzyme activity, combinatorial association of α and β subunits could, in theory, give rise to at least four different isoforms. But, in fact, only one form ($\alpha_1\beta_1$) has been shown to occur *in vivo*. In expression experiments, the α_2 subunit was able to form a catalytically active enzyme when

coexpressed with the β_1 subunit [9]. The α_2 subunit shares 48 % identical amino acids with the α_1 subunit. Whereas the homology is most pronounced in the C-terminal catalytic region, which is conserved in all subunits of sGC, the N-terminal regions of the α subunits are quite diverse (27 % identical amino acids) [9]. The α_2 subunit was shown to occur in an alternatively spliced catalytically inactive form (α_{2i}) which is able to function as a dominant negative protein when coexpressed with the α_2 and β_1 subunits [11]. Reverse transcription PCR (RT-PCR) analysis of the tissue distribution of sGC subunits suggests an almost ubiquitous expression of the α_2 subunit [11]. However, the natural occurrence of the α_2 subunit has not been demonstrated on the protein level, and it is not known whether the $\alpha_2\beta_1$ heterodimer is formed under physiological conditions.

Here, we show the physiological existence of the $\alpha_2\beta_1$ heterodimer in human placenta. Although the α_1 and α_2 subunits share only 27 % identical amino acids in the N-terminal region, a comparison of the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ isoforms, expressed and purified from cells of the *Spodoptera frugiperda* ovary cell line Sf 9, did not reveal any considerable difference. Both isoforms contain equivalent amounts of haem, show comparable kinetic properties and exhibit the same sensitivity towards NO. As has been observed with the $\alpha_1\beta_1$ isoform, ODQ inhibited and YC-1 stimulated and sensitized the new enzyme towards NO and CO.

EXPERIMENTAL

RT-PCR analysis

The following primers were used in PCR: α_2 -subunit (GenBank accession number: X63282): α_2 -549, 5'-TGT ACA CCA GAT TTG ACC ACC AGT-3' and α_2 -680, 5'-ACG AGA CCG CGG AAT GAA TG-3', product 392 bp; α_{2i} -subunit (Z50053): α_{2i} -

Abbreviations used: DEA-NO, 2,2-diethyl-1-nitroso-oxyhydrazine sodium salt; DTT, D,L-dithiothreitol; ODQ, ³H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; RT-PCR, reverse transcription PCR; Sf 9, *Spodoptera frugiperda* ovary cell line; sGC, soluble guanylyl cyclase; TEA, triethanolamine; YC-1, 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole.

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619 5'-TTT TCT CCT TTC CTG TTT CCA TCC-3' and α_{21} -711 5'-ACG AGA CCG CGG AAT GAA TG-3', product 275 bp; α_1 -subunit (X54014): α_1 -146 5'-TGY TAY GAR GAR GAY GAR TAY AT-3' and α_1 -354 5'-TTY TTC ATISWR TTR TCC CA-3', product 647 bp; β_1 -subunit (Y00770): β_1 -1 5'-ATG TAY GGI TTY GTI AAY CAY GC-3' and β_1 -187 5'-TCY TCR TAR AAR TCY TCY TCY TT-3, product 581 bp; β_2 -subunit (M57507): β_2 -105 5'-GAY ATG AAY GAR GAR GTI GA-3' and β_2 -305 5'-GGI SWR CAC ATR AAI ATC ATR CA-3', product 623 bp. The primer designations refer to the corresponding location in the human α_2/α_{21} , bovine α_1 , bovine β_1 and rat β_2 protein sequences. PCRs were performed for 35 cycles (94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min) using the wax-mediated hot-start technique (Perkin-Elmer). cDNA library solution (10^6 – 10^7 plaque-forming units) was used as template.

Antisera

Antibodies directed against the C-terminal peptide of the α_1 -subunit (KKDVEEANANFLGKASGID), α_2 -subunit (KKVSYNIGTMFLRETSL) and β_1 -subunit (SRKNTGTETEEDEN) were obtained as described by Koesling et al. [12].

Coupling of the β_1 antibody

Serum of immunized rabbits (1.5 ml of serum per ml of Sepharose), adjusted to pH 8 with 0.1 M Tris buffer, was incubated with Protein A-Sepharose (fast flow, Pharmacia) for 1 h at room temperature using an overhead rotator. Subsequently, the Sepharose was washed in a column with 10 bed vols. of 0.1 M Tris, pH 8, 0.01 M Tris, pH 8, and 0.2 M sodium borate, pH 9. The Sepharose was resuspended in 10 bed vols. of 0.2 M sodium borate, pH 9, and covalent coupling of the antibodies was achieved by the addition of dimethylpimelimidate (final concentration 20 mM) and a 30 min incubation at room temperature. The reaction was stopped by washing the Sepharose with 0.2 M ethanolamine, pH 8.

Biotinylation of antibodies

For purification of IgG, antisera adjusted to pH 8 with 0.1 M Tris were applied to a Protein A column; after washing with 10 vols. of 100 mM Tris, pH 8, and 10 mM Tris, pH 8, antibodies were eluted with 100 mM glycine, pH 3, directly into vials containing one-tenth of the fraction volume of 1 M Tris, pH 8. Antibody-containing fractions were pooled and dialysed against polyethylene glycol (molecular mass = 35000). Biotinylation was carried out with 50 μ g of D-biotin-*N*-hydroxysuccinimide ester (Boehringer Mannheim) per mg of IgG for 3 h at ambient temperature. After the reaction, unbound D-biotin-*N*-hydroxysuccinimide ester was removed on a PD 10 column (Pharmacia).

Immunoprecipitation and immunoblot analysis of human placental cytosol

Human placentas obtained from normal deliveries were dissected in a food processor and homogenized in a buffer containing 75 mM NaCl, 2 mM D,L-dithiothreitol (DTT), 2 mM EDTA, 0.2 μ M benzamidine, 0.5 mM PMSF, 1 μ M pepstatin A and 50 mM triethanolamine (TEA)/HCl, pH 7.4. Cytosol preparation and anion-exchange chromatography were performed as described previously [5]. Precipitation of sGC activity was performed in a 500 μ l aliquot overnight at 4 °C, using an overhead rotator with final concentrations of 2 g/l protein of the sGC-containing fraction after anion-exchange chromatography, a 1:10 dilution of preimmune-serum or the respective antiserum

and 25 g/l Protein A-Sepharose CL-4B in buffer A containing 150 mM NaCl, 2 mM DTT, 2 mM EDTA, 0.2 μ M benzamidine and 50 mM TEA/HCl, pH 7.4. After overnight incubation, samples were centrifuged for 1 min in a tabletop centrifuge (2000 g) and the pellets were washed twice with 1 ml of buffer A. Enzyme activity was determined in the supernatant and the resuspended pellet. Immunoprecipitations for Western blot analysis were performed with an antibody covalently coupled to Protein A-Sepharose (see above). Precipitation was performed in an 8 ml aliquot with 400 μ l of antibody Protein A-Sepharose and 10 g/l protein of the sGC-containing fraction after anion-exchange chromatography. The precipitated proteins in the pellets were separated on 7.5% (w/v) SDS/PAGE gels, and immunoblot analysis was performed as described by Harteneck et al. [3], except that biotinylated antibodies were used. Detection was carried out with a streptavidin-peroxidase complex (Boehringer Mannheim) at a 1:1000 dilution.

Expression and purification of the recombinant heterodimers from Sf 9 cells

Recombinant subunits of sGC were cloned as described [11,13]. For purification of recombinant sGC, spinner cultures [TNM-FH insect culture medium (Sigma) supplemented with 10% (v/v) fetal calf serum] grown to 1.5×10^6 cells per ml were co-infected with the appropriate recombinant baculoviruses at a multiplicity of infection of 1 for each virus. The medium was supplemented with 4 mg/l haemin and additional 10% (v/v) fetal calf serum (final content 20%). Sixty-two hours after infection, the cells were collected by centrifugation, resuspended in 80 ml of buffer (2 mM DTT, 2 mM EDTA, 0.2 μ M benzamidine and 50 mM TEA/HCl, pH 7.4) and lysed by shear stress using a glass Teflon homogenizer. The homogenate was centrifuged at 200 000 g for 60 min at 4 °C, and purification of the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ heterodimers from the cytosol was performed as described [5] using antibody-coupled Sepharose prepared as described above. Protein concentrations were determined by the method of Bradford [14] with BSA as standard.

Determination of sGC activity

sGC activity of the purified proteins (approx. 0.1 μ g per assay tube) was determined by incubation for 10 min at 37 °C in the presence of 1 mM cGMP, 0.5 mM [α - 32 P]GTP (about 0.2 μ Ci), 3 mM MgCl₂, 0.5 g/l BSA and 50 mM TEA/HCl, pH 7.4, in a total volume of 0.1 ml. All experiments were performed in the presence of 3 mM DTT, with the exception of the ODQ experiment in which 3 mM GSH was used [7]. All but the CO experiments were started by the addition of 2,2-diethyl-1-nitrosooxyhydrazine sodium salt (DEA-NO; NCI Chemical Carcinogen Repository). For the CO experiments, solutions saturated with defined CO gases were prepared in gas-tight tubes with rubber stoppers at 37 °C and the reactions were started by addition of sGC with a syringe. All experiments were stopped by ZnCO₃ precipitation, and purification of the enzyme-formed cGMP was performed as previously described [15]. ODQ and YC-1 were dissolved in DMSO; the final concentration of DMSO in the samples did not exceed 2%, which does not affect sGC activity at all at this concentration. Results are presented as means $\pm$ S.D. of three independent experiments performed in duplicate.

Determinations of sGC activities in crude fractions were performed with the addition of 0.25 g/l creatine kinase, 5 mM creatine phosphate and 1 mM 3-isobutyl-1-methylxanthine.

Spectral studies

Approx. 10 μg of sGC in a maximum volume of 20 μl was injected onto a column (0.5 $\times$ 10 cm) of Sephadex G50 fine with a running buffer containing 175 mM NaCl and 50 mM TEA/HCl, pH 7.4 (flow rate 0.15 $\text{ml} \cdot \text{min}^{-1}$). Absorbance spectra were recorded with a photodiode array detector with an 8 μl sample cell (Waters 990, Millipore).

RESULTS

Existence of two sGC isoforms in human placenta

In placenta, mRNAs coding for the α_2 , α_1 and β_1 subunits were identified by RT-PCR analysis (Figure 1). Messenger RNAs for the alternatively spliced form of the α_2 or for the β_2 subunit were not detectable. Using anion-exchange chromatography, we enriched sGC from placental cytosol about 10-fold. Antibodies directed against the respective C-terminal region of the α_1 , α_2 and β_1 subunits, which have been shown to specifically precipitate enzyme activity, were used to investigate the presence of the different subunits in the sGC-enriched fraction. The anti- β_1

Figure 1 RT-PCR analysis of sGC subunits in placenta

RT-PCR was performed using primer pairs specific for the indicated sGC subunit and a cDNA library from placenta (10^5 – 10^7 plaque-forming units) as template. Products were separated on a 1% (w/v) agarose gel and stained with ethidium bromide.

Figure 2 Coprecipitation of the β_1 subunit with the α_1 and α_2 subunits in human placental cytosol

sGC, enriched from placental cytosol by anion-exchange chromatography, was immunoprecipitated with an antibody directed against the β_1 -subunit. The precipitated proteins were separated on a 7.5% (w/v) SDS/polyacrylamide gel. After transfer to nitrocellulose membranes, sGC α subunits were detected using antibodies against C-terminal peptides of the α_1 and α_2 subunits in the absence (—) and presence (+) of the respective antigenic peptide (0.1 g/l).

Figure 3 SDS/PAGE of sGC isoforms purified from Sf 9 cells

sGC isoforms were purified from Sf 9 cells by immuno-affinity chromatography (see Experimental section) and separated on a 7.5% (w/v) SDS/polyacrylamide gel (3 μg of the proteins per lane), and stained with Coomassie Blue. The molecular masses, corresponding to 70 kDa, 73 kDa and 82 kDa for the β_1 , α_1 , α_2 subunits respectively, of the indicated isoforms are shown. For comparison the enzyme purified from bovine lung ($\alpha_1\beta_1$) is shown.

antibody precipitated enzyme activity almost completely. In contrast, the anti- α antibodies precipitated only about two-thirds (anti- α_1 antibody) and one-third (anti- α_2 antibody) of enzyme activity. This is consistent with the notion that the β_1 subunit dimerizes with either subunit in human placenta. In order to confirm this hypothesis, we identified the α subunits dimerizing with the β_1 subunit in Western blots. sGC heterodimers were precipitated with the β_1 -antibody out of the sGC-enriched fraction. Subsequently, coprecipitated subunits were detected with specific antibodies against the α_1 and α_2 subunits as can be seen in Figure 2. A specific α_2 -immunoreactive protein with the appropriate molecular mass was identified in the β_1 -antibody precipitate. Preincubation of the antisera with the antigenic peptide abolished the signal. In accordance with the data on the precipitation of sGC activity, the anti- α_1 antibody produced a

Figure 4 Absorbance spectra of purified $\alpha_2\beta_1$ and $\alpha_1\beta_1$ isoforms

Absorbance spectra of 10 μg of purified $\alpha_2\beta_1$ (solid line) and $\alpha_1\beta_1$ (dotted line) enzymes were recorded in a photodiode array detector as described in the Experimental section.

Figure 5 Comparison of the NO sensitivity of the sGC heterodimers

Enzymic activities of the $\alpha_2\beta_1$ (■) and the $\alpha_1\beta_1$ (●) heterodimers were determined in the presence of increasing concentrations of the NO donor DEA-NO.

stronger signal. The data demonstrate the coexistence of the $\alpha_1\beta_1$ and the $\alpha_2\beta_1$ heterodimers in human placenta.

The novel $\alpha_2\beta_1$ isoform contains haem

In order to be able to perform functional analysis of the two isoforms, we expressed both heterodimers in Sf 9 cells. With the immunoaffinity purification procedure described in the Experimental section, the expressed isoforms were purified to apparent homogeneity as judged by SDS/PAGE analysis (Figure 3). Purification from approx. 1 g of cytosolic Sf 9 protein yielded between 1 and 2.5 mg of the isolated enzymes with an approx. 250-fold enrichment of specific activity for either heterodimer. As the $\alpha_1\beta_1$ heterodimer has been shown to contain a prosthetic haem group, we analysed the haem content of both enzymes by UV/VIS spectroscopy (Figure 4). Both heterodimers displayed a peak at 431 nm (Soret band), indicating a pentacoordinate ferrous haem group with a histidine as the axial ligand. Thus, although the α_2 subunit differs considerably from the α_1 subunit in the proposed haem-binding region [16], the haem-binding properties of the two heterodimers appear to be identical.

Functional properties of the isoforms

Under non-stimulated conditions, enzyme activities of both isoforms were comparable ($60 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein). Both isoforms were stimulated up to 300-fold by the NO donor DEA-NO ($10 \mu\text{M}$), yielding maximal catalytic rates of around $18 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$, and exhibited similar EC_{50} values ($690 \pm 110 \text{ nM}$ and $510 \pm 92 \text{ nM}$ NO for the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ heterodimers respectively). The results indicated equally high affinities for the activator NO (Figure 5). Moreover, both enzymes revealed a similar substrate optimum with K_m values of $90 \pm 18 \mu\text{M}$ for $\alpha_1\beta_1$ and $106 \pm 24 \mu\text{M}$ for $\alpha_2\beta_1$ under non-stimulated conditions, and a comparable shift to lower MgGTP concentrations upon activation with DEA-NO ($14 \pm 4 \mu\text{M}$ and $24 \pm 5 \mu\text{M}$ respectively) (results not shown). The results obtained are in good agreement with published data for the $\alpha_1\beta_1$ heterodimer [17].

We investigated the sensitivity of the $\alpha_2\beta_1$ heterodimer to ODQ, a new specific inhibitor of sGC activity [7]. As shown in

Figure 6 Inhibition of NO-stimulated sGC activity by ODQ

NO-stimulated activities (100 nM DEA-NO) of $\alpha_2\beta_1$ (■) and $\alpha_1\beta_1$ (●) isoforms were determined with different concentrations of the inhibitor of the NO-stimulated sGC activity, ODQ.

Figure 7 Effect of YC-1 on NO-stimulated activity of the $\alpha_2\beta_1$ heterodimer

DEA-NO concentration–response curves for the $\alpha_2\beta_1$ heterodimer were obtained in the absence (□) and presence (■) of $200 \mu\text{M}$ YC-1.

Figure 6, ODQ inhibited the stimulated activity (100 nM DEA-NO) of both isoforms with similar sensitivities.

Recently, we have shown that the substance YC-1 stimulates the $\alpha_1\beta_1$ enzyme from bovine lung about 10-fold, potentiates the NO-induced stimulation and turns CO into an effective activator of the enzyme [8]. Analogous experiments revealed that YC-1 stimulated the $\alpha_2\beta_1$ heterodimer about 17-fold, led to an 18% increase in maximal NO-induced activity, and shifted the concentration–response curve for NO to the left (EC_{50} 340 nM versus 50 nM DEA-NO) (Figure 7). In order to investigate the effect of YC-1 on the CO-induced stimulation, we prepared solutions of the indicated concentrations using gases with defined CO contents. In the presence of YC-1, saturating concentrations of CO ($860 \mu\text{M}$) led to maximal activation of both isoforms (250 ± 29 -fold and 190 ± 19 -fold for the $\alpha_1\beta_1$ and $\alpha_2\beta_1$

Figure 8 CO-induced stimulation of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ isoforms in the presence of YC-1

Enzymic activities of the isoforms were determined in solutions saturated with CO gases (10, 100, 1×10^3 , 1×10^4 , 1×10^5 p.p.m. CO in N_2 and 100% CO) in the absence ($\alpha_2\beta_1$ □, $\alpha_1\beta_1$ ○) and presence ($\alpha_2\beta_1$ ■, $\alpha_1\beta_1$ ●) of 200 μ M YC-1.

isoforms respectively) (Figure 8). Even at a 10-fold lower concentration (87 μ M), CO already yielded an approx. 200-fold increase in activity (220 ± 24 -fold, 160 ± 18 -fold for the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ isoforms respectively). Half-maximal effects were observed at 20 μ M CO.

DISCUSSION

In this report, we show for the first time the physiological existence of the α_2 subunit of sGC on the protein level and its dimerization with the β_1 subunit *in vivo*. The resulting novel isoform $\alpha_2\beta_1$ contains enzyme-bound haem with the same absorbance characteristics as the $\alpha_1\beta_1$ heterodimer. Regarding kinetic properties and responsiveness towards NO, both isoforms were indistinguishable. ODQ, the specific inhibitor of the NO-stimulated activity of the $\alpha_1\beta_1$ isoform, also decreased the activity of the new heterodimer, requiring about the same concentration for half-maximal inhibition. As has been shown for the $\alpha_1\beta_1$ heterodimer, the new isoform was stimulated by YC-1 and exhibited increased sensitivity towards NO. Moreover, in the presence of YC-1, the $\alpha_2\beta_1$ heterodimer was stimulated as much as 200-fold by CO, the EC_{50} for CO in the presence of YC-1 being around 20 μ M for both heterodimers.

There is an overall similarity of about 48% identical amino acids shared by the α_1 and α_2 subunits. With 87% identical amino acids the similarity is most pronounced in the C-terminal catalytic domains and the central parts of the subunits which are probably involved in dimerization. In contrast, the N-terminal regions are less well conserved (27% identical amino acids) [9]. Deletion mutants revealed that the N-terminal regions of the α_1 and β_1 subunits are involved in haem binding [16], and although the α_2 subunit differs considerably from the α_1 subunit in this haem-binding region, the spectral studies and the comparable responsiveness to NO suggest similar binding and coordination of the prosthetic haem group in both isoforms. Either the residues critical for haem binding and coordination are conserved within the α subunits, or, alternatively, residues located on the β_1 subunit may determine the spectral properties of the prosthetic haem group. Indeed, histidine 105 of the β_1 subunit has been

identified as the possible haem-coordinating residue, as point-mutation of this amino acid led to an NO-insensitive, haem-deficient enzyme [13]. Moreover, an enzyme with a truncated α_1 subunit (131 N-terminal amino acids), although greatly reduced in haem-binding capacity, retained some responsiveness towards NO, indicating correct haem coordination, whereas deletion of the 64 N-terminal amino acids of the β_1 subunit abolished NO sensitivity [16,18].

The amount of sGC in human placenta is comparable to that of bovine lung. The enzyme has been purified from placenta [19]. The reported molecular mass of the purified enzyme on SDS/PAGE is compatible with that of the $\alpha_2\beta_1$ heterodimer, although the other results obtained in that study do not match the characteristics of our enzyme. In addition to the two different isoforms of sGC described in the present study, the endothelial NO synthase and cGMP-dependent protein kinase type I β have been shown to exist in human placenta. Thus, the NO-cGMP-dependent signalling cascade may have an important function in the maintenance of the low vascular tone in feto-placental vessels [20–22].

In summary, we have shown the existence of a new isoform of sGC in human placenta. As a comparison of the two isoforms does not reveal any functional differences, the physiological relevance of these isoforms remains unknown, although post-translational modification or different sensitivity towards so far unknown modulators cannot be excluded. On the other hand, expression under the transcriptional control of cell-type-specific or developmental promoters may be a plausible explanation for the existence of two functionally similar isoforms and should be the subject of further studies.

This work was supported by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie. We are indebted to Jürgen Malkewitz for excellent technical assistance. We thank Dr. Günther Schultz and Dr. Andreas Friebe for critical reading of the manuscript and helpful discussions.

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Received 12 May 1998/6 July 1998; accepted 27 July 1998