Functional expression and sites of gene transcription of a novel α subunit of the GABA_A receptor in rat brain

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Two α subunits of the GABA_A receptor in rat brain have been identified by molecular cloning. The deduced polypeptide sequences share major characteristics with other chemically gated ion channel proteins. One polypeptide represents the rat homologue of the α 3 subunit previously cloned from bovine brain [14], while the other polypeptide is a yet unknown subunit, termed α 5. When coexpressed with the β 1 subunit in *Xenopus* oocytes the receptors containing the α 5 subunit revealed a higher sensitivity to GABA than receptors expressed from α 1 + β 1 subunits or α 3 + β 1 subunits ($K_a = 1 \mu M, 13 \mu M$ and $14 \mu M$, respectively). The α 5 subunit was expressed only in a few brain areas such as cerebral cortex, hippocampal formation and olfactory bulb granular layer as shown by in situ hybridization histochemistry. Since the mRNA of the α 5 subunit was colocalized with the α 1 and α 3 subunits only in cerebral cortex and in the hippocampal formation the α 5 subunit may be part of distinct GABA_A receptors in neuronal populations within the olfactory bulb.

GABA_A receptor heterogeneity; GABA_A receptor isoform $\alpha 5/\beta 1$; GABA_A receptor isoform $\alpha 3/\beta 1$; Hybridization histochemistry, in situ; Subunit expression; (*Xenopus* oocytes)

1. INTRODUCTION

GABA_A receptors, the major synaptic targets of the neurotransmitter GABA in the CNS, constitute gated chloride channels. By virtue of their allosteric modulation by drugs such as barbiturates and benzodiazepines, they serve as pharmacological control elements for the regulation of anxiety, vigilance, muscle tension and epileptiform activity [1,2]. Based on photoaffinity labeling [3,4] and receptor purification [5-7] two polypeptides, the α and β subunit, were originally identified as major constituents. Molecular cloning of subunit cDNAs, however, revealed an unexpected subunit heterogeneity comprising at least four classes of subunits $(\alpha, \beta, \gamma, \delta)$ which share an amino acid sequence identity between 35 and 40% [8-13]. Each class of subunits may comprise several variants which share a close sequence homology (70-75%). So far, three α subunits $(\alpha 1, \alpha 2, \alpha 3)$ [14], three β subunits $(\beta 1, \beta 2, \beta 3)$ [9], one γ subunit (γ 2) [11,12] and one δ subunit [10] have been identified. We now report on the cloning, functional expression and mRNA distribution of a further α subunit variant in rat brain.

2. MATERIALS AND METHODS

2.1. Isolation of cDNA clones

A λ gt11 rat brain cDNA library [13] was screened with a 2700-bp 32 P-nick-translated fragment encoding the entire bovine GABA_A

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receptor $\alpha 1$ subunit [8]. The positive cDNA clones were subcloned into the M13RF vector mp19 (Pharmacia) before sequencing. An overlapping set of deletions was generated using the exonuclease III method described by Henikoff [15]. DNA sequencing was performed by the chain-termination technique of Sanger et al. [16]. Sequence analysis was performed on a Vax terminal using the GCG program (Genetics Computer Group, University of Wisconsin).

2.2. Expression of cloned subunits in Xenopus oocytes

The rat $\alpha 1$ (1503 bp), $\alpha 3$ (3800 bp), $\alpha 5$ (2000 bp) and $\beta 1$ (1941 bp) cDNAS were cloned into the pSpT19 vector (Pharmacia) for transcription. The cRNAs were capped and polyadenylated as previously described [13]. Follicle cells from Xenopus laevis were mechanically isolated, maintained in culture, and, on the following day, microinjected with about 50 nl of a solution containing each cRNA at a concentration of 200 nM. The follicular cell layers were removed as described [17] 1 day before the electrophysiological measurements. Oocytes were placed on a nylon grid in a 0.4 ml bath, which was perfused throughout the experiment at 6 ml/min with 90 mM NaCl/1 mM KCl/1 mM MgCl₂/1 mM CaCl₂/5 mM Hepes-NaOH (pH 7.4). The effect of diazepam was tested using a fast perfusion technique, which permitted medium exchanges in the subsecond time range [12]. All experiments were carried out at room temperature (22-26°C). For the current measurements the oocytes were impaled with two microelectrodes and the membrane potential voltageclamped at -100 mV as described [17]. Current amplitudes were read as the peak currents. Dose-response curves were fitted using a nonlinear least-squares method (Gauss-Newton-Marquardt).

2.3. In situ hybridization histochemistry

2.3.1. Tissue preparation

Male rats (specific pathogen-free (SPF) albino, Füllinsdorf, Switzerland) weighing 120–130 g were decapitated, the brains removed, then immediately frozen on dry ice and stored at -80° C until used. Cryostat sections (12 μ m) were mounted on slides, previously coated twice with 0.5% gelatin + 0.5% CrK(SO₄)₂, then fixed in 4% paraformaldehyde (in PBS pH 7.4) for 40 min followed by three 5 min washes in PBS, then stored at -20° C until used.

2.3.2. Oligonucleotide labelling

Subunit-specific synthetic oligodeoxyribonucleotide probes (Mcd prob A.S.) representing sense and antisense sequences of the rat α 1 subunit (bp 1144–1197) [13], the α 3 subunit (bp 1535–1585) and the α 5 subunit (bp 1486–1537) were labelled at the 3' end using terminal deoxynucleotidyl transferase (BRL) and ³⁵S-dATP (New England Nuclear). The reaction mixture (30 μ l total) contained 20 μ l ³⁵S-dATP, 6 μ l tailing buffer (BRL: 500 mM potassium cacodylate pH 7.2, 10 mM CoCl₂, 1.0 mM DTT) and 1 μ l terminal deoxynucleotidyl

transferase (15 U/ μ l). The cocktail was transferred to 37°C for 5 min, then the reaction was stopped by adding 70 μ l spun-column buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.5) to a final volume of 100 μ l. The labelled probe was separated from unincorporated nucleotides with a Sephadex G50 spun-column (4 min at $1600 \times g$, Sorvall SW 24).

2.3.3. In situ hybridization

Brain sections were brought to room temperature for 1 h before

1 GAATTCCCTTTTTTGATGTACAGTTTTTAGGAGCTCATTATTATCCTTGGAGATGGAACGGAGCAGACAAGATTGTCAAACCTTTATTTGGAGCCTGGG TTAGAAGGCACGCCCATAGAGATGGAAGGGAGAAAAAGGAAAAAAGGAGGAAGCTCACACTTTGGTCCTGAAGAGGGAAGTAAGCATCTCACAGCGGCTT 101 -28 I S I L P G T T G V Q G E S R R Q E P G D F V K Q D I G G L S P K H -9 GATTAGTATTCTACCTGGAACCACTGGCCAAGGGGAGTCAAGACGACAAGAACCTGGGGACTTTGTGAAGCAAGATATTGGAGGCCTCTCTCCCAAGCAT 301 25 A P D I P D D S T D (N) I T I F T R I L D R L L D G Y D N R L R P G L GCCCCAGATATTCCCGACGATAGTACAGATÃACATCACTATCTTCACTAGAATCTTGGATCGGCTTCTGGATGGCTATGACAACCGACTGCGACCTGGGC G D A V T E V K T D I Y V T S F G P V S D T D M E Y T I D V F F R 59 501 TTGGAGATGCAGTGACTGAAGTGAAGACAGATATCTATGTGACCAGTTTTGGCCCTGTGTCAGACACTGATATGGAATATACTATTGATGTGTTTTTTAG Q T W H D E R L K F D G P M K I L P L N N L L A S K I W T P D T F ACAGACATGGATGAAGACTGGAAATTTGATGGACCAATGAAGATCCTTCCACTGAATAACTTCTCGGCTAGTAAGATATGGACTCCAGATACCTTC 92 125 F H N G K K S V A H ® M T T P N K L L R L V D ® G T L L Y T M R L T TTCCACAACGGTAAAAAATCAGTGGCTCACÄATATGACCACCCCAACAAGGTGCTCAGACTGGTAGACÄATGGGACCCTCCTCTATACAATGAGGTTAA 159 I H A E C P M H L E D F P M D V H A C P L K F G S Y A Y T K A E V CANTACATGCTGAATGCCCTATGCATTTAGAAGATTTCCCCATGGATGTGCATGCCTGTCCACTGAAGTTTGGAAGCTATGCCTATACCAAAGCTGAAGT 801 192 I Y S W T L G K N K S V E V A Q D G S R L N Q Y D L L G H V V G T E I I R 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 L P C
GAGATAATCCGGTCTAGTACAGGAGAATATGTCGTCATGACAACCCACTTTCATCTGAAGAGAAAAATTGGCTACTTTGTCATCCAGACCTACTTGCCAT 225 1001 259 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 T V L T 1101 GTATCATGACTGTCATTCTGTCACAAGTTTCTTTCTGGCTTAATAGAGAATCTGTCCCTGCTCGCACAGTCTTTTGGTGTCACCACTGTTCTCACCATGAC •M3• T L S I S A R N S L P K V A Y A T A M D W F M A V C Y A F V 292 CACCTTGAGTATCAGTGCCAGAAACTCTTTACCTAAAGTGGCATACGCGACGGCCATGGACTGGTTCATGGCCGTCTGTTATGCTTTTTTCTGCA 1201 LIEFAT V[°]NYFT KRSWAWEGKKVPEALEMKKKTPA 325 1301 359 A P T K K T S T T F N I V G T T Y P I N L A L D T E F S T I S K A CAGCCCCAACCAAGAAAACAAGCACCACCTTCAACATAGTGGGAACCACCTATCCTATCAACCTTGGCCTTGGATACTGAGTTCTCCACCATCTCCAAGGC 1401 392 A A A P S A S S T P T V I A S P K T T Y V Q D S P A E T K T Y N S TGCTGCTGCTCCCAGTGCTTCTTCAACTCCAACAGTGATTGCTTCCCCCAAGACCATTATGTGCAAGACAGTCCTGCTGAGACCAAGACCTACAACAGT – M4 – 425 V S K V D K I S R I I F P V L F A I F N L V Y W A T Y V N R E S A I 1601 GTCAGCAAGGTTGACAAAATTTCCCGCATCATCTTCCCTGTGCTCTTTGCCATATTCAATCTTGTCTATTGGGCCACATATGTGAACAGGGAATCCGCTA 459 KGMIRKQ* 1701 TCAAGGCATGATCCGCAAACAGTAGATAATAGTGGCAGCACAGCAACCAGATCACCCATGAAGCATCCAAATCCCAAACCCCAGAGCTCCC

Fig.1. Nucleotide and deduced amino acid sequence of the α 3 subunit (a) and the α 5 subunit (b) of the GABA_A receptor in rat brain. Potential sites of N-linked glycosylation are encircled. The proposed membrane spanning domains (M1-M4) are indicated by solid lines. The arrow points to the putative signal sequence cleavage site.

GARCARCA ACCOMPROMISOCIARO CONTROCO CONTROCO CONTRAGRACA TORONO CONTROLO CO

carrying out a prehybridization step consisting of a 1 h incubation at room temperature in 50 μ l of a solution containing 4×SSC (1×SSC is 0.15 M NaCl, 0.015 M trisodium citrate pH 7.0), 2 mg/ml BSA, 10% dextransulfate, 1 mg/ml t-RNA (Boehringer Mannheim), 1 mg/ml Poly A (Sigma), 50% deionized formamide (Bethesda Research Laboratories) and 10 mM dithiothreitol (Fluka) under Parafilm coverslips. For the hybridization step, labelled probe

 $(1.5 \times 10^6 \text{ cpm in } 50 \,\mu\text{l} \text{ per section})$ was added and incubated in a moist chamber at 30°C for 20–24 h. After rinsing the sections briefly in $2 \times \text{SSC}/50\%$ deionized formamide and 10 mM dithiothreitol (first for 15 min at room temperature, then three times for 15 min at 40°C) and afterwards in $2 \times \text{SSC}$ and 10 mM dithiothreitol (three times for 1 h at room temperature), the sections were dehydrated in ethanol, exposed to sheet film (Hyperfilm, β/Max , Amersham) and then dipped

b 1	GAGGAGGAAGGCCTCCTGGCGAGCCTCAGCCGGCCGCTCAGGGGGGCTAAGGAGGTCTGGAGGGCTCGGCGCGCACTAAAGGCCCGGACCACTAGAGGTATC
	GGGTGGGGCTGCGGCAGCAGCAGCTGGGGGTGGGGGAGGCTGCGGGCTCCAGTGCCATCCCTTATTCCACCTGCGCTGTCAGCATGCACCTTGCAGCATGCACCTTGCAGCATGCACCTTGCAGCATGCACCTTGCAGCAACCAGGAACCAGGAACCAGGATGCTGCTGCGCTTTTTCTTAAATTCACACATCAAGATCAAGATCAAGATCAAGATGAAGGAAG
201	GAAGCAGCTTGCTGGCTTTGAACGTGTGGCAAATATTTCAMAAAGCTTCAMATCAAATTGAAGAAAGGACGTTTTCTTCTAAATTGATTGCTTCA
-31	
301	ACTATTATTCTTACTGGGAATGGACAATGGAATGCTCTCTAGATTTATCATGACCAAAACGCTCCTTGTCTTCTGCATTTCCATGACCTTATCCAGTCAC
	F G F S Q M P T S S V Q D E T N D (N) I T I F T R I L D G L L D G Y D
-4	F G F S V Q M P T S S V Q D E T N D (N) I T I F T R I L D G L L D G I D TTTGGCTTTTCACAAATGCCAACTAGTTCTGTACAAGATGAGACCATGACATCACAATATTCACCAGGATCTTGGACGGCTCTTGGATGGCTATG
401	TTTGGCTTTTACAAATGCCAACTAGTTCTGTACAAGATCATCACAATATTCACCAGGATCTTGGACGGGCTCTTGGATGGCTAG
31	NRL R P G L G E R I T Q V R T D I Y V T S F G P V S D T E M E Y
501	ACAACAGACTGCGGCCTGGGAGGGGGAATCACGCAGGTGCGAACAGACATCTATGTTACCAGCTTTGGCCCAGTGTCCGACACGGAAATGGAATA
	TIDVFFRQSWKDERLRFKGPHQRLPLNNLLARK
	TACCATAGATGTATTTTCCCTCAAAGCTGGAAAGATGAAAGGCTGCGGTTTTAGGGGCCTATGCAACGTCTCCTCCTACAACATCTTTCCCAGGAAA
002	
	I W T P D T F F H N G K K S I A H ® M T T P N K L L R L E D D G T L
701	ATCTGGACCCCAGACACATTCTTCCACAATGGGAAGAAGTCCATTGCGCACĀACATGACGACACCCAACAAGCTGCTGAGGGCTGAGGATGATGGCACAC
131	LYTMRLTISAECPMQLEDFPMDAHACPLKFGSY
	TECTURACACCANGGOCCTURACGATCTCTGCTGAGTGTCCAATGCAGCTTGAGGACTTTCCGATGGATG
164	AYPNSEVVYVWT®GSTKSVVVAEDGSRLNQYHL
901	TGCTTACCCTAATTCGGAAGTTGTCTATGTTTGGACCAATGGTTCCACCAAGTCTGTGGTGGCAGAAGATGGCTCCAGACTCAACCAGTACCACCTC
197	M G Q T V G T E (R) I S T S T G E Y T I M T A H F H L K R K I G Y F V
1001	ATGGGGCAGACAGTAGGCACTGAGAACATCAGCACCAGCACGGTGAATATACAATCATGACTGCTCATTTTCACCTGAAGAGGAAGATCGGGTACTTTG
	M1 M2
231	M1 — M2
231 1101	IQTYLPCIMTVILSQVSFWLNRESVPARTVFGV
	I Q T Y 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 TCATCCAGACGTACCTTCCCTGCATCATCACAGTCATCTTATCCCAGGTGTCTTTTTGGCTTAATCGAGAATCTGTCCCAGCTAGGACAGTTTTTGGAGT
1101	I Q T Y 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 TCATCCMACGTACCTTCCCTGCATCATGACAGTCATCTTATCCCAGGTGTCTTTTTGGCTTAATCGAGAATCTGTCCCAGCTAGGACAGTTTTTGGAT
1101	I Q T Y 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 TCATCCMACGTACCTTCCCTGCATCATGACAGTCATCTTATCCCAGGTGTCTTTTTGGCTTAATCGAGAATCTGTCCCAGCTAGGACAGTTTTTGGAT T 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 V C
1101	T 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 V C GACCACAGTGCCTGACCATGACAACCCTCAGCATGACCAGTGCCCGGAATTCGCCCCAAAGTGGCCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACCATGACTGCCCCGGAATTCGCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGGTTCATTGCTGTCTGC
264 1201	T 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 V C GACCACAGTGCCTGACCATGACCACCAGCATGCCTCCTGCCCCAAAGTGGCCTATGCCCACAGCCATGACCATGCTCCTGCCCCAAAGTGGCCTATGCCACAGCCATGACCACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGACTGGTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCTGCCACAAAGTGGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGCTGCACAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGCTGCCACAGCCATGGACTGGTTCATTGCTGCTGCCACAGCCATGGACTAGCACAGCCATGGACTAGCACAAGCCCACAGCCATGGACTAGCACAAGCCCACAGCCATGGACTAGCACAAAAAAAA
264 1201 297	T 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 V C GACCACAGTGCTGACCATGACACCCTCAGCATCAGTGCCCGGAATTGCCGCCCAAAGTGGCCTATGCCACAGCCATGGACTGCTCTCTCC X A F V F S A L I E F A T V N Y F T K R G W A W D G K K A L E A A K
264 1201 297	T 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 V C GACCACAGTGCCTGACCATGACCACCAGCATGCCTCCTGCCCCAAAGTGGCCTATGCCCACAGCCATGACCATGCTCCTGCCCCAAAGTGGCCTATGCCACAGCCATGACCACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTCTCTGCCCCAAAGTGGCCTATGCCACAGCCATGACTGCTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGACTGGTTCATTGCTGTCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGTCTGCTGCCACAAAGTGGCCATGGACTGGTTCATTGCTGTCTGCCACAGCCATGGACTGGTTCATTGCTGCTGCACAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGCTGCCACAGCCATGGACTGGTTCATTGCTGCTGCCACAGCCATGGACTAGCACAGCCATGGACTAGCACAAGCCCACAGCCATGGACTAGCACAAGCCCACAGCCATGGACTAGCACAAAAAAAA
264 1201 297	T 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 V C GACCACAGTGCTGACCATGACACCCTCAGCATCAGTGCCCGGAATTGCCGCCCAAAGTGGCCTATGCCACAGCCATGGACTGCTCTCTCC X A F V F S A L I E F A T V N Y F T K R G W A W D G K K A L E A A K
264 1201 297 1301	T 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 V C GACCACATGACATCATGACCACCACCACCACCACCACCACCACCACCACCACCACC
264 1201 297 1301	T 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 V C GACCACAGTGCTAGCCATGACCATCAGCACTCAGTGCCCGGAATTCGCCCCCAAAGTGGCCTATGCCACAGCCATGGACTGCTCTCTCCC Y A F V F S A L I E F A T V N Y F T K R G W A W D G K K A L E A A K TATGCATTTGCTTCTCTCTCCCCTGATTGAATTTGCCACAGTCAACTACTTTACAAAGAGAGGATGGCCTGGGATGGCAAGAAGGCCTTGGAAGCAGCTA I K K K E R E L I L N K S T N A F T T G K L T H P P N I P K E Q L
264 1201 297 1301	T 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 V C GACCACATGACATCATGACCACCACCACCACCACCACCACCACCACCACCACCACC
264 1201 297 1301 331 1401	T 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 V C GACCACATGACATCATCACAACATCCCAGCTAGCACATCCACAGCAGCAGCATGCCTCCCACAAGAGAGCCTAGCCACAGCATGCTCTCCCAGCATGCTCTCCCAAAGAGAGGCCTAGCCACAGCATGCTCATGCTCTCCCAAAGAGAGGCCTAGCCACAGCCATGACCATCCCTAGCATCATTGCTCTCCCCAAAGAGGGGCTAGCCCACAGCCATGACTGCTCATTGCTGCTCTCCCAAAGAGAGGAGTGCTCTCTCT
264 1201 297 1301 331 1401	T 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 V C GACCACAGTGCTACCATGACAGCCTCAGCATCATGCCCGGAATTCGCCCCAAAGTGCCTATGCCCAAGCATGGCTCTCTCCC M3 Y A F V F S A L I E F A T V N Y F T K R G W A W D G K K A L E A A K TATGCATTTGCTTCTCTCCCCTGATTGAATTTGCCACAGTCAACTACTTTACAAAGAGAGAG
264 1201 297 1301 331 1401	T 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 V C GACCACATGACATCATCACAACATCCCAGCTAGCACATCCACAGCAGCAGCATGCCTCCCACAAGAGAGCCTAGCCACAGCATGCTCTCCCAGCATGCTCTCCCAAAGAGAGGCCTAGCCACAGCATGCTCATGCTCTCCCAAAGAGAGGCCTAGCCACAGCCATGACCATCCCTAGCATCATTGCTCTCCCCAAAGAGGGGCTAGCCCACAGCCATGACTGCTCATTGCTGCTCTCCCAAAGAGAGGAGTGCTCTCTCT
264 1201 297 1301 331 1401	TOTOLOGGIACOTTCCCTGCATCATGACAGCATCAGCATCAGCACCAGCAGGAGGAGCAGCATCGCAGAGAGCAGCATCGCAGCAGGAGGAGCAGCATCGCAGCAGGAGGAGCAGCATCGCAGCAGGAGGAGCAGCATCGCAGCAGGAGGAGCAGCAGGAGGAGCAGGAGGAGCAGGAGG
264 1201 297 1301 331 1401 364 1501	T 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 V C GACCACAGTGCTACCATGACAGCCTCAGCATCATGCCCGGAATTCGCCCCAAAGTGCCTATGCCCAAGCATGGCTCTCTCCC M3 Y A F V F S A L I E F A T V N Y F T K R G W A W D G K K A L E A A K TATGCATTTGCTTCTCTCCCCTGATTGAATTTGCCACAGTCAACTACTTTACAAAGAGAGAG
264 1201 297 1301 331 1401 364 1501	TOT Y 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 TCATCCMACGTACCTCCCTGCATCATGACAGTCATCTTATCCCAGGTGTCTTTTTGGCTTAATCGAGAATCTGTCCCAGCTAGGACMGTTTTTGGAGT T 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 V C GACCACAGTGCTGACCATGACAACCCTCAGCATCAGTGCCCGGAATTCGCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGC WA F V F S A L I E F A T V N Y F T K R G W A W D G K K A L E A A K TATGCATTTGTCTTCTCTGCCCTGATTGAATTTGCCACAGTCAACTACTTTACAAAGAGAGGAGTGGGCCTGGGATGGCAAGAAGGCCTTGGAAGCAGCTA I K K K E R E L I L N K S T N A F T T G K L T H P P N I P K E Q L AAATCAAGAAAAAAGAACGTGAACTCATACTAAATAAGTCAACAATGCTTTTTACAACTGGGAAGTTGACCCATCCTCCAAACATCCCAAAGGAGCAGCT P G G T G N A V G T A S I R A S E E K T S E S K K T Y N S I S K I TCCAGGGGGGGACTGGGAATGCTGTGGGTACAGCCTCAAATCAGCAACAGCATCAGCAAGAGACCTCAAACAGCATCAGCAAGAGACCTCAAACAGCATCAGCAAGAGACCTCAAACAGCATCAGCAAGAGACCTCAAACAGCATCAGCAAGAGACCTCAAACAGCATCAGCAAGAGACCTCAAACAGCATCAGCAAGAGACCTCCAAACAGCATCAGCAAGAGACCTCCAAACAGCATCAGCAAGAGACCTCCAAACAGCATCAGCAAGAGCCTCAAACAGCATCAGCAAGAGCCTCCAAACAGCATCAGCAAGAGCCTCCAAACAGCATCAGCAAGAGCCTCCAAACAGCATCAGCAAGAGCCTCCAAACAGCATCAGCAAGAGCCTCCAAACAGCATCAACAACAGCATCAAGCAAG
264 1201 297 1301 331 1401 364 1501	TOTATCCAGACCATCCCTCCCTGCATCATCAGCACCATCTTATCCCAGGTGTCTTTTTGGCTTAATCGAGAATCTGTCCCAGCTAGGACAGTTTTTTGGAGT TOULT MOTE LSISARNS LPKVAYA TAM DWFIAVC GACCACAGTGCTGACCATCAGCACCCTCAGCATCAGTGCCCGGAATTCGCTGCCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGC M3 YAFVFSALIEFATVNYFTKRGWAYWOODGCCTGGAATCGCTGCCCCAAAGTGGCCTTGGCAGCCATGGACTGGTTCATTGCTGTCTGC IKKKERELILNKSTNAFTCAACAATGCTTTTACAAAGAGAGGAGTGGCCTGGAAGAAGCCTTGGAAGCAGCATCAACAAGAGAGAG
264 1201 297 1301 331 1401 364 1501	T 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 V C GACCACAGTGCTAGCATGACAGCCTCAGCATCAGTGCCCGGAATTCGCCGCCAAAGTGGCCTAGCCAGCC
264 1201 297 1301 331 1401 364 1501 397 1601	TOTYLPCIMTVILLSQVSFWLNRESVFARTVFGV TCATCCAGACGTACCTTCCCTGCATCATGACAGCATCTTATCCCAGGTGTCTTTTTGCTTAATCGAGAAACTGTCCCAGCTAGGACAGTTTTTGGAGT TTVLTMTTLSISARNSLPKVAYAATAAMDWFIAVC GACCACAGTGCTGACCAGACCACTCAGCATCAGTGCCCGGAATTGCCGCCCAAAGTGGCCTATGCCACAGCCATGGACTGGTTCATTGCTGTCTGC M3 YAFVFSALIEFATVNYFTKRGWAWAWDGKKAALEAAAK TATGCATTTGCTTCTCTCCCCTGATTGAATTAGCCACAGTCAACTACTTTACAAAAAGAGAGGATGGCCTGGGATGGCAAGAAGCCTTTGGAAGCAGCTA IKKKERELILNKSTNAATAAGTCAACAAATGCTTTTACAACTGGGAAGTTGACCCCATCCTCCAAAACATCCCAAAAGGAGCAGCT PGGTGNAVGGACTCATACTAAATAAGTCAACAAATGCTTTTACAACTGGGAAGTTGACCCATCCTCCAAACATCCCAAAAGGAGCAGCT PGGTGNAVGGACTGGGATGCTTGGGTACAGCCTCAATCAGGAACATCTGAAGGAGCAGCTCTGAGAGAACATCCCAAAAGGAGCATCAGCAACAGCATCAGCAACAGCATCAGCAACAGCATCAGCAAGAGACCTCAAAAAAGACCTCCAAAAAGGAGCCTCAATCAGCAACATCTGAAGGAGCATCAGCAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAACATCAGCAACAAAAAGGGGCTA DKMSRIVFPILLFGTTTGGCACCTTCAATCTAGGTTTACTGGGCAACAATATTGAATAAGGGAGCCCGTGATAAAAAGGGGCTA SPK*
264 1201 297 1301 331 1401 364 1501 397 1601 431 1701	T 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 V C GACCACAGTGCTGCACCATCAGCACCACCAGGGGAATTCGCCCCAAAAATGGCCTATGCCCACAGCCATGGACAGTTCATGCGGCAACACCCTCGCCCAAAATGGCCCCAAAAGGGCCTTGGAAGCAGCATGGCTGACCATGCCTCAAACATGCCTCACCAAAAAGACAAAAAAAA
264 1201 297 1301 331 1401 364 1501 397 1601 431 1701 1801 1901	TOT Y 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 TCATCCAGACGTACCTTCCCTGCATCAGACAGCATCTTATCCCAGGTGTCTTTTTGGCTTAATCGAGAATCTGTCCCAGCTAGGACAGTTTTTGGAGT T 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 V C GACCACAGTGCTGACCAGCATGACCACCAGCAATGGCCCGGAATTCGCCCCAAAGTGGCCTAGCCAGCC
264 1201 297 1301 331 1401 364 1501 431 1701 1801 1901 2001	TOTAL POLIMIT VILS Q V S F W L N R E S V F A R T V F G V TOTAL CONSCINATION CONTINUATION CONTINU
264 1201 297 1301 331 1401 364 1501 431 1701 1801 1901 2001 2101	TO TO Y 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 TEATCCAGACGACCTCCCCCCCCCCCCCCCCCCCCCCCCC
264 1201 297 1301 331 1401 364 1501 397 1601 431 1701 1801 1901 2001 2101 2201	TOTAL 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 TEATCCAGACGTACCTCCCTGCATCAGACAGTCTTTTGGAGT TOTAL T N 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 V C GACCACAGTGCTCAGACCATCAGCACCAGGCATCAGTCCTCACAGGACTCTTCCTCCCAGACTGCCCCAAAGTGCCCACAGCCATGGACCATGCTCATTGCTGTCTCCCCCAAAGTGCCCACAGCCATGGACCATGGACCATGCTCATTGCTGTCTCCCCCAAAGTGCCCTTAGCCACAGCCATGGACCATGGACCATGCTCTCTCCCCCCAAAGTGCCCTTGCAGCCATGGACCATGCTCTCTCT
264 1201 297 1301 331 1401 364 1501 431 1701 1801 1901 2001 2101 2201 2301	TO TYLFCIM TVILS QVSFWLNRESSVPARTVFGVFGVTTCATCCAGGAGTCATCTCCAGCAGGACAGTTTTTGGGGTTCATTCGGGATCACCAGGACAGTTTTTGGGGTTCATTCGGGATCACCAGGACAGTTTTTGGGGTTCATTCGGGATCACCAGGACAGTTTTTGGGGTTCATTCGGGATCACCAGGACAGTTCCCAGGACAGTTCCCAGGACAGTTCCCAGGACAGTTCCCAGGACAGTTCCCAGACCACGGAATCCCCCAAAGTGGCCCAAAGTGGCCAAAGAGGCCTTGGAAGCAGCACAGTCACCACAGGACACAGTGGCCCAAAGTGGCCCAAAGTGGCCCAAAGTGGCCACAGCCATGGCAAGAAGAGGCCTTGGAAGCAGCACATTACCAAAGAGAAAAAGACATCCCCAAAACACACCCTTACAACAGGAACAATTCTCAGAATTACCAAAGAGAAAATGCCCTTAAAACAAGACAAATTCTCAACAAGAAAATGCCCTTAAAAACAAAATGCCTTTCAAACAAGAAAAATGCCCTTAAAAACAAAATGCCTTTCAATCTAAGAATTACTTCAAAGTAAAAAAAA
264 1201 297 1301 331 1401 364 1501 431 1701 1801 2001 2101 2201 2301 2401	TOTAL 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 TEATCCAGACGTACCTCCCTGCATCAGACAGTCTTTTGGAGT TOTAL T N 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 V C GACCACAGTGCTCAGACCATCAGCACCAGGCATCAGTCCTCACAGGACTCTTCCTCCCAGACTGCCCCAAAGTGCCCACAGCCATGGACCATGCTCATTGCTGTCTCCCCCAAAGTGCCCACAGCCATGGACCATGGACCATGCTCATTGCTGTCTCCCCCAAAGTGCCCTTAGCCACAGCCATGGACCATGGACCATGCTCTCTCCCCCCAAAGTGCCCTTGCAGCCATGGACCATGCTCTCTCT

in a nuclear track emulsion (Ilford (Warnington, PA) K5, diluted 1:1 with distilled water) to reveal the regional and cellular localization of the α subunit subtypes mRNAs.

3. RESULTS AND DISCUSSION

Screening of a rat brain cDNA library with the bovine $\alpha 1$ subunit cDNA probe containing the entire coding sequence [8] resulted in the isolation of six positive clones, of which two $\lambda oT\alpha 2F$ 3800 bp and $\lambda oTR\alpha F3$ 3000 bp hybridized only weakly but displayed distinct patterns in restriction mapping. The clone $\lambda oT\alpha 2F$ (1792 bp) contained an open reading frame of 1468 nucleotides encoding a protein of 493 amino acid residues (fig.1a). Its deduced amino sequence revealed a 96% sequence identity with bovine $\alpha 3$ subunit suggesting that this clone is the rat homologue of the $\alpha 3$ subunit. The sequence homology with the rat $\alpha 1$ and bovine $\alpha 2$ subunits amounted to 71% and 72%, respectively.

The clone $\lambda o Tr \alpha F3$ (2591 bp; fig.1b) encoded a polypeptide of 433 amino acid residues (48 kDa). It displayed a striking sequence homology to the known α subunit variants. Its sequence identity with the rat $\alpha 1$ subunit [8] was 70%, with the bovine $\alpha 2$ subunit [14] 74% and with the rat $\alpha 3$ subunit 69%. These findings suggest that the isolated clone codes for a novel α subunit variant, which was termed $\alpha 5$.

The structural features of the deduced polypeptides of the $\alpha 3$ and $\alpha 5$ subunits conformed with those of other subunits of the GABA_A-receptor [19]. Potential N-linked glycosylation sites (four each in $\alpha 3$ and in $\alpha 5$) and potential disulfide-bonded loops are located on the presumed extracellular domain. Hydropathy analysis revealed four putative membrane-spanning regions (M1-M4). The domains of M1-M3 are virtually iden-

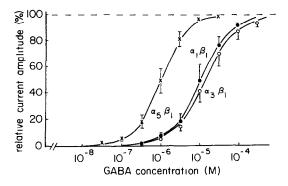


Fig.2. GABA dose-response curves recorded from *Xenopus* oocytes injected with RNAs coding for either $\alpha 1 + \beta 1$ subunits ($\bullet - \bullet$), $\alpha 3 + \beta 1$ subunits ($\circ - \bullet$) or $\alpha 5 + \beta 1$ subunits ($\times - \times$). For each subunit combination the dose-response curve was obtained at least three times in oocytes exhibiting different degrees of channel expression. Each curve was fitted with the equation given in the text. Maximal current was arbitrarily set at 100% and the current amplitudes were expressed in relative values. The curves shown were obtained by reapplying the fit to the standardized averaged values.

tical between the rat $\alpha 1$, $\alpha 3$ and $\alpha 5$ subunits with only one conservative amino acid change ($I \rightarrow M314$) in the $\alpha 3$ sequence. The lowest degree of sequence homology was found in the signal peptides [20], in the most extracellular N-terminus and in the presumed cytoplasmic loops connecting the M3 and M4 domains.

In order to test whether the new α 5 subunit variant differed functionally from the known members of the α subunit family, the α 5 subunit RNA was coexpressed with the rat β 1 subunit RNA in *Xenopus* oocytes. The resulting receptors were compared to those coexpressed from either $\alpha 1 + \beta 1$ or $\alpha 3 + \beta 1$ subunit RNAs. Upon superfusion with GABA, inward currents were recorded under voltage clamp conditions. Dose- response studies revealed (fig.2) that the receptor expressed from α 5 + β 1 RNAs had a higher sensitivity to GABA than the receptors expressed from either $\alpha 1 + \beta 1$ RNAs or $\alpha 3 + \beta 1$ RNAs. The dose-response curves were fitted with the equation $I(c) = I_{\text{max}}c^{\text{n}}/(c^{\text{n}} + K_{\text{a}}^{\text{n}})$, where I_{max} is the maximal current amplitude, c is the GABA concentration, n the Hill coefficient, and K_a the GABA concentration that elicits half-maximal current response. The best fit indicated K_a values of 13 μ M, 14 μ M and $0.97 \,\mu\text{M}$ for the combinations of the β 1 subunit with the







Fig. 3. In situ hybridization with 35 S-labeled antisense probes of the α 1 subunit (a), α 3 subunit (b) and α 5 subunit (c) in parasagittal sections of rat brain. White areas represent regions fo hybridization Bar = 2 mm.

 $\alpha 1$, $\alpha 3$ and $\alpha 5$ subunits, respectively. Thus, the $\alpha 5$ subunit appears to confer a differential sensitivity to GABA-gating of the channel. However, for all subunit combinations studied some variability in the individual K_a values determined in different oocytes was observed even when oocytes from the same batch of cells were used. The variability was most prominent for the combination of $\alpha 3 + \beta 1$ subunits. In this case a K_a value of µM was deduced from three individual dose-response curves as averaged in fig.2. Two additional dose- response curves indicated a K_a in the submicromolar range. The reason for this diversity is not clear. Possibly different oocytes assemble ion channels with different subunit stoichiometries. When GABAgated channels are expressed from a triple-combination of subunits, $\alpha 1 + \beta 1 + \gamma 2$, no such variability in the dose-response curves is observed [12].

The limiting slopes in log/log plots of the dose-response curves shown in fig.2 revealed Hill coefficients of 0.9, 0.9 and 1.2 for the combinations of the β 1 subunit with the α 1, α 3 and α 5 subunits, respectively. The current response elicited by GABA was not potentiated by 1 μ M diazepam in any of the subunit combinations tested.

Injection of $\beta 1$ subunit RNA leads to the formation of anion channels which are open in the absence of GABA [21]. Co-injection with either $\alpha 1$, $\alpha 3$ or $\alpha 5$ subunit RNA prevented the formation of these channels. Thus, the assembly of heteroligomeric channels appears to be favoured over the formation of homomeric channels.

Among the known α subunit variants, the α 1 subtype has a very wide distribution in rat brain. This was shown earlier by in situ hybridization histochemistry using a cRNA probe [18] and was now confirmed using a subunit-specific oligonucleotide probe (fig.3a). The brain regions containing α 1-mRNA include the olfactory bulb mitral cells, cerebral cortex (layers II, III, V, VI), anterior olfactory nucleus, hippocampal pyramidal cells, dentate gyrus granule cells, various thalamic nuclei, colliculi, cerebellar Purkinje cells, stellate, basket and granule cells, deep cerebellar nuclei (n. interpositus), brainstem nuclei (e.g. vestibular, facial, motor trigeminal). In order to explore the physiological relevance of the α 3 and α 5 subunit variants in rat brain, their pattern of gene expression was visualized in brain sections using sequence specific 35S-labeled sense and antisense oligonucleotide probes. While the sense probes resulted only in background labeling, the antisense probes revealed a striking hybridization pattern. The mRNA for the α 3 subunit (fig.3b) was codistributed with $\alpha 1$ in most brain regions, although the intensity of the hybridization signal was considerably lower than that of $\alpha 1$. Notable exceptions which did not reveal an α 3-signal include the cerebellar Purkinje cells and cells

in the molecular layer, the pallidum and substantia nigra pars reticulata. A weak $\alpha 3$ hybridization signal but no $\alpha 1$ signal was observed in caudate-putamen and substantia nigra zona compacta. The $\alpha 5$ mRNA (fig. 3c) was more restricted in its distribution than $\alpha 3$ mRNA. Its hybridization signal codistributed with the $\alpha 1$ and $\alpha 3$ signal only in cerebral cortex layers (particularly layer VI) and in the hippocampal formation. A weak hybridization signal was additionally observed in the olfactory bulb granular layer in which neither $\alpha 1$ nor $\alpha 3$ signals were detected. Our results show that the genes of the $\alpha 3$ and $\alpha 5$ subunits can be differentially regulated and may contribute to distinct GABA_A-receptors in different neuronal populations. These receptors may display a differential sensitivity to GABA.

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