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Molecular cloning of the cDNAs coding for the two subunits of soluble guanylyl cyclase from human brain

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Complementary DNA clones corresponding to the 70 and 82 kDa subunits of soluble guanylyl cyclase from human adult brain have been isolated and sequenced. Their respective open reading frames correspond to 619 amino acids (M_r 70,469) and 717 amino acids (M_r 81,324). Southern blots of human genomic DNA using these clones as probes give patterns which might be compatible with the presence of more than one copy per gene, or pseudogenes, for each subunit in the human genome. Comparison of the protein sequence of the large subunit from adult brain with the subunit cloned from human fetal brain (Harteneck, C., Wedel, B., Koesling, D., Malekewitz, J., Böhme, E. and Schultz, G. (1991) FEBS Lett. 292, 217–222) revealed only 34% homology. This result demonstrates the existence of a novel large subunit isoform for soluble guanylyl cyclase in human tissues.

Soluble guanylate cyclase; Human brain; cDNA library

1. INTRODUCTION

Cyclic GMP is formed from GTP by guanylyl cyclases which exist in both membrane-bound and soluble forms (GC-S) [1]. The membranous form is a single protein which is the receptor for a family of natriuretic peptides. The GC-S is composed of a large (α) and a small (β) subunit and contains a heme binding site. This soluble form can be activated by nitric oxide (NO) [2], some porphyrins [3], fatty acid hydroperoxides [4], prostaglandin endoperoxides [5] and dehydroascorbate [6].

The β-subunit has been cloned from rat [7] and bovine [8] lung, and an other isoform has been isolated from rat kidney [9]. In human only the partial sequence for two β-subunits of lung GC-S has been published [10]. These two β-subunits result from alternative splicing and are related to the isoforms isolated from rat and bovine lung. The α-subunit has also been cloned from bovine [11] and rat [12] lung; more recently another isoform has been isolated from human fetal brain [13].

In this article, we report the isolation and nucleotide sequence of two cDNA clones coding for the 70 and 82 kDa subunits of GC-S from human adult brain. This is the first characterization of a complete β-subunit of human GC-S, as well as the identification of a novel human GC-Sα related to the α-subunits, isolated from bovine and rat lung [11,12].

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2. MATERIALS AND METHODS

2.1. *cDNA construction and screening*

A λ-gt 10 cDNA library (containing 3×10^6 unique recombinants), derived from mRNA purified from the human frontal lobe, was kindly provided by Dr. Yves Le Bouc (INSERM U142, Paris) with the approval of Transgene SA. The library was analyzed as already described [14] using the coding region of the cDNAs corresponding to the 70 and 82 kDa subunit of rat lung GC-S [7] as probes. The inserts of the positive clones, selected under stringent conditions, were subcloned into PGEM 7Zf(+).

2.2. *DNA sequencing*

DNA sequencing was done on the longest clones corresponding to the β- and the α-subunits of GC-S. The nucleotide sequence of the cDNA clone corresponding to the β-subunit was determined by Genset (Paris). The cDNA clone corresponding to the α-subunit was submitted to a series of progressive unidirectional deletions according to Henikoff [15] using the Erase-a-Base System (Promega). Both strands of the different constructs were sequenced manually by the Sanger's method modified for double stranded DNA [16] by using Sequenase (US Biochem. Corp.) [17].

2.3. *Southern blot*

High molecular weight DNA was extracted as previously described [18]. Aliquots (40 μg) were digested overnight at 37°C with *Eco*R1, *Bam*H1 and *Kpn*I restriction enzymes (4–12 U/μg) (Promega) and blotted as reported [20]. The blots were prehybridized, and hybridized with the insert of clones GC-Sβ_3 and GC-Sα_3, according to [20]. Blots were washed for 15 min at room temperature in 0.2×SSC, 0.1% SDS, followed by two 45 min washes at 68°C in the same solution. The membranes were exposed to hyperfilm MP (Amersham) at −80°C for 3 days with two intensifying screens.

3. RESULTS

3.1. *Cloning of the cDNAs corresponding to the 70 and 82 kDa subunits of GC-S*

The human brain cDNA library was screened using the coding region of the 70 and 82 kDa subunit of rat

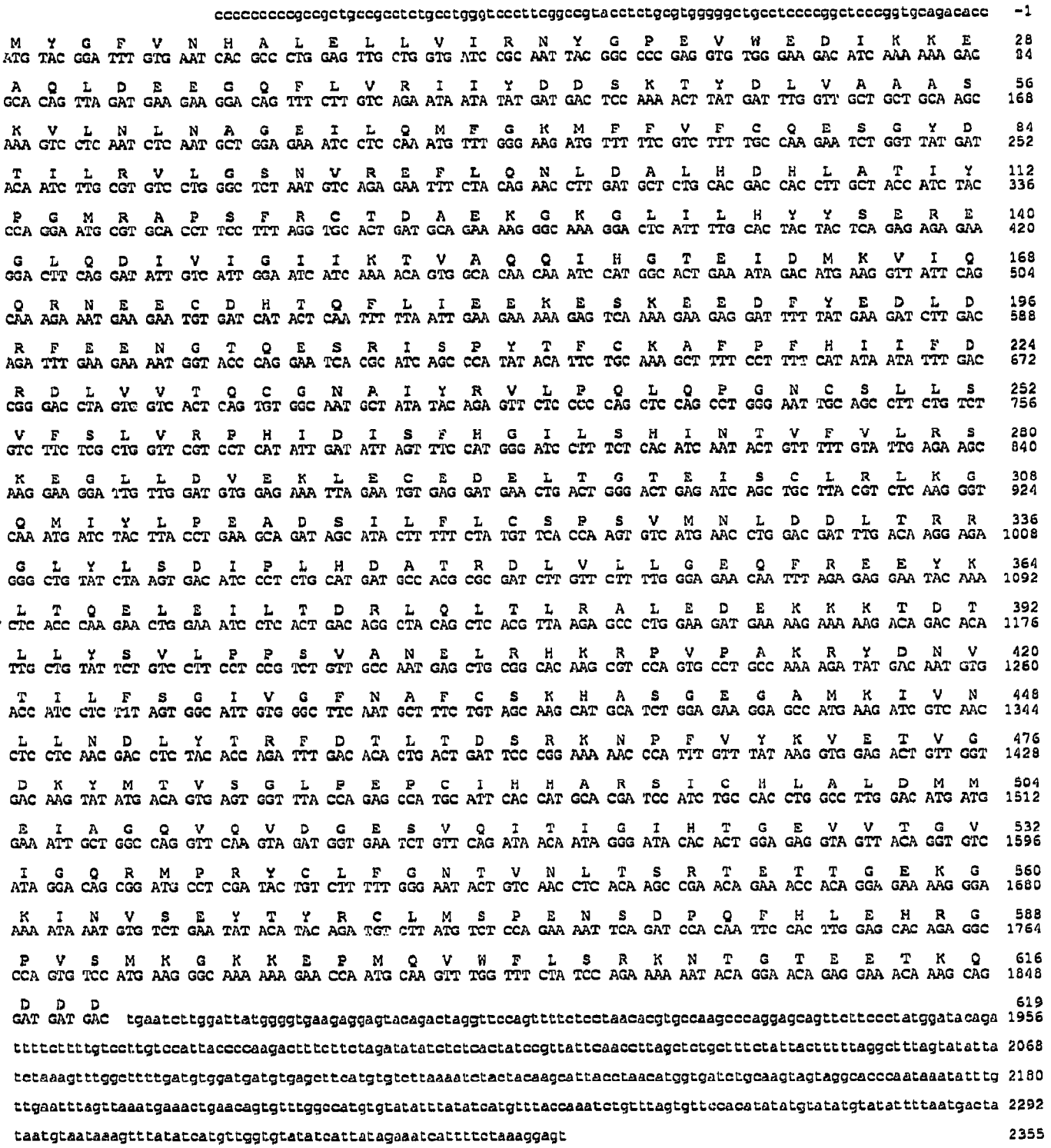

Fig. 1. Nucleotide sequence and corresponding protein sequence of GC-Sβ_3.

lung GC-S [7]. After repeated screening, one positive clone for each subunit, which encoded the entire protein, was isolated. They were designated GC-Sβ_3 and GC-Sα_3, respectively, according to the nomenclature suggested by Yuen et al. [9]. The nucleotide sequences of the cDNAs, and the deduced amino-acid sequences for the 70 and 82 kDa subunits, are shown in Fig. 1 and Fig. 2, respectively. The open reading frame of GC-Sβ_3 (1,857 bp) encodes 619 amino acids. The calculated molecular weight of the protein is 70,469 Da. For GC-Sα_3

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                                                    ccctta tggcgattgggcggctgcagagaccaggactcagttcccctgccctagtctg -465
agcctagtgggtgggactcagctcagagtcagttttcagaagcaggtttcagttgcagagttttcctacacttttcctgcgctagagcagcgagcagcctggaacagacccaggcg -349
gaggacacctgtgggggagggagcgcctggaggagcttagagaccccagccgggcgtgatctcaccatgtgcggatttgcgaggcgcgccctggagctgctagagatccggaagca -233
cagccccgaggtgtgcgaagccaccaagactgcggctcttggagaaagcgtgagcaggggggccaccgcggtctccggcctgtctgcaccctgtcgcctgagctgcctgacagtgac -117
aatgacatcccagttaccagtgtccttgaattgatagtggcttctgtttgtcagtctcatataagaactacagctcatcaggaggagatcgcagcagggtaagagacaccaacacc -1

 M   F   C   T   K   L   K   D   L   K   I   T   G   E   C   P   F   S   L   L   A   P   G   Q   V   P   N   E   S     29
ATG TTC TGC ACG AAG CTC AAG GAT CTC AAG ATC ACA GGA GAG TGT CCT TTC TCC TTA CTG GCA CCA GGT CAA GTT CCT AAC GAG TCT    87

 S   E   E   A   A   G   S   S   E   S   C   K   A   T   V   P   I   C   Q   D   I   P   E   K   N   I   Q   E   S     58
TCA GAG GAG GCA GCA GGA AGC TCA GAG AGC TGC AAA GCA ACC GTG CCC ATC TGT CAA GAC ATT CCT GAG AAG AAC ATA CAA GAA AGT   174

 L   P   Q   R   K   T   S   R   S   R   V   Y   L   H   T   L   A   E   S   I   C   K   L   I   F   P   E   F   E     87
CTT CCT CAA AGA AAA ACC AGT CGG AGC CGA GTC TAT CTT CAC ACT TTG GCA GAG AGT ATT TGC AAA CTG ATT TTC CCA GAG TTT GAA   261

 R   L   N   V   A   L   Q   R   T   L   A   K   H   K   I   K   E   S   R   K   S   L   E   R   E   D   F   E   K    116
CGG CTG AAT GTT GCA CTT CAG AGA ACA TTG GCA AAG CAC AAA ATA AAA GAA AGC AGG AAA TCT TTG GAA AGA GAA GAC TTT GAA AAA   348

 T   I   A   E   Q   A   V   Q   Q   S   P   V   E   L   S   K   N   L   L   V   K   R   F   L   K   Y   V   T   R    145
ACA ATT GCA GAG CAA GCA GTG CAG CAG AGT CCA GTG GAG TTA TCA AAG AAT CTC TTG GTG AAG AGG TTT TTA AAA TAT GTT ACG AGG   435

 K   M   K   T   S   L   G   W   L   E   A   P   L   K   I   F   K   Q   L   Q   Y   P   S   E   T   E   Q   P   L    174
AAG ATG AAA ACA TCC TTG GGG TGG TTG GAG GCA CCC TTA AAG ATT TTT AAA CAG CTT CAG TAC CCT TCT GAA ACA GAG CAG CCA TTG   522

 P   R   S   R   K   K   G   Q   L   E   D   A   S   I   L   C   L   D   K   E   D   D   F   L   H   V   Y   Y   F    203
CCA AGA AGC AGG AAA AAG GGG CAG CTT GAG GAC GCC TCC ATT CTA TGC CTG GAT AAG GAG GAT GAT TTT CTA CAT GTT TAC TAC TTC   609

 F   P   K   R   T   T   S   L   I   L   P   G   I   I   K   A   A   A   H   V   L   Y   E   T   E   V   E   V   S    232
TTC CCT AAG AGA ACC ACC TCC CTG ATT CTT CCC GGC ATC ATA AAG GCA GCT GCT CAC GTA TTA TAT GAA ACG GAA GTG GAA GTG TCG   696

 L   M   P   P   C   F   H   N   D   C   S   E   F   V   N   Q   P   Y   L   L   Y   S   V   H   M   K   S   T   K    261
TTA ATG CCT CCC TGC TTC CAT AAT GAT TGC AGC GAG TTT GTG AAT CAG CCC TAC TTG TTG TAC TCC GTT CAC ATG AAA AGC ACC AAG   783

 P   S   L   S   P   S   K   P   Q   S   S   L   V   I   P   T   S   L   F   C   K   T   F   P   F   H   F   M   F    290
CCA TCC CTG TCC CCC AGC AAA CCC CAG TCC TCG CTG GTG ATT CCC ACA TCG CTA TTC TGC AAG ACA TTT CCA TTC CAT TTC ATG TTT   870

 D   K   D   M   T   I   L   Q   F   G   N   G   I   R   R   L   M   N   R   R   D   F   Q   G   K   P   N   F   E    319
GAC AAA GAT ATG ACA ATT CTG CAA TTT GGC AAT GGC ATC AGA AGG CTG ATG AAC AGG AGA GAC TTT CAA GGA AAG CCT AAT TTT GAA   957

 Y   F   E   I   L   T   P   K   I   N   Q   T   F   S   G   I   M   T   M   L   N   M   Q   F   V   V   R   V   R    348
TAC TTT GAA ATT CTG ACT CCA AAA ATC AAC CAG ACC TTT AGC GGG ATC ATG ACT ATG TTG AAT ATG CAG TTT GTT GTA CGA GTG AGG  1044

 R   W   D   N   S   V   K   K   S   S   R   V   M   D   L   K   G   Q   M   I   Y   I   V   E   S   S   A   I   L    377
AGA TGG GAC AAC TCT GTG AAG AAA TCT TCA AGG GTT ATG GAC CTC AAA GGC CAA ATG ATC TAC ATT GTT GAA TCC AGT GCA ATC TTG  1131

 F   L   G   S   P   C   V   D   R   L   E   D   F   T   G   R   G   L   Y   L   S   D   I   P   I   H   N   A   L    406
TTT TTG GGG TCA CCC TGT GTG GAC AGA TTA GAA GAT TTT ACA GGA CGA GGG CTC TAC CTC TCA GAC ATC CCA ATT CAC AAT GCA CTG  1218

 R   D   V   V   L   I   G   E   Q   A   R   A   Q   D   G   L   K   K   R   L   G   K   L   K   A   T   L   E   Q    435
AGG GAT GTG GTC TTA ATA GGG GAA CAA GCC CGA GCT CAA GAT GGC CTG AAG AAG ACG CTG GGG AAG CTG AAG GCT ACC CTT GAG CAA  1305

 A   H   Q   A   L   E   E   E   K   K   K   T   V   D   L   L   C   S   I   F   P   C   E   V   A   Q   Q   L   W    464
GCC CAC CAA GCC CTG GAG GAG GAG AAG AAA AAG ACA GTA GAC CTT CTG TGC TCC ATA TTT CCC TGT GAG GTT GCT CAG CAG CTG TGG  1392

 Q   G   Q   V   V   Q   A   K   K   F   S   N   V   T   M   L   F   S   D   I   V   G   F   T   A   I   C   S   Q    493
CAA GGG CAA GTT GTG CAA GCC AAG AAG TTC AGT AAT GTC ACC ATG CTC TTC TCA GAC ATC GTT GGG TTC ACT GCC ATC TGC TCC CAG  1479

 C   S   P   L   Q   V   I   T   M   L   N   A   L   Y   T   R   F   D   Q   Q   C   G   E   L   D   V   Y   K   V    522
TGC TCA CCG CTG CAG GTC ATC ACC ATG CTC AAT GCA CTG TAC ACT CGC TTC GAC CAG CAG TGT GGA GAG CTG GAT GTC TAC AAG GTG  1566

 E   T   I   A   M   P   I   V   W   L   G   G   L   H   K   E   S   D   T   H   A   V   Q   I   A   L   M   A   L    551
GAG ACC ATT GCG ATG CCT ATT GTG TGG CTT GGG GGA TTA CAC AAA GAG AGT GAT ACT CAT GCT GTT CAG ATA GCG CTG ATG GCC CTG  1653

 K   M   M   E   L   S   D   E   V   M   S   P   H   G   E   P   I   K   M   R   I   G   L   H   S   G   S   V   F    580
AAG ATG ATG GAG CTC TCT GAT GAA GTT ATG TCT CCC CAT GGA GAA CCT ATC AAG ATG CGA ATT GGA CTG CAC TCT GGA TCA GTT TTT  1740

 A   G   V   V   G   V   K   M   P   R   Y   C   L   F   G   N   N   V   T   L   A   N   K   F   E   S   C   S   V    609
GCT GGC GTC GTT GGA GTT AAA ATG CCC CGT TAC TGT CTT TTT GGA AAC AAT GTC ACT CTG GCT AAC AAA TTT GAG TCC TGC AGT GTA  1827

 P   R   K   I   N   V   S   P   T   T   Y   R   L   L   K   D   C   P   G   F   V   F   T   P   R   S   R   E   E    638
CCA CGA AAA ATC AAT GTC AGC CCA ACA ACT TAC AGA TTA CTC AAA GAC TGT CCT GGT TTC GTG TTT ACC CCT CGA TCA AGG GAG GAA  1914

 L   P   P   N   F   P   S   E   I   P   G   I   C   H   F   L   D   A   Y   Q   Q   G   T   N   S   K   P   C   F    667
CTT CCA CCA AAC TTC CCT AGT GAA ATC CCC GGA ATC TGC CAT TTT CTG GAT GCT TAC CAA CAA GGA ACA AAC TCA AAA CCA TGC TTC  2001

 Q   K   K   D   V   E   D   A   S   Q   F   F   R   Q   S   I   R   N   R   L   A   T   Y   I   P   I   Y   K   S    696
CAA AAG AAA GAT GTG GAA GAT GCA AGC CAA TTT TTT AGG CAA AGC ATC AGG AAT AGA TTA GCA ACC TAT ATA CCT ATT TAT AAG TCT  2088

 L   G   F   D   S   L   K   M   C   R   A   S   E   S   T   L   G   I   V   D   G                                 717
TTG GGG TTT GAC TCA TTG AAG ATG TGT AGA GCC TCT GAA AGC ACT TTA GGG ATT GTA GAT GGC  taacaagcagtattaaaatttcaggagccaa 2182
gtcacaatctttctcctgtttaacatgacaaaatgtactcacttcagtacttcagctcttcaagaaaaaaaaaaaaccttaaaaagctacttttgtgggagtatttctattatat 2298
aaccagcacttactacctgtactcaaaattcagcaccttgtacatatatcagataattgtagtcaattgtacaaactgatggagtcacctgcaatctcatatcctggtggaatgcc 2414
atggttattaaagtgtgtttgtgatagttgtcgtcaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa                                                 2482
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Fig. 2. Nucleotide sequence and corresponding protein sequence of GC-Sα_3.

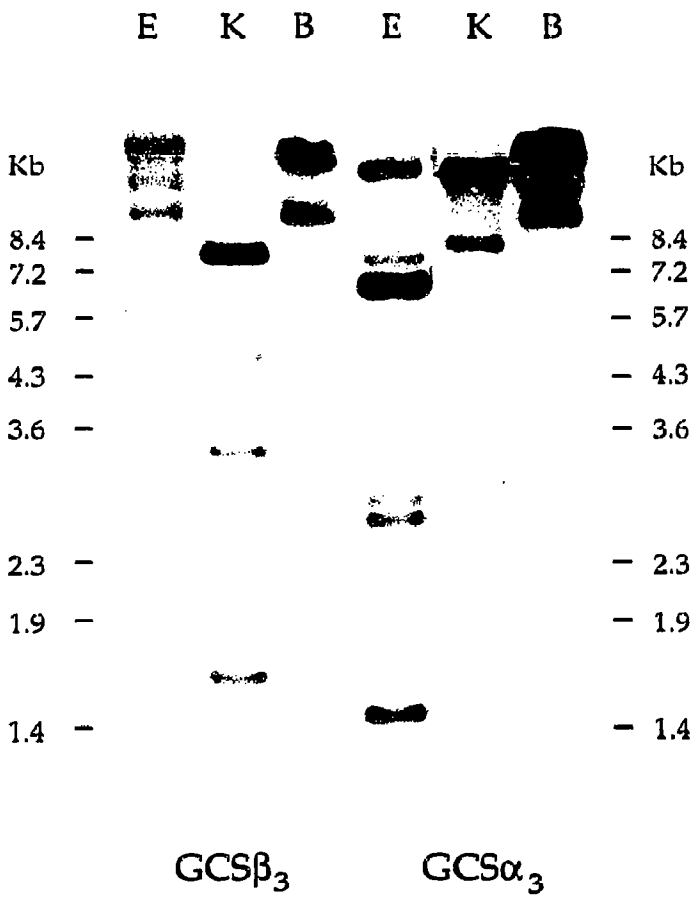

Fig. 3. Southern blot of human genomic DNA. Genomic DNA was digested with restriction enzymes *Eco*RI (E), *Kpn*I (K) and *Bam*HI (B) and the blots prepared as described in section 2. The blots were hybridized with either GC-Sβ_3 or GC-Sα_3 as probes. The position and the size of the markers run in parallel are indicated on both sides of the figure.

the open reading frame (2,151 bp) codes for 717 amino acids. The calculated molecular weight is 81,324 Da.

3.2. *Southern blot analysis*

Southern blot analysis of human genomic DNA, digested with *Eco*RI, *Bam*HI and *Kpn*I were hybridized with the insert of both clones as probes. This hybridization revealed a number of discrete hybridized bands (Fig. 3) with different patterns for the α_3- and β_3-subunits. These results suggest either that the genes coding for these subunits might be present in more than one copy in the human genome, or the presence of pseudogenes.

3.3. *Comparison of the α_3- and β_3-subunits with the known sequences of soluble guanylyl cyclase subunits*

There is a considerable homology (i.e. identical plus similar amino acids) between the predicted amino acid sequences of the 70 and 82 kDa subunits of human adult brain GC-S. The homology, in 548 amino acid residues of both chains, is of 34% and rises to 46% if one considers only the last 300 residues of the C-terminal parts (Fig. 4).

We compared the amino acid sequences of the α_3- and β_3-subunits with sequences of other GC-S subunits. The comparison of the sequence of various a subunits (four bottom lines of each block, Fig. 4) reveals that the α_3 was 78.5% homologous to the bovine α_1-subunit and 81% homologous to the rat α_1-subunit in a 684 amino acid overlap [11,12]. Interestingly, the comparison of α_3 with α_2 from human fetal brain [13] reveals a very limited degree of homology in the N-terminal region but a 72% homology between the 310 amino acids corresponding to the C-terminal sequence of both subunits. Comparison of the protein sequence of β_3 with those of β_1-subunits from bovine and rat lung [7,8] exhibits an homology of 99 and 98.5%, respectively, in a 619 amino acid overlap (three upper lines, Fig. 4). The homology between β_3 and β_2 from the rat kidney is less pronounced, with only 39% in a 418 amino acid overlap mainly located in the carboxy terminal region of both subunits. From the analysis of Fig. 4 it is clear that a sequence of 268 amino acids of the C-terminal segments (amino acids 428–696) is highly conserved between the α- and the β-subunits of GC-S from various species.

The comparison of the protein sequence of β_3 with the partial sequences, HSGC-1 and HSGC-2, cloned from human lung [10] reveals a 99.5% homology between amino acid 1–209 of β_3 and amino acids 337–545 of HSGC-1, and a 98.3% homology between amino acids 425–545 of β_3 and amino acids 56– 176 of HSGC-2 (data not shown). The remaining sequences of HSGC-1 and HSGC-2 are not available.

4. DISCUSSION

Recently, Harteneck et al. described the cloning and expression of a new α_2-subunit of GC-S isolated from a human fetal brain cDNA library [13]. The comparison of the deducted amino acid sequence of GC-Sα_3 with those of this α_2-subunit reveals only a 34% homology predominantly in the C-terminal segment. It is highly

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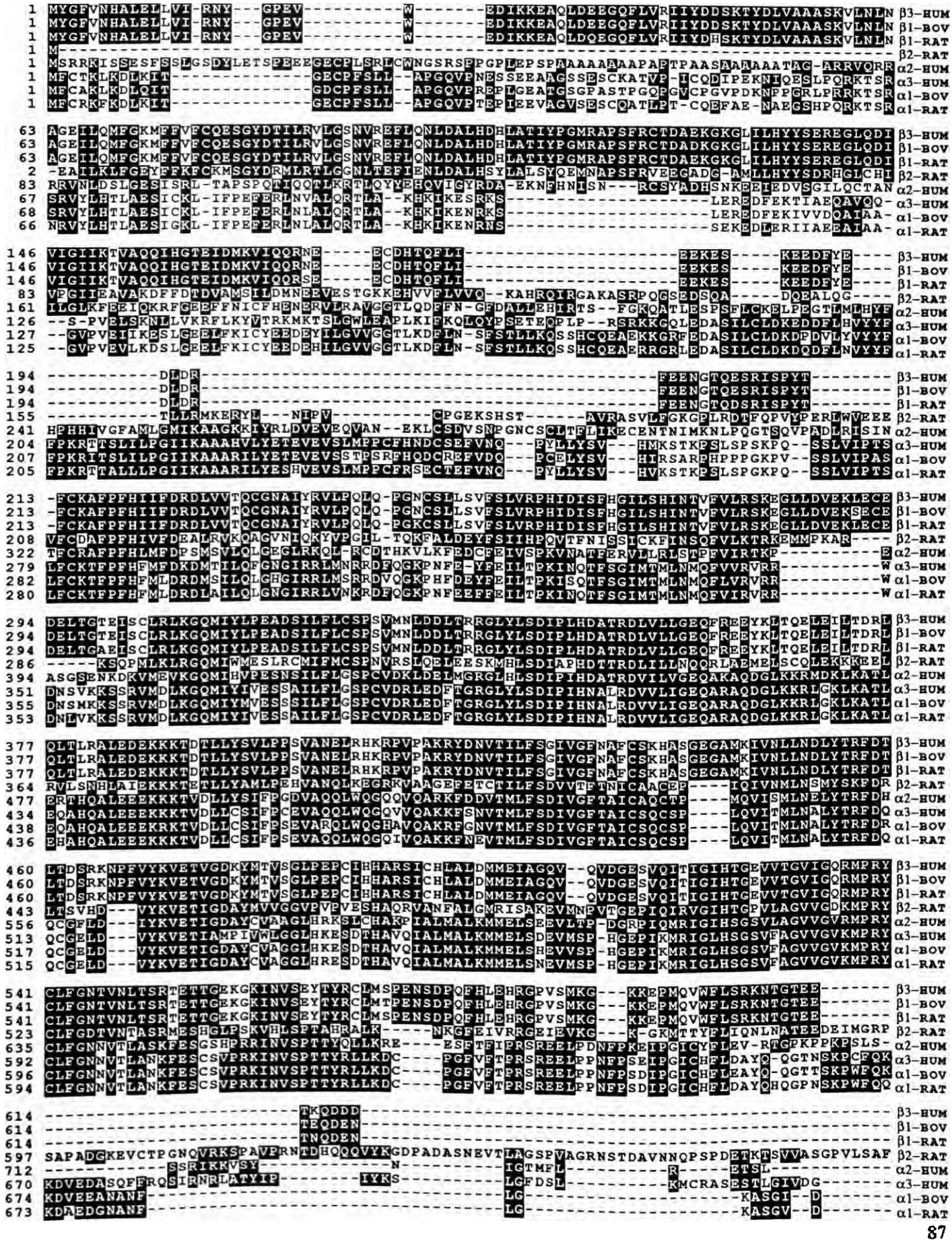
Fig. 4. Comparison of the protein sequence of the large and small subunits of various GC-S's. The protein sequences of GC-S for the small subunits from human brain (β3-hum), bovine lung (β1-bov), rat lung (β1-rat), and large subunits from human fetal brain (α2-hum), human adult brain (α3-hum), bovine lung (α1-bov) and rat lung (α1-rat), were compared on VAX computer [21] using the clustal alignment method [22]. Similar groups of amino acids were defined as follows : {A,G,S,T} {N,D,Q,E,B,Z} {R,K} {I,L,M,V}{F,W,Y}{C}{X}{H}{P}. Identical and similar residues are boxed using the program Boxshade [21].



probable that such a difference reflects the expression of two different genes for these two human GC-Sα-subunits. The possible expression of two GC-Sα-subunit isoforms at different stages of development of the human brain raises the interesting possibility of specific regulation of GC-S expression during ontogeny. The GC-S might also be expressed in a tissue-specific manner. It has been shown by Yuen et al. [9] that two different small subunits of GC-S were present in kidney and liver cells, apparently encoded by two different genes. Recently, Chhajlani et al. [10] reported the heterogeneity of two human GC-S clones isolated from a human lung cDNA library, and which seem to be generated by alternative splicing. Altogether, these results might argue for a tissue-specific expression of GC-S isoforms.

The two GC-S subunits that we have cloned exhibit strong homologies with other known GC-S subunits. This might reflect the existence of a common ancestor for all these proteins, but also the pressure of selection for the maintenance of the active site. Using transfection experiments it as been shown that both subunits are indispensable for the expression of the catalytic activity [13]. The putative catalytic domain has been localized in the C-terminal part of both subunits and corresponds to the region where all subunits exhibit the highest homology.

From the diversity within the GC-S enzyme family one might expect, in a near future, new interesting observations on the expression and regulation of guanylyl cyclase, leading to a better understanding of the role of cyclic GMP in the cells and, more precisely, in nervous tissue.

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