Human soluble guanylate cyclase: functional expression and revised isoenzyme family

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Soluble guanylate cyclase (sGC), a heterodimeric (α/β) haem protein that converts GTP to cGMP, functions as the receptor for nitric oxide (NO) and nitrovasodilator drugs. Three distinct cDNA species of each subunit (α1–α3, β1–β3) have been reported from various species. From human sources, none of these have been expressed as functionally active enzyme. Here we describe the expression of human α/β heterodimeric sGC in SF9 cells yielding active recombinant enzyme that was stimulated by the nitrovasodilator sodium nitroprusside or the NO-independent activator 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1). At the protein level, both α and β subunits were detected in human tissues, suggesting co-expression also in vivo. Moreover, resequencing of the human cDNA clones [originally termed α3 and β3; Giuili, Scholl, Bulle and Guellaen (1992) FEBS Lett. 304, 83–88] revealed several sequencing errors in human α3; correction of these eliminated major regions of divergence from rat and bovine α1. As human β3 also displays more than 98% similarity to rat and bovine β1 at the amino acid level, α3 and β3 represent the human homologues of rat and bovine α1 and β1, and the isoenzyme family is decreased to two isoforms for each subunit (α1, α2; β1, β2). Having access to the human key enzyme of NO signalling will now permit the study of novel sGC-modulating compounds with therapeutic potential.

INTRODUCTION

Guanylate cyclases catalyse the conversion of GTP to cGMP in response to various extracellular stimuli, thereby providing an important second messenger for the regulation of protein kinases, phosphodiesterases and ion channels [1,2]. Soluble guanylate cyclase (sGC) is the main receptor for vasodilating nitric oxide (NO)-releasing compounds (nitrovasodilators), which have been used for over 100 years in the treatment of coronary heart disease and angina pectoris. Since the discovery of endogenous, L-arginine-derived NO as an intercellular second messenger [3,4], it became clear that sGC is the physiological receptor for this important signalling molecule [5,6] and presumably the most relevant molecular target for NO-releasing drugs in human cardiovascular therapy [7].

sGC has been purified from various tissues as a heterodimeric haem protein composed of a larger α and a smaller β subunit [8–10]. One [11] or two [12] prosthetic haem groups are essential for stimulation of sGC by NO, and function as its molecular receptor [13–15]. Corresponding α and β cDNA clones have been isolated from rat and bovine lung and brain cDNA libraries and have been designated α1 and β1 [16–19]. Moreover the existence of additional isoforms has been reported: (1) α2 cDNA was isolated from human fetal brain [20], (2) α2i, an alternatively spliced variant of α2, has been detected in several cell lines and tissues at the mRNA level [21], and (3) a β2 cDNA clone was isolated from rat kidney [22]. Because heterodimerization of α and β subunits is essential for basal and stimulated enzyme activity [17,23], the existence of multiple α and β isoforms implies regulatory mechanisms for sGC activity in living cells via alternative heterodimerization. In fact, the human α2 isoform is active when co-expressed with bovine β1 in mammalian cells [20], whereas α2i is inactive under the same conditions [21]. In a mammalian expression system, rat α1 and β2 form an active heterodimer; however, this is less sensitive than the α1/β1 heterodimer to NO [24].

However, human sGC has neither been purified from native tissue sources nor been expressed as functional recombinant enzyme (Table 1). Moreover, no human homologues of rat and bovine α1/β1 and rat β2 have been detected, and the physiological complementary subunit(s) of the human α2 and α2i isoforms are unknown. Instead, additional cDNA clones coding for further sGC isoforms were isolated from an adult human brain library and termed α3 and β3 [25]. Thus the sGC family seems to comprise at least six isoenzymes (α1–α3, β1–β3). In the present study we expressed and characterized for the first time α3 and β3.

Table 1 Present terminology of human NO receptor cyclase cDNA species

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Subunit . . . α</th>
<th>β</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Not detected</td>
<td>β1*</td>
</tr>
<tr>
<td>2</td>
<td>cDNA; active when co-expressed with bovine β1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Not detected</td>
<td>cDNA†</td>
</tr>
<tr>
<td>† Reference [20].</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Reference [20].</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: IBMX, 3-isobutyl-1-methylxanthine; rhsGC, recombinant human sGC; sGC, soluble guanylate cyclase; SNP, sodium nitroprusside; YC-1, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole; KRB, Krebs/Ringer buffer.

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The nucleotide sequence data reported for the human soluble guanylate cyclase α1 subunit (new terminology) will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession no. Y15723.
functional heterodimeric human sGC, composed of α1 and β1, as an NO receptor, and revised the isoenzyme family of sGC α and β subunits.

**EXPERIMENTAL**

**Reagents**

All chemicals were obtained from Sigma (Deisenhofen, Germany) unless stated otherwise. [α-32P]GTP (1.48 x 10^{13} Bq/mmol) was obtained from Amersham. For the sGC assay, sodium nitroprusside (SNP) and 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1) were prepared as 10-fold concentrated stock solutions in 1 mM sodium acetate, pH 5.0, and 10% (v/v) DMSO respectively. The saturated CO solution (1 mM at 20 °C) was prepared by bubbling water with CO gas (99.97% purity; Linde, Unterschleisheim, Germany) for 30 min. For cGMP determination in Sf9 cells, SNP and YC-1 were prepared as 100-fold concentrated stock solutions in 40 mM sodium acetate buffer, pH 5.0, and DMSO respectively. sGCx3 and β3 cDNA species were provided by G. Guellaen [25].

**Baculovirus construction**

cDNA species, originally designated α3 and β3 cDNA species [25] but later renamed α1 and β1 (see the Results section), comprising the complete coding and 270 bp (α1) and 500 bp (β1) of 3′ non-coding sequences, and lacking 5′ untranslated regions, were cloned into the pVL1393 baculovirus transfer vector (Pharmergen, San Diego, CA, U.S.A.). For this purpose, BamHI sites (underlined in the sequences below) were introduced by PCR immediately upstream of the translational start sites. Fragments were amplified with the primer pairs for α1 (nucleotide numbering in accordance with the corrected α1 cDNA sequence; see the Results section), 5′-AAAGCTTCAATGTCCTGACG-CAGGCG-3′ (nt 524-541) and 5′-ATTATGGAAGCCGGA-GG-3′ (nt 1249-1232), and for β1, 5′-AAAGATCCATTGCTGATC-CAGGCTC-3′ (nt 89-106) and 5′-ATCGTGATTTCTTGTA-3′ (nt 711-692). Products were cut with BamHI/BsaAI (α1) or BamHI/KpnI (β1) and ligated with BsaAI/EcoRI (1193-3015, α1) or KpnI/EcoRI (692-2441, β1) cDNA fragments to the BamHI/EcoRI-cleaved vector. Recombinant baculoviruses, obtained by co-transfection of transfer vectors and BaculoGold DNA (Pharmergen), were plaque-purified from the culture medium. For preparation of high-titre virus stocks, Sf9 cells were spinner-cultured to a density of 2.5 ml, infected at a multiplicity of infection of 0.1 plaque-forming unit per cell, and harvested 6 days after infection.

**Sf9 cell culture and production of recombinant human sGC (rhsGC)**

Sf9 cells were cultured in IPL-41 medium supplemented with 10% (v/v) fetal calf serum, 4% (w/v) tryptose phosphate broth, 1% (v/v) Pluronic F68, 0.5 %, Amphotericin B (all Gibco BRL), and 80 µg/ml gentamycin sulphate (Merck) at 27 °C. Monolayer cultures (2.5 x 10^{10} cells per Petri dish, 90 mm in diameter) or spinner cultures (2 x 10^6 cells/ml) were infected with hsGCα1 and/or hsGCβ1 viruses at a multiplicity of infection of 1–2 plaque-forming units per cell and cultured at 27 °C. Cells were harvested 72 h after infection, when protein production, basal and NO-stimulated enzyme activity were maximal (results not shown). All subsequent procedures were performed at 4 °C. Cells were homogenized by sonication in lysis buffer [25 mM triethanolamine (pH 7.8)/50 mM NaCl/1 mM EDTA/5 mM dithiothreitol/1 mM leupeptin/0.5 mg/ml soybean trypsin inhibitor]. Crude supernatant and particulate fractions were separated by centrifugation (20000 g) for 15 min at 4 °C; the pellet was resuspended in an equal volume of lysis buffer. Aliquots were subjected to SDS/PAGE [26] followed by Coomassie Blue protein staining or protein immunoblot (see below). For storage, fractions were brought to a final concentration of 50% (v/v) glycerol and kept at −20 °C. Protein concentrations were determined by the method of Bradford [27].

**sGC activity assay**

sGC activity in crude soluble fractions of Sf9 cells was measured at 37 °C for 10 min by the conversion of [α-32P]GTP to [32P]cGMP as described [28], in a total volume of 100 µl, containing 50 mM triethanolamine/HCl, pH 7.4, 3 mM MgCl₂, 3 mM dithiothreitol, 1 mM 3-isobutyl-1-methylxanthine (IBMX), 1 mM cGMP, 5 mM creatine phosphate, 0.25 mg/ml creatine kinase and 500 µM GTP. Reactions were started by the simultaneous addition of the enzyme-containing Sf9 fraction and the sGC activators SNP, CO or YC-1. [32P]GMP was separated and measured as described [28].

**cGMP formation in intact Sf9 cells**

Sf9 monolayer cultures (see above) were co-infected with hsGCα1 and hsGCβ1 viruses and cultured for 48 h. Medium was replaced by 10 ml Krebs/Ringer buffer [KRB: 119 mM NaCl/4.74 mM KCl/2.54 mM CaCl₂/1.19 mM MgSO₄/1.19 mM KH₂PO₄/25 mM NaHCO₃/0.1 µg/ml gentamycin sulphate (Merck)] containing 1 mM IBMX, and cells were incubated for 1 h at 27 °C. For measurement of cGMP, stock solutions of SNP or YC-1 were added to the KRB. After 2 min at room temperature, cells were washed with ice-cold KRB and harvested. The cGMP content of ethanol-extracted cells was determined with a radioimmunoassay kit (Biotech, Köln, Germany), in accordance with the manufacturer’s protocol.

**DNA sequencing and amino acid sequence analysis**

PCR products and the original sGC cDNA clones were sequenced in an ABI1200 DNA sequencer (Applied Biosystems). The deduced amino acid sequences were aligned by using the Clustal method (Lasergene Multiple Sequence Alignment; DNASTAR, Madison, WI, U.S.A.).

**Generation of antisera**

Primary antisera were generated by the immunization of rabbits against unique synthetic peptides corresponding to human α1 (Phe-Thr-Pro-Arg-Ser-Arg-Glu-Leu-Pro-Pro-Asn-Phe-Pro, residues 634–647) and β1 (Lys-Gly-Lys-Lys-Glu-Pro-Met-Gln-Lys-Glu-Glu-Leu-Pro-Pro-Asn-Phe-Pro, residues 593–614) sequences, coupled to keyhole limpet hemocyanin via an additional C-terminal (α1) or N-terminal (β1) cysteine residue. Antisera were affinity-purified against the respective peptides coupled to epoxy-activated Sepharose (Pharmacia, Freiburg, Germany) in accordance with the manufacturer’s protocol.

**Immunodetection of sGC in human tissues**

Human lung tissue was obtained from the tumour-free area of lung resections; human cortex and cerebellum were from a normal autopsy procedure. All tissues were immediately frozen in liquid nitrogen and kept at −70 °C. For protein extraction, the frozen tissue was crushed in a liquid-nitrogen-cooled stainless steel mortar. The resulting powder was resuspended in 2 x concentrated Laemmli buffer [26] preheated to 95 °C, incubated
for 10 min at 95 °C, centrifuged for 20 min at 20000 g, and the supernatant was subjected to SDS/PAGE [8 % or 10 % (w/v) gel] [26]. Proteins were transferred to Hybond ECL nitrocellulose membranes (Amersham) in 48 mM Tris base gel [26]. Proteins were transferred to Hybond ECL nitrocellulose supernatant was subjected to SDS-PAGE [8].

Statistics

Results are expressed as means ± S.E.M. for at least n = 3 experiments. Values of P < 0.05 (analysis of variance) were considered statistically significant.

RESULTS

sGC α3 and β3 are the human homologues of rat and bovine sGC α1 and β1

In a homology-based screening for human sGC subunits, Giuili et al. [25] isolated α3 and β3 cDNA clones from a human brain cDNA library derived from the frontal lobe. Whereas human β3 is almost identical with bovine and rat β1 at the amino acid level, human α3 contains two main areas of divergence (designated S1 and S2; see Figure 1A) from rat and bovine α1. Because α3 and β3 have not been studied at the protein level, it is unknown whether they are physiologically co-expressed and constitute active heterodimeric enzyme. To address this question we performed PCR experiments to study sGC expression in human tissues (results not shown). However, we were unable to amplify α3-specific fragments from different human cDNA libraries. All fragments obtained with α-specific primers showed consistently identical discrepancies from the published α3 sequence (results not shown). Therefore either α3 was not expressed and a novel subunit had been detected, or the α3 sequence is genetically polymorphic, or the published sequence contained sequencing errors. Accordingly we re-sequenced the original α3 and β3 cDNA clones, kindly provided by the authors, and found 19 incorrect sites within the α3 sequence. The reported β3 cDNA sequence was correct. Sequence correction of the α3 subunit led to several frame shifts in S1 and S2, resulting in predicted peptide sequences highly similar to the corresponding sequences in rat and bovine α1 (Figure 1B). In addition, a further small stretch of divergence (residues 526–532 of α3, corresponding to residues 529–535 in α1 terminology; see below) disappeared after correction, and an additional glutamic residue was inserted (between Glu-319 and Tyr-320 of α3, corresponding to Glu-322 in α1 terminology; see below), which is also found in rat and bovine α1 (results not shown). A small N-terminal stretch of decreased similarity between human α3 and rat/bovine α1 (residues 27–60) is also less conserved between rat and bovine α1 (results not shown).

![Figure 1](image-url)

**Expression of human soluble guanylate cyclase**

(A) Domain organization of α isoforms (reviewed in [53]). A putative C-terminal catalytic cyclase domain (hatched) is shared by the sGC α1 and β1 subunits, particulate guanylate cyclases and adenylate cyclase [54]. A central sGC homology region is common to sGC α1 and β1 subunits, whereas the N-termini are unique and essential for NO activation [29]. S1 (residues 121–187) and S2 (residues 670–717) are amino acid sequences unique to α3, with no similarity with the α1 isoforms given in [25]. (B) Correction of α3 cDNA sequence eliminates S1 and S2 regions of divergence. Alignments of deduced amino acid sequences in human α3 S1 (top) and S2 (bottom) with the corresponding sequences after cDNA sequence correction (now termed α3), and with rat [17] and bovine α1 [19] are shown. Conserved amino acids are shaded.
and 99% similarity to rat/bovine α/β, and therefore represents its human homologue. hsGC1 and hsGC/β1 (human soluble GCα1 and β1) is suggested as the new nomenclature for the former α3 and β3 subunits.

Characterization of rhsGC

To examine whether the human α1 and β1 subunits constitute enzymatically active heterodimeric sGC, we expressed both subunits in Sf9 insect cells by using the baculovirus expression system. As revealed by SDS/PAGE of fractionated Sf9 cell extracts (Figure 2A), recombinant α1 and β1 proteins migrated with apparent molecular masses of 79.5 and 68.5 kDa respectively, which is close to the molecular masses predicted from the respective cDNA species (77.5 and 70.5 kDa). The identities of the respective bands were confirmed by immunoblotting with subunit-specific antisera raised against peptide sequences near the C-termini of hsGCα1 and hsGC/β1 subunits (lanes 1–4); subsequently the same blot was re-analysed with anti-hsGCβ1 polyclonal antiserum without stripping the α1-specific antibody (lanes 5–8). The asterisk indicates a degradation product of α1.

Table 2 rhsGC activity in Sf9 cellular extracts

<table>
<thead>
<tr>
<th>Conditions</th>
<th>rhsGC activity</th>
<th>Stimulation factor</th>
</tr>
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<tbody>
<tr>
<td>Basal</td>
<td>1487±139</td>
<td></td>
</tr>
<tr>
<td>Basal+100 µM SNP</td>
<td>7966±1314</td>
<td>5.4</td>
</tr>
<tr>
<td>Basal+100 µM YC-1</td>
<td>5522±1251</td>
<td>3.7</td>
</tr>
<tr>
<td>Basal</td>
<td>607±50</td>
<td></td>
</tr>
<tr>
<td>Basal+100 µM SNP</td>
<td>6099±1077</td>
<td>10.0</td>
</tr>
<tr>
<td>Basal</td>
<td>1361±255</td>
<td></td>
</tr>
<tr>
<td>Basal+100 µM SNP</td>
<td>2974±8747</td>
<td>21.8</td>
</tr>
</tbody>
</table>

Figure 2 Expression and subcellular distribution of rhsGC α1 and β1 subunits in Sf9 cells

(A) Sf9 monolayer cultures were either non-infected controls (Co) or infected with hsGCα1 or hsGC/β1 baculoviruses, and then cultured for 72 h. The 20000 g soluble (S) and particulate (P) fractions of the cell homogenates were analysed by SDS/PAGE [10% (w/v) gel] and Coomassie Blue protein staining. Large amounts of the recombinant polypeptides were found in the pellet fraction. (B) Sf9 monolayer cultures were either non-infected controls (Co) or co-infected with hsGCα1 and hsGC/β1 baculoviruses. S and P fractions (see [A]) were analysed by protein immunoblotting. Proteins were detected first with anti-hsGCβ1 polyclonal antiserum (lanes 1–4); subsequently the same blot was re-analysed with anti-hsGCα1 polyclonal antiserum without stripping the β1-specific antibody (lanes 5–8). The asterisk indicates a degradation product of α1.

Figure 3 Effects of SNP and YC-1 on rhsGC in intact Sf9 cells

Sf9 monolayers co-infected with hsGCα1 and hsGC/β1 baculoviruses were cultured for 48 h and treated for 2 min with 10, 100 or 1000 µM SNP (columns 2–4), 10 or 100 µM YC-1 (columns 5 and 6) or 10 or 100 µM YC-1 in the presence of 100 µM SNP (columns 7 and 8), or were left untreated (column 1) in the presence of the phosphodiesterase inhibitor IBMX. The total content of cGMP was determined by radioimmunoassay. Results are means ± S.E.M. for a representative experiment performed in triplicate.

showed). Taken together, correction of the erroneous α3 cDNA sequence eliminated all coherent stretches of divergence at the amino acid level between human α3 and rat/bovine α1. Therefore we suggest a novel isofrom classification for α3 as the human homologue of rat and bovine α1. Likewise, β3 displays 98.5%
Expression of human soluble guanylate cyclase

Figure 4  Effect of YC-1 on NO- and CO-stimulated rhsGC in Sf9 cellular extracts

Sf9 spinner cultures co-infected with hsGC\(\alpha_1\) and hsGC\(\beta_1\) baculoviruses were harvested 72 h after infection. sGC activity of the soluble fraction was assayed in the absence (control) or presence of 100\(\mu\)M YC-1, 100\(\mu\)M SNP, 100\(\mu\)M SNP plus 100\(\mu\)M YC-1, 200\(\mu\)M CO, or 200\(\mu\)M CO plus 100\(\mu\)M YC-1. Results are means±S.E.M. for at least four independent experiments. *Significant difference (\(P<0.05\)) from control reaction; **significant difference (\(P<0.05\)) from 100\(\mu\)M YC-1.

The measured sGC activity. This could be due to misfolding of the protein in insect cells or lack of essential modifications, or could reflect a loss of haem because of suboptimal lysis conditions.

NO-induced sGC activation causes enzyme desensitization or, with nitrovasodilators, tolerance. With YC-1 there is now a promising lead compound to influence sGC activity independently of NO. YC-1 was first described as a specific NO-independent activator of sGC in rabbit platelets [30,33], and also potentiates the activation of purified bovine sGC by NO [32,34]. Here we investigated whether human \(\alpha_1/\beta_1\) heterodimer is also a target of YC-1. Indeed, in Sf9 cellular extracts, rhsGC activity was stimulated up to 3.7-fold by 100\(\mu\)M YC-1 (Table 2). Similar stimulation factors have been reported for rat and human platelets [30,33], which might also contain \(\alpha_1/\beta_1\) heterodimers, and purified bovine sGC [32,34]. The YC-1 effect was concentration-dependent with an \(EC_{50}\) of 20\(\mu\)M (results not shown). Moreover YC-1 also stimulated NO-activated rhsGC (Figure 4). It is noteworthy that, in intact cells, 10\(\mu\)M YC-1 was sufficient to reach maximum effects on basal and NO-stimulated sGC activity (Figure 3), compared with 100\(\mu\)M in cellular extracts. Thus the activation of sGC by YC-1 in vivo might involve metabolic potentiation. It has been reported that CO, a putative physiological activator of sGC [35], and YC-1 also act synergistically on sGC [34]. However, CO (200\(\mu\)M) had no effect on rhsGC, and the activation by the combination of 200\(\mu\)M CO and 100\(\mu\)M YC-1 was not significantly more effective than by YC-1 alone (Figure 4).

Detection of sGC \(\alpha_1\) and \(\beta_1\) in human tissues

To investigate the distribution of \(\alpha_1\) and \(\beta_1\) in human tissues, we performed protein immunoblots with antibodies against synthetic \(\alpha_1\) and \(\beta_1\) peptides (Figure 5). SGC immunoreactive polypeptides were detected only in brain and lung. Neither \(\alpha_1\) nor \(\beta_1\) polypeptides were found in kidney, liver and pancreas under our conditions (results not shown). The detected polypeptides co-migrated with the respective subunits expressed in insect cells, indicating no major modification differences between the native and recombinant enzyme subunits. Subunit \(\alpha_1\) was found mainly in cortex and lung; a small amount was also detected in cerebellum (Figure 5A, filled arrowhead). Some faster-migrating faint bands detected in cortex and cerebellum competed fully with the corresponding peptide and might represent degradation.
products of α1. Interestingly, a high-molecular-mass protein (more than 150 kDa) was specifically detected by anti-hsGCα1 in human cortex (arrow in Figure 5A). It is as yet unknown whether this polypeptide has an accidental overlap with the hsGCα1 peptide sequence, or whether it represents an sGC-related protein. Specific signals for β1 were detected in human cortex, cerebellum and lung (Figure 5B, open arrowhead). Taken together, these results indicate that both human α1 and β1 proteins are expressed in vivo, occur together in the same organs and therefore might constitute the active NO receptor in humans.

DISCUSSION

The NO-cGMP signalling pathway, with sGC as the central enzyme, has an important role in maintaining blood pressure, inhibiting platelet adhesion and aggregation, modulating neuronal transmission and many other physiological processes [36]. Therefore sGC is an important target protein for therapeutic drugs such as nitrovasodilators [37,38] or new antithrombotic agents [39]. In this paper we present for the first time the expression and initial characterization of an active rhsGC and the detection of the corresponding sGC polypeptides in human tissues. Furthermore we found that the sGC subunits previously described as α3 and β3 in fact represent the human homologues of rat and bovine α1 and β1. Taken together with rhsGC activity and human sGC expression in vivo, this implies that the human α1/β1 heterodimer is also the functional homologue of rat or bovine sGC. The resulting new terminology of the revised isoenzyme family is depicted in Table 3.

The enzyme activity of expressed rhsGC is sensitive to SNP and the NO-independent activator YC-1, as reported for purified bovine sGC [32,34]. However, the responsiveness of rhsGC to NO seemed to decline on the lysis of cells, as intracellular cGMP levels of rhsGC-expressing cells increased markedly in response to SNP, whereas the stimulation factors observed in cellular extracts were only moderate (up to 20-fold). This suggests that either NO sensitivity declined and/or basal rhsGC activity increased on extract preparation, which also seemed to contribute to the observed variation in stimulation factors. Loss of NO sensitivity could be due to loss of haem, which has been proposed to increase basal sGC activity as well [40]. Alternatively, an increase in basal activity could be due to autoactivation of sGC, the exact mechanism of which is unknown but seems to involve redox processes [41,42].

Previous studies have shown that transcription of sGC subunits and presence of mRNA is regulated in a manner specific to cell type [43–45] and development [46], and influenced by extracellular and intracellular messenger molecules [47,48]. In contrast, it was reported that rat α1 and β1 as well as human α2 mRNA species are widely expressed in different tissues [17,21]. Here we show that large amounts of α1 and β1 subunits were restricted to only a few of the human tissues so far analysed. Human sGC was found in cortex and cerebellum, consistent with the role of NO and possibly CO in neurotransmission [35,49], and also in lung, a prominent source of native sGC in other species [8,10]. Interestingly, the relative concentrations of α1 were high in lung and low in cerebellum, whereas much more β1 was found in cerebellum than in lung, suggesting that complementary sGC subunits are not necessarily expressed in a 1:1 ratio. However, the physiological significance of this finding remains to be elucidated.

It would be of great therapeutic interest to have a non-NO-releasing activator of sGC, because all known nitrovasodilators are prone to tolerance and cross-tolerance to other nitrovasodilators [50,51]. YC-1 is a good candidate for a lead substance for such drugs. Here we show that YC-1 is also an activator of human sGC and increased the effectiveness of submaximally stimulating concentrations of NO. Therefore YC-1 or related substances might be able to potentiate the effects of NO-releasing nitrovasodilators or, more importantly, of endogenous endothelium-derived NO. At present there is also discussion of whether CO is a physiological ligand of sGC [13,15,52]. Because YC-1 was reported to potentiate the activation of sGC by CO, it was proposed that a putative endogenous YC-1-like molecule or sGC binding protein might enhance weak effects of CO in vivo [34]. However, we were unable to stimulate rhsGC by CO, and activation by the combination of YC-1 and CO was not significantly more effective than by YC-1 alone. This might be explained by the overall lower responsiveness of the rhsGC preparation used here, compared with the bovine enzyme analysed by Friebe et al. [34]. Importantly, our findings show that the pharmacological principle of YC-1 is fully transferable to human sGC. However, the mechanism of action of YC-1 is not understood. Although YC-1 seems to act directly on sGC, we found that in rhsGC-expressing S9 cells, YC-1 was 10-fold more potent than in cellular extracts, suggesting that additional factors or metabolic events might be involved when YC-1 elevates the cGMP concentration in living cells.

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