

Sequence and expression of human GABA_A receptor α 1 and β 1 subunits

Peter R. Schofield*, Dolan B. Pritchett, Harald Sontheimer⁺, Helmut Kettenmann⁺ and Peter H. Seeburg

Laboratory of Molecular Neuroendocrinology, Zentrum für Molekulare Biologie, Im Neuenheimer Feld 282, University of Heidelberg, 6900 Heidelberg and ⁺Department of Neurobiology, Im Neuenheimer Feld 364, University of Heidelberg, Heidelberg, FRG

Received 2 January 1989

The deduced amino acid sequences of cDNA clones encoding human GABA_A receptor α 1 and β 1 subunits are presented. The human subunits display very high levels of sequence identity with the corresponding bovine receptor subunits. The cloned human GABA_A receptor subunits induce the formation of GABA-gated chloride channels when expressed in mammalian cells.

γ -Aminobutyric acid A receptor; Ligand-gated ion channel; Electrophysiology; cDNA cloning; (Human brain)

1. INTRODUCTION

Receptors for the major inhibitory neurotransmitter GABA (γ -aminobutyric acid) are present on the majority of neurons in the mammalian brain. The GABA_A receptor contains an intrinsic chloride ion channel which is opened (gated) by the neurotransmitter, thus stabilizing the neuron's resting potential [1]. Channel activity can be allosterically modulated by therapeutically useful drugs e.g. barbiturates and benzodiazepines [1]. The primary structure of the cloned bovine α and β subunits has shown the existence of a ligand-gated ion channel receptor superfamily [2]. Coexpression of these subunits in *Xenopus* oocytes produces receptors with many functional characteristics of native GABA_A receptors [2]. The clinical importance of drugs that act at this ubiquitous brain receptor makes it desirable to characterize the structure of

the human receptor subtypes. To this end we have cloned and sequenced the human GABA_A receptor α 1 and β 1 subunits and expressed them in mammalian cells.

2. MATERIALS AND METHODS

Full-length GABA_A receptor α 1 and β 1 subunit cDNAs were isolated from a human fetal brain cDNA library, constructed in λ gt10 by standard methods. 2×10^6 pfu of this library were screened with subunit-specific radiolabelled oligonucleotides based on the bovine cDNA sequences: α 1 subunit, 5'-ACCCTGGCCAGATTAGGTGTGTAGCTGGTTGCTGTTGGA-3'; β 1 subunit, 5'-TCCCACGCCCGTGAGCACTTCAGAGGCCGCTCGTCTCGTTCCTGATCTCCGGGTACTGAGGAG-AATGTTGCCGTG-3'. Three α 1 subunit cDNA clones were analyzed and the largest (in λ hGR α 28), 4.25 kb in size, was sequenced across the coding, and into the 3'-untranslated region. Eight cloned β 1 subunit cDNAs were obtained and rescreened with specific 5'- and 3'-oligonucleotides based on the known bovine β 1 cDNA sequence. One (λ hGR β 19) which hybridized to both probes was sequenced. Two β 1 cDNAs positive only to the 3'-probes (λ hGR β 18) contained an additional T at position 1220, unlike λ hGR β 19. This missing nucleotide was added to the sequence derived from λ hGR β 19 by site-directed mutagenesis, using the oligonucleotide 5'-ATGTACTCCTA-TGACGAGCG-3', thus restoring the reading frame to complete alignment with the bovine β 1 subunit cDNA. DNA sequences were obtained using the chain-termination method [4] and M13 vectors.

Correspondence address: P.H. Seeburg, Laboratory of Molecular Neuroendocrinology, Zentrum für Molekulare Biologie, Im Neuenheimer Feld 282, University of Heidelberg, 6900 Heidelberg, FRG

* Present address: Pacific Biotechnology Ltd, 72–76 McLachlan Avenue, Rushcutters Bay 2011, Australia

1 GTGAATCTTCAGAAAGGAGCAGCGAGAGTCCATGATGGCTCAGACCAAGTGTAGAGGGCAGAGGAGGACGCCCTCTGCTCTG
89 GCGCCCGCGGACTCGGACTCGGACTCGGCTGGCTCCAGTCTCTCCAGGATCTCTCTCCAGACTTTTCCCGGGTCTTAAGAGATCC
-27 *** K M R K S P L I S D C L W A W T I L L
179 TGTGTCAGAGGGGGCTTAGCTGCTCCAGCCCGGATGAGAAAAGTCCAGGTCTGTGTGACTGCTTTGGGGCTGGATCTCTCTG
-9 S T L T G R S Y G Q P S L Q D E L K D N T V F T R I L D R
269 AGCAGACTGACTGGAAGAAGCTATGGACGGCTATTACAAGATGAACCTAAGACAAATACCACCTGCTTCCACCAAGATTTGCGAGA
22 L L D G Y D N R L R P G L G E R V T E V K T D I F V T S F G
359 CTCTAGATGGCTATGACAATCGCTGAGACAGGATGGGAGAGCGTGAACCGAAGTGAAGACTGATATCTTGGTCCAGGATTTGCGA
52 P V S D H D M E Y T I D V F F R Q S W K D E R L K F K G P M
449 CCGCTTTCAGACCATGATGGAATATACAATAGATGATTTTCCGTCAAGCTGGAAGGATGAAAGTTAAATTTAAAGACCTATG
82 T V L R L N L M A S K I R T P D T F F H N G K K S V A H N
539 ACAGTCTCCGGTTAAATAACCTAATGGCAAGTAAATCCGGACTCCGGACACATTTTCCACAATGGAAGAAGTACGGTCCGCCAAC
112 M T M P N K L L R I T E D G T L L Y T M R L T V R A E C P M
629 ATGACCTGCCAACAACTCTCGGATCACAGAGGATGGCACCTTGGCTGACACCATGAGGCTGACAGTGAAGGCTGAATGTCGGA
142 W L E D F P M D A W A C P L K F G S Y A Y T R A E V V Y E W
719 CATTGGAGACTTCCCTATGGATGCCACTGTGCCACTAAAATTTGGAAGTTATGCTTATACAAGACGAGAAGTTGTTATGAATGG
172 T R E P A R S V V A E D G S R L N Q Y D L L G Q T V D S G
809 ACCAGAGGCCAGCACCGCTCAGTGGTTGTAGCAGAAGTGGATCAGCTTAAACAGTATGACCTTCTGGACAAACAGACTGACTGGGA
202 I V Q S S T G E Y V V M T T H F H L K R K I G Y F V I Q T Y
899 ATTTCCAGTCAAGTACAGAGAATATGTTGTTATGACCACTTCCACTTGAAGAGAAGATGGCTACTTGTATTCAAACTAC
232 L P C I M T V I L S Q V S F W L N R E S V P A R T V F G V T
989 CTGGCTGCATATGACAGTATTCTCTCAAGTCTCTCTGGCTCAACAGAGTCTGTACCAGCAAGACTGCTTTGGAGTAACA
262 T V L T M T T L S I S A R N S L P K V A Y A T A M D W F I A
1079 ACTGTCTACCATGACAACATGAGCATCGCCAGAACTCCCTCCCTAAGGTGGCTTATGCAACAGCTATGGATGCTTTATGGC
292 V C Y A F V F S A L I E F A T V N Y F T K R G Y A W D G K S
1169 GTGCTATGCTTGTGTCTCAGCTCTGATGAGTTGGCCAGTAACTATTTCACTAGAGAGGTTATGCTGGATGGCAAAAGT
322 V V P E K P K K V K D P L I K K N N T Y A P T A T S Y T P N
1259 GTGGTCCAGAAAAGCAGAAAGTAAAGGATCCCTTATTAGAAAAACAACACTTAGGCTCCAACAGCAACCCAGTACCCCTAAT
352 L A R G D P G L A T I A K S A T I E P K E V K P E T K P P E
1349 TTGGCCAGGGGCGACCCGGCTTAGCCACCATGCTAAAGTGCACCACTAGAACCTAAGAGGCTCAAGCCGAAAACCAACAGAA
382 P K K T F N S V S K I D R L S R I A P P L L F G I F N L V Y
1439 CCAAGAAAACCTTAAAGTGTACGAAATGACCGACTGCAAGAAATAGCTTCCCGTCTTATGGAATCTTAACTTAGTCTAC
412 W A T Y L N R E P D L K A P T P H Q ***
1529 TGGGCTACGTTTAAACAGAGGCTCAGCTAAAAGCCCCACACACATCAATAGACTTTTACTACACTTGTGTTGACGTCC
1619 TGACGGGAATTTATGTTCTCAAGCAGTAAATCCCATCTGCTTTATGGCTCTGTCTTAAAGAAATTTGAAAGTTCCCTATTT
1709 TCATAATTCATTAAGACAAGGACCCCTGTCTG

-25 *** M W T V Q N R E S L G L L S F P V M I
1 GAAAAGACAATCTTTAATCAGAGTATGATGGACAGTACAAAATCGAGAGAGTCTGGGGCTCTCTCTTCCCTGTGATGATT
A S
-6 T M V C C A H S T N E P S N M P Y V K E T V D R L L K G P
89 ACCATGGTCTGTGTGCACACAGCACCATGAACCCAGCAACATGCCATAGCTGAAAGACAGTGGACAGATGCTCAAAGATATGAC
25 I R L R P D F G G P P V D V G M R I D V A S I D M V S E V N
179 ATTCGCTTGGGCGGACTCGGAGGGCCCCCGTGCAGCTTGGATGGGATCGATGCTGCCAGCATAGACTGCTTCCGAGGAT
55 M D Y T L T M Y E Q Q S W K D K R L S Y S G I P L N L T L D
269 ATGATTTACACTCACCATGATTTCCAGCAGTCTGGAAGACAAAAGGCTTCTTATCTGGAATCCCAGTACACTCACCCTAGAC
85 N R V A D Q L W V P D T Y F L N D K K S F V H G V T V K N R
359 AATAGGCTAGCTGACCAACTGCGGTACCAGACACTACTTCTGATGACAAGAAATCATTGTCATGGGTCACAGTGAATAATCGA
115 M I R L H P D G T V L Y G L R I T T T A A C M M D L R R Y P
449 ATGATTCAGCTGACTCGTGAACAGTCTCTATGACTCCGAATCACACCAGCAGTGCATGATGATGATGCTGCGAATGCTGCA
145 L D E Q N C T L E I E S Y G Y T T D D I E F Y W N G G E G A
539 CTGGATGACAGACTGACCTGGAGATCGAAAGTTATGGCTATACCAGTATGACATGAAATTTACTGGAATGGAGGAGGGGCA
175 V T G V N K I E L P Q F S I V D Y K M V S K K V E F T T G A
629 GTCACTGGTGTAAATAAATGGAATTCCTCAATTTCAATTTGTTGACTACAAGATGGTGTCTAAGAAGTGGAGTTCACACAGGAGGG
205 Y P R L S L S F R L K R N I G Y F I L Q T Y M P S T L I T I
719 TATCCAGACTGCTCACTAAGTTTGTCTAAAGAGAAACATGGTACTTCAATTTGCAACCTACATGCTCTYACACTGATTAACAAT
235 L S W V S F W I N Y D A S A A R V A L G I T T V L T H T I
809 CTGCTCTGGGTCTTTTGGTCAACTATGATGCTGACCCAGACTGACACTAGGAATCAGCAGGCTGCTTCAACTAGCAACCATC
265 S T H L R E T L P K I P Y V K A I D I Y L N G C F V F V F L
899 AGCACCACCCTCAGGAGACCTGCCAAAGATCCCTATGCTCAAAGGATGATATTATCTGATGGTGGCTTGTGTTGTTGCTTCTG
295 A L L E Y A F V N Y I F F G K G P Q K K G A S K Q D Q S A N
989 GCTCTGCTGGAGTATGCTTTGTAATTAATCTCTTTGGAAAGGCCCTCAGAAAAGGGAGCTAGCAAAACAGACAGGATGCCAAT
325 E K N K L E M N K V Q V D A H G N I L L S T L E I R N E I S
1079 GAGAGAATAAATCGGAGTGAATAAAGTCCAGGTCAGCCGCCAGGTAACTTCTCCAGCACCCTGGAATAACCCGAAACAGAGGAT
355 G S E V L T S V S D P K A T M Y S Y D S A S I Q Y R K P L S
1169 GGCTCGAAGTGTCTCAGAGGCTGAGCGCCCAAGGCCACCATGACTCTATGACAGCCGACATCCAGTCCGACAGCCCTGAGG
385 S R E A Y G R A L D R H G V P S K G R I R R A S Q L K V K
1259 AGCCGCGAGGCTADGGGCGCCCTGGACCGCACGGGATACCAGCAAGGGGGCATCCGAGGCTGCCCTCCAGCTCAAGTCAAG
415 I P D L T D V N S I D K W S R M F F I T F S L F N V V Y W
1349 ATCCCGACTTGACTGATGTAATCCATAGACAAGTGGTCCGAAATGTTTTCCCATCAGCTTTTCTTTTAAATGCTGCTTATGG
445 L Y V V H ***
1439 CTTTACTATGTACACTGAGGCTGTCTAATGGTCCATTTAGACTACTTTCCTCTCTATGTTTTTAACTTACAGTCCCAACAG
1529 CGATCTGCTGTTCTCAGGTAAAGACTCAGCCATCAATGGTTTGGCTTCTGATCAGTTTAACTGACAGGATTTACTT
1619 CAAAAGACAAAACAAAATAATTTTCCAGTCTACCGTGGTCCAGTTATCAGCTCTTTAAGAGCTCTAATAATGGCATGTT
1709 ACAACAACAGCAAGAGAGAGTGTAGACAGGTAGACTTTAGCAGTCTTTCTAGTTCCCTGGATTCAGCTGATTTAATTTAGGGA
1799 AATGAAAAGAGGACTTGCCTCCGCTGCATGCTTCCGTGAACTATAACAACCTATGCTGCCAAAAA

Fig.1. DNA and predicted amino acid sequence of the human GABA_A receptor $\alpha 1$ (a) and $\beta 1$ (b) subunit cDNAs. Amino acid numbering starts at the proposed mature N-terminal residues. The putative signal sequence cleavage site is indicated by an arrow and the signal sequence is shown in negative numbering. The proposed membrane-spanning hydrophobic sequences are highlighted by overscoring, the disulfide-bonded loop region is indicated by a dotted line and the putative extracellularly located *N*-glycosylation sites are boxed. Asterisks denote both 3'-terminal stop codon and the first upstream in-frame stop codon in the 5'-untranslated sequence. Amino acids that differ from the bovine sequences are indicated above the human polypeptide sequence.

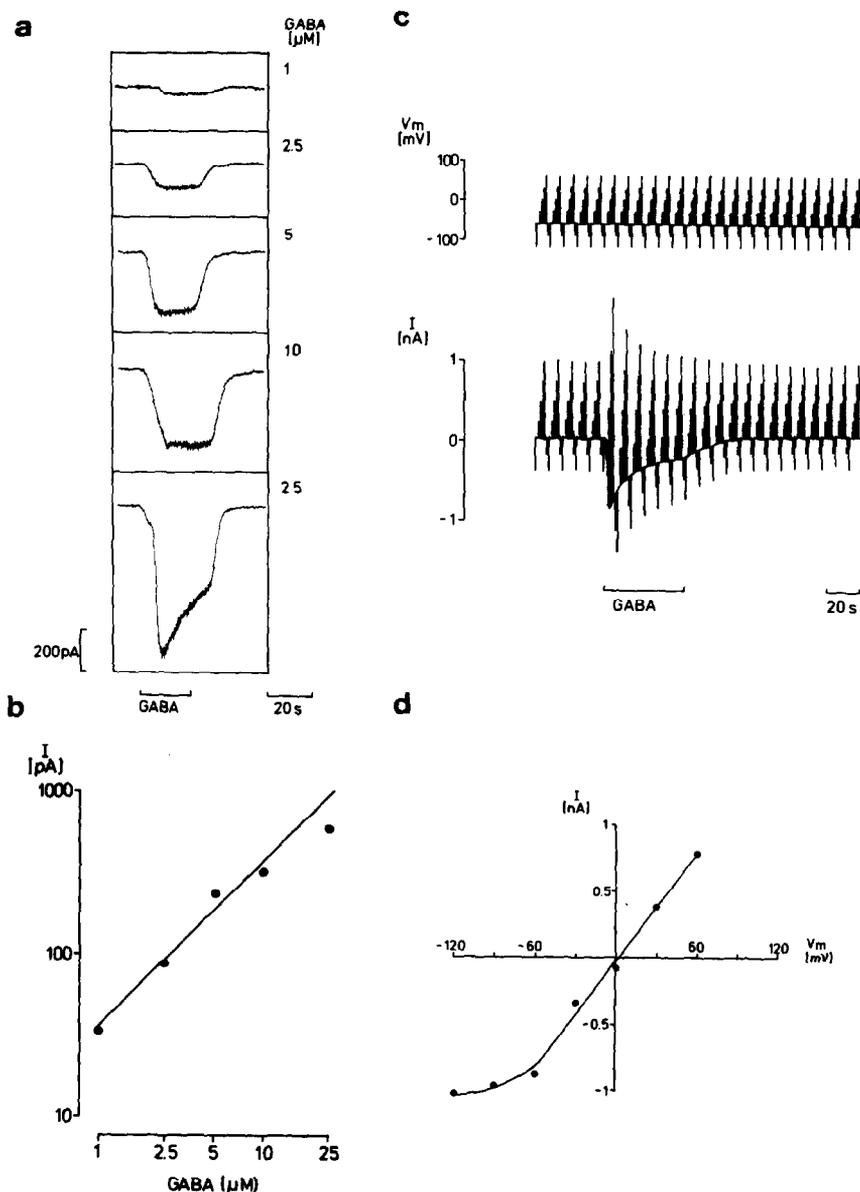


Fig.2. Dependence of the GABA-induced current on ligand concentration. (a) The membrane current at the resting potential (~ 60 mV) was recorded from a cell transfected with the $\alpha 1 + \beta 1$ subunits using the patch-clamp technique in the whole cell configuration. GABA was applied as indicated by the bar at the given concentrations. A dose-dependent inward current was observed. (b) Dose-response curve of GABA-induced currents (I) for the experiment shown in a, log-log scale (Hill plot). The slope of the curve at low GABA concentrations is approx. 1, suggesting a single-binding site for the ligand. (c) The membrane current was measured while briefly clamping membrane potential (V_m) from the resting value (-60 mV) to 120, -90 , -30 , 0, 30 and 60 mV for a period of 200 ms with 1 s intervals. This series of voltage jumps was continuously applied. GABA was added as indicated by the bar at a concentration of 10^{-4} M. (d) Currents (I) before application of GABA were subtracted from currents in the presence of GABA for the experiments shown in c and plotted as a function of membrane potential (V_m). $V_m\text{Cl}^- = 0$ mV, $V_m\text{K}^+ = -84$ mV and $V_m\text{Na}^+ \geq 80$ mV.

The $\alpha 1$ - and $\beta 1$ -subunit cDNAs were expressed and analysed in mammalian tissue culture cells as previously described [3]. Membrane currents were recorded, using the patch-clamp technique in the whole cell configuration.

3. RESULTS AND DISCUSSION

Using oligonucleotide probes, cloned human GABA_A receptor $\alpha 1$ and $\beta 1$ subunit cDNAs were isolated from a human fetal brain cDNA library. One of three $\alpha 1$ cDNAs was sequenced and found to be full-length. Rescreening of eight $\beta 1$ cDNA clones with 5'- and 3'-sequence-specific oligonucleotide probes identified a single full-length cDNA encoding this subunit. The DNA and predicted polypeptide sequence of the $\alpha 1$ and $\beta 1$ cDNAs (fig.1) reveal a very high degree of conservation with the corresponding bovine sequences [2]. The 456 amino acid $\alpha 1$ subunit which contains a 27 residue signal peptide displays 99% sequence identity (five amino acid differences) with the bovine $\alpha 1$ subunit. However, of these, four are located within the signal sequence. The only difference in this mature protein sequence occurs in the extracellular domain and is a non-conservative replacement. The human sequence contains Trp 95 while this residue is an Arg in the bovine sequence. The 474 amino acid $\beta 1$ subunit displays 98% sequence identity (11 amino acid differences). In this case, only one of the differences is located within the 25 residue signal peptide. Of the remainder, two non-conservative replacements are found at the extreme N-terminus and the other 8 are located within the intracellular loop located between transmembrane domains 3 and 4. Of these, only two are conservative in nature. The concentration of sequence differences within the intracellular loop is consistent with this region of all members of this receptor superfamily being the most divergent. The results obtained suggest that the GABA_A receptor subunits are very highly conserved among the mammalian species.

The cloned human $\alpha 1$ and $\beta 1$ subunit cDNAs have recently been expressed in mammalian cells [3]. Using this technique, we transfected human cells as described and obtained electrophysiological recordings 48 h after transfection. GABA_A receptors expressed in mammalian cells have many of the properties of the native receptors [3]. They conduct chloride ions, are specifically inhibited by picrotoxin and the competitive GABA_A receptor

antagonist bicuculline and are potentiated by barbiturates. They do not display benzodiazepine responsiveness [3,5]. Single cell dose-response curves of GABA-induced currents (fig.2) had an average slope of 1 (SD = 0.1, N = 5), indicating that binding of only one GABA molecule per receptor caused a channel opening. This is at variance with the observed cooperativity of neurotransmitter gating of GABA_A receptor channels [6-8], but in agreement with results obtained by coexpression of α - and β -subunits in *Xenopus* oocytes [2,5].

Our results suggest a more complex composition of neuronal GABA_A receptors. Other hitherto unknown subunits may coassemble with $\alpha 1$ and $\beta 1$ subunits to generate a full display of all known receptor properties. The recent isolation in our laboratory of cDNAs which encode proteins with partial sequence identity to the α - and β -subunits should facilitate the experimental evaluation of the design of this important neurotransmitter receptor.

Acknowledgements: The authors thank Jutta Rami for secretarial assistance. This work was supported in part by the Deutsche Forschungsgemeinschaft, SFB 317 grant B/9 and by the Bundesministerium für Forschung und Technologie grant BCT 0381/5 to P.H.S.

REFERENCES

- [1] Olsen, R.W. and Venter, J.C. (1986) Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties, A.R. Liss, New York.
- [2] Schofield, P.R., Darlison, M.G., Fujita, N., Burt, D.R., Stephenson, F.A., Rodriguez, H., Rhee, L.M., Ramachandran, J., Reale, V., Glencorse, T.A., Seeburg, P.H. and Barnard, E.A. (1987) *Nature* 328, 221-227.
- [3] Pritchett, D.B., Sontheimer, H., Gorman, C.M., Kettenmann, H., Seeburg, P.H. and Schofield, P.R. (1988) *Science* 242, 1306-1308.
- [4] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
- [5] Levitan, E.S., Schofield, P.R., Burt, D.R., Rhee, L.M., Wisden, W., Koehler, M., Fujita, N., Rodriguez, H., Stephenson, F.A., Darlison, M.G., Barnard, E.A. and Seeburg, P.H. (1988) *Nature* 335, 76-79.
- [6] Barker, J.L. and Ransom, B.R. (1978) *J. Physiol. (Lond.)* 280, 331-354.
- [7] Sakmann, B., Hamill, O.P. and Bormann, J. (1983) *J. Neural Transm. suppl.* 18, 83-95.
- [8] Borman, J. and Clapham, D.E. (1985) *Proc. Natl. Acad. Sci. USA* 82, 2168-2172.