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Cloning of a glycine receptor subtype expressed in rat brain and spinal cord during a specific period of neuronal development

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Complementary (c) DNAs encoding a glycine receptor (GlyR) isomer were cloned from libraries constructed in λZAPII with poly (A)$^+$ RNA of neonatal rat spinal cord. Northern blot analysis revealed that RNA hybridized to the cloned cDNA is detectable only for a period of late embryonic/early postnatal stage of the spinal cord. Moreover, other central nervous tissues, such as hippocampus and cerebral cortex, in the infant rats are also rich in this message. The 'neonatal (N) GlyR' has 71% homology to that of another GlyR isoform in which adult rad cord is rich (AGlyR). Injection of a single RNA transcribed from the NGlyr-cDNA into *Xenopus* oocyte induced functional formation of glycine-gated Cl^- channels, however, its pharmacological property differed from that of AGlyR.

Glycine receptor; Chloride channel; Messenger RNA; cDNA cloning; *Xenopus* oocytes; Heterogeneity; Northern blot; Neuronal development

1. INTRODUCTION

Glycine, the structurally simplest amino acid, is a major neurotransmitter in the vertebrate nervous system, especially in the spinal cord and brainstem of mammals [1]. On spinal motoneurons, glycine exerts an inhibitory action due to increase in Cl^- permeability via activation of a specific receptor/ion channel complex [1,2]. Molecular cloning work with the glycine receptor (GlyR) has been carried out using rat and human cDNA libraries, and so far 3 species of the receptor subunit cDNAs (referred to as rat and human α1, human α2 and rat β subunits) have been isolated [3–6]. In the expression systems using *Xenopus* oocytes and embryonic human kidney cell lines, the α1 or α2 protein itself has the function of a glycine-gated Cl^- channel, whereas the ability of the β protein was much less [4–8]. Northern blot analysis shows that mRNAs hybridizing to the α1 or β subunit cDNA are present abundantly in the spinal cord of matured rats (older than 20 days), but rather less abundantly in infant animal cords [3,9]. In addition to these GlyR-mRNAs, one of the authors (H.A.) has provided some evidence for the existence of another species of GlyR-mRNA, production of which is made preferentially in the growing spinal cord [9,10]. Thus, we attempted cDNA cloning of this 'juvenile' glycine receptor subunit, and identified the target.

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Abbreviations: NGlyR, neonatal glycine receptor; AGlyR, adult glycine receptor; E_{Cl}, equilibrium potential for chloride ion; IC_{50}, concentration giving 50% inhibition.

2. MATERIALS AND METHODS

2.1. *Isolation and purification of infant rat spinal cord mRNAs*

Poly (A)$^+$ RNAs were extracted from 10-day-old rat spinal cord by the phenol/chloroform method and purified by oligo-dT cellulose chromatography. The poly (A)$^+$ RNA was further purified by ultracentrifugation in a linear sucrose-density-gradient solution (32–10% w/v; 10 ml) as described previously [9,10]. The solution was collected in 0.4 ml fractions, from which the RNAs were precipitated with ethanol and dried. The RNA fractions were suspended in sterilized water (20 μl), and 50 nl of each was injected into oocytes in order to assess its ability to express functional glycine receptors by electrophysiological detection (see section 2.4.).

2.2. *Construction and screening of cDNA library*

The RNA fraction that had the most potent expressional potency for the glycine receptor (fraction No. 14, shown by the stippled bar in Fig. 1A) was used as template for the construction of a cDNA library using a λZAPII cDNA Cloning Kit and Gigapack Gold (Stratagene). The titer of the library was 2.6×10^6 pfu/ml. A mixture of oligonucleotides (5′-GT$\begin{matrix}G\\T\end{matrix}$AGTCAT$\begin{matrix}G\\T\end{matrix}GT\begin{matrix}G\\C\end{matrix}AG\begin{matrix}G\\C\end{matrix}AC\begin{matrix}G\\T\end{matrix}GT\begin{matrix}G\\T\end{matrix}$AGT-3′), which encodes an octapeptide conserved in almost all of $GABA_A$ and glycine receptor subunits so far cloned (cf. ref. 11, but for an exception see ref. 5) was used as probe for screening of the library. For sequencing the cDNAs and subsequent in vitro synthesis of RNAs, the positive λZAPII clones were excised directly into a plasmid, Bluescript SK(-) in vivo by the use of a helper phage R408. The nucleotide sequence was determined by the chain termination method [12]. Both sense and antisense strands of the cloned cDNA were analyzed.

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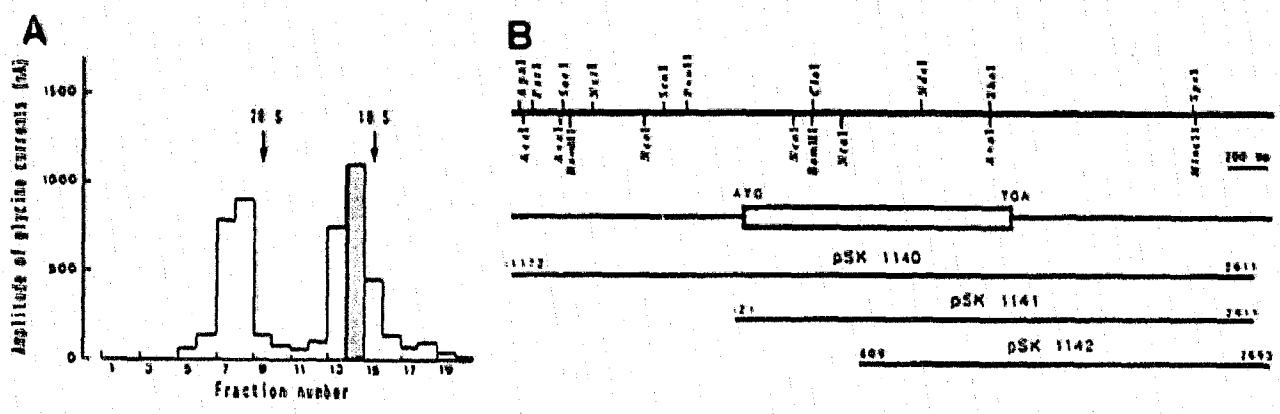

Fig. 1. (A) Expression of glycine receptors by sucrose density fractions of mRNAs from 10-day-old rat spinal cord. The fractionated mRNA was injected into *Xenopus* oocytes to examine the expressional potency for the glycine receptor. Glycine at 1 mM was applied by perfusion to oocytes which were voltage-clamped at −70 mV. Each column represents an average amplitude of glycine-induced inward currents ($n = 3$–8). (B) Size and relative position of cloned GlyR-cDNAs. The restriction enzyme map is shown on the top. The open reading frame is indicated by an open box.

2.3. *Northern blot analysis*

The RNAs (10 μg) were gel-electrophoresed in a 1% agarose gel, onto which a nylon membrane (Dupont, GeneScreen plus) was overlaid in order to transfer the RNAs by the capillary method with 10 × SSC. The blotted RNA was hybridized with the ^{32}P-labeled cDNAs (the *Bam* HI–*Xho* I fragment of pSK1141, see Fig. 1B; and the *Hinc* II–*Hinc* II fragment of pGRA1, see [6]) in a solution containing 1 M NaCl and 50% formamide at 42°C. After the hybridization for 28–36 h, the membrane was washed with 2 × SSC at 60°C. Autoradiography was carried out for 16–25 h with an intensifying screen (Dupont) at −80°C.

2.4. *Synthesis of the cloned RNA and functional expression of the cloned receptor in* Xenopus *oocytes*

The RNA was synthesized in vitro from the cDNA insert in the pBluescript SK(−) by the use of the SP6 promoter, and was injected into *Xenopus* oocytes at a concentration of 2.5–5 ng/50 nl/cell. The methods for culturing oocytes and following electrophysiological experiments were as described previously [6,9,10,13].

3. RESULTS AND DISCUSSION

Poly(A)$^+$RNAs extracted from the spinal cord of 10-day-old rats were purified by sucrose-gradient sedimentation, and each fraction was assessed for its expressional potency of functional glycine receptors using a *Xenopus* oocyte translation system. As reported previously [9,10], the rat GlyR-mRNA is categorized into 2 subclasses according to the sedimentation profile; one heavy and the other light. The adult rat spinal cord is richer in the heavy mRNA whereas the newborn one (3-days-old) has mainly the light mRNA. In the 10-day-old rat spinal cord, as shown in Fig. 1A, both types of mRNAs coexist abundantly, providing the advantage of cloning different species of GlyR-cDNA from single source of mRNA. In order to identify the juvenile isoform of GlyR, we used the light GlyR-mRNA fraction (Fraction 14) as template for the construction of a cDNA library. The heavy GlyR was already analyzed and we found it to be identical to the α1 (or 48 kDa) subunit [3,6].

The library was screened with synthetic oligonucleotides, and 3 clones were isolated (Fig. 1B). Sequential analysis revealed that these clones (pSK1140 to 1142) had overlapping nucleotide sequences in the DNA insert, and 2 clones (pSK1140 and pSK1141) possessed a full-length open reading frame (ORF, see Fig. 1B). The nucleotide and deduced amino acid sequences are indicated in Fig. 2. The reconstructed cDNA consists of 3865 base pairs (bp) of nucleotides in which the size of the ORF was 1356 bp (corresponding to 452 amino acids). There is a potent polyadenylation signal (AATAAA, nucleotides 2594–2599) in the 3'-noncoding region, but poly (A) clusters are not included downstream of the signal.

Fig. 3 shows the autoradiograms obtained by Northern blot analysis of mRNAs extracted from rat spinal cord. When the cDNA (the 908 bp *Bam* HI–*Xho* I fragment of pSK1141) was used as a probe, an intense band at a position of ~3.8 kb was detected in mRNAs derived from 0 and 3-day-old rat spinal cord (Fig. 3Ai). The size of the positive mRNA was in good agreement with that of the cloned cDNA (Fig. 2). As would be expected from previous results by electrophysiological analysis with adult and neonatal spinal cord mRNAs in oocytes [9,10], there was no detectable band in 25, 50 and 90-day-old rat spinal cords (Fig. 3Ai). This contrasts with the hybridization pattern obtained using the fragment of adult cord-derived cDNA (the 732 bp *Hinc* II–*Hinc* II fragment of pGRA1, see [6]) as a probe, in which the intensity of the main band at ~10 kb increases along with animal age (Fig. 3Aii, see also [3,9]). Thus, the mRNA hybridizing to the pSK1141 clone would be expressed during a restricted period of the neuronal development, and presumably the amount of translated receptor protein would parallel that of the

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-1172 TTTTTTTTCACACCAAGCAGAGTTTATTGATGCCGTGGGCGGGGCCCACGTACACAGCAGGTGGCGCACGCAGGGCAGATACTCACTGGCTGGACTGCAGGGG
-1069 TGTTAGCAGCGGCAAAAGCAGATGACCTGTGGGGAGCACCCTTTGGGGGGTGCCCTCCTCCTAGCAGCATTGGGGGCTTTGGGTGAGCATCAAGAACCACCTA
 -966 GTCACCCTAAGGTTCTCTTGGAAGCCCCTCCCCCTCCTTCCCGAGCTCCTTGTGAGTTGGGGGCTAGGATCCCTTCCTGTGATCAAAGGTGGGATGATCCCTT
 -863 GAGATTCACTATAAAAATTAAAATTCATTTATAAATCACAAGAGAAGGGCAGGAGGGAACACCGGGGAAACCTGACTTCATCGGTTACCTTGAGCCTACTCCC
 -760 CTCCCTCAGGTTCTAACTCCAAAAGAGAGAGCAAAGAGCTAGGTCTACAGGCACAGAAAGGGGCGAGAGACAGAGGAAAGGCAGTCGGAAGAACGGGACGAGA
 -657 GAGGAAAAAGATGCATCGGAAGAAAAGTATGGCTGCTGAGGCTCCTCTCCCCAACAGCTCCCGAAAGCCATGACCTGGGGATAAAGGAGAAAAGTTCCCAGCC
 -554 CAGAGACTCCTACAAGGTAAGGTTCCAAATGTCCCCGATCCGGTCCTTGCTCAAGCCATGGCGGGCCTTGAGATACTCAGAGCTGTGTGGATGGGAGCTGAGG
 -451 GAGGCGAGTGCTACTCCAGGTAGATATCTTCTGTGGGGCAGGCGAATGCCAGGTGCTCCTGGTAGTACTCCAGGAAGGCCCGGCCCACGCTCCTTGCCACTGG
 -348 CCTCATAGACTCCTGGGACACAAAGACGTGGCAGGCAAAGCGGCTTAGCAGCGGGTGTTTCGTAATGAAGCCAAAATAGCAGCTGTTTCGGGGATGACAGCCA
 -245 CAGAAGGAGATGTTCTTCATCTGGAAAAAGTGGCTGCAACGCCCTGGTTTGACCTGACCATGATGCCCAAGACTGGCACTTTTTGTTTTTTCTCAGCAAACTG
 -142 TACAACCCCAAATCTCTTTTTGATTTTCAAGGAAACTAGATTCCTGCCAGAATTTGAGTCTGGACAATAAATAGACACTTTGTCCTAGTCTTCTTTCTGGAAT
  -39 CATTTCGGGATATTTTCCACAAGCATCACGGAAACAGGA ATG AAC CGG CAG CTA GTG AAC ATT TTG ACA GCC TTG TTT GCA TTT TTC
                                            Met Asn Arg Gln Leu Val Asn Ile Leu Thr Ala Leu Phe Ala Phe Phe
                                              1                                 10

  +49 TTA GGG ACA AAC CAC TTC AGG GAA GCA TTC TGC AAA GAC CAT GAC TCC AGG TCT GGA AAA CAT CCC TCG CAG ACC CTG
      Leu Gly Thr Asn His Phe Arg Glu Ala Phe Cys Lys Asp His Asp Ser Arg Ser Gly Lys His Pro Ser Gln Thr Leu
                    20                                  30                                  40

 +127 TCT CCT TCA GAT TTC TTG GAT AAA CTA ATG GGA AGG ACA TCA GGA TAT GAT GCA AGA ATC AGG CCA AAT TTT AAA GGT
      Ser Pro Ser Asp Phe Leu Asp Lys Leu Met Gly Arg Thr Ser Gly Tyr Asp Ala Arg Ile Arg Pro Asn Phe Lys Gly
                                50                                  60

 +205 CCT CCA GTA AAC GTT ACT TGC AAT ATT TTT ATC AAC AGT TTT GGA TCG GTC ACA GAA ACC ACC ATG GAC TAC CGA GTG
      Pro Pro Val Asn Val Thr Cys Asn Ile Phe Ile Asn Ser Phe Gly Ser Val Thr Glu Thr Thr Met Asp Tyr Arg Val
        70                                  80                                  90

 +283 AAC ATT TTT CTG AGA CAG CAG TGG AAC GAT TCA CGG CTG GCA TAC AGT GAG TAC CCA GAT GAT TCC CTG GAT TTG GAT
      Asn Ile Phe Leu Arg Gln Gln Trp Asn Asp Ser Arg Leu Ala Tyr Ser Glu Tyr Pro Asp Asp Ser Leu Asp Leu Asp
                      100                                 110                                 120

+361 CCA TCG ATG CTG GAT TCG ATT TGG AAA CCA GAT TTG TTC TTT GCC AAT GAG AAA GGA GCC AAT TTC CAT GAT GTC ACC
     Pro Ser Met Leu Asp Ser Ile Trp Lys Pro Asp Leu Phe Phe Ala Asn Glu Lys Gly Ala Asn Phe His Asp Val Thr
                                    130                                 140

+439 ACT GAT AAC AAG TTG CTG CGG ATT TCC AAA AAT GGC AAA GTG CTC TAC AGT ATT AGA CTC ACC TTG ACT TTA TCC TGT
     Thr Asp Asn Lys Leu Leu Arg Ile Ser Lys Asn Gly Lys Val Leu Tyr Ser Ile Arg Leu Thr Leu Thr Leu Ser Cys
                  150                                 160                                 170

+517 CCC ATG GAC CTG AAG AAC TTT CCA ATG GAT GTC CAG ACC TGT ACA ATG CAG CTG GAG AGT TTT GGG TAC ACC ATG AAT
     Pro Met Asp Leu Lys Asn Phe Pro Met Asp Val Gln Thr Cys Thr Met Gln Leu Glu Ser Phe Gly Tyr Thr Met Asn
                              180                                 190

+595 GAC CTG ATA TTT GAG TGG TTA AGT GAT GGT CCA GTA CAA GTT GCT GAA GGA CTC ACC CTG CCT CAG TTT ATT TTG AAA
     Asp Leu Ile Phe Glu Trp Leu Ser Asp Gly Pro Val Gln Val Ala Glu Gly Leu Thr Leu Pro Gln Phe Ile Leu Lys
        200                                 210                                 220

+673 GAA GAG AAG GAA CTT GGC TAT TGC ACA AAG CAT TAC AAC ACT GGC AAG TTT ACC TGC ATT GAG GTC AAG TTT CAC CTG
     Glu Glu Lys Glu Leu Gly Tyr Cys Thr Lys His Tyr Asn Thr Gly Lys Phe Thr Cys Ile Glu Val Lys Phe His Leu
                    230                                 240                                 250

+751 GAA CGC CAG ATG GGC TAT TAT TTG ATC CAG ATG TAT ATC CCC AGC CTG CTG ATA GTC ATT TTG TCC TGG GTC TCC TTT
     Glu Arg Gln Met Gly Tyr Tyr Leu Ile Gln Met Tyr Ile Pro Ser Leu Leu Ile Val Ile Leu Ser Trp Val Ser Phe
                                    260                                 270

+829 TGG ATA AAC ATG GAT GCA GCT CCT GCC AGG GTT GCC CTT GGC ATC ACA ACC GTC CTG ACA ATG ACT ACC CAG AGT TCA
     Trp Ile Asn Met Asp Ala Ala Pro Ala Arg Val Ala Leu Gly Ile Thr Thr Val Leu Thr Met Thr Thr Gln Ser Ser
                  280                                 290                                 300

+907 GGT TCC AGG GCA TCT CTG CCA AAG GTC TCA TAT GTG AAA GCA ATT GAC ATC TGG ATG GCA GTG TGC CTT CTG TTT GTG
     Gly Ser Arg Ala Ser Leu Pro Lys Val Ser Tyr Val Lys Ala Ile Asp Ile Trp Met Ala Val Cys Leu Leu Phe Val
                              310                                 320

+985 TTT GCT GCC TTA CTG GAA TAT GCA GCA GTG AAC TTT GTC TCC AGG CAA CAC AAG GAG TTC CTT CGT CTC CGG AGA CGA
     Phe Ala Ala Leu Leu Glu Tyr Ala Ala Val Asn Phe Val Ser Arg Gln His Lys Glu Phe Leu Arg Leu Arg Arg Arg
        330                                 340                                 350

+1063 CAG AAG AGG CAG AAT AAG GAA GAA GAT GTT ACT CGT GAA AGC CGT TTT AAC TTC AGC GGT TAT GGG ATG GGT CAC TGC
      Gln Lys Arg Gln Asn Lys Glu Glu Asp Val Thr Arg Glu Ser Arg Phe Asn Phe Ser Gly Tyr Gly Met Gly His Cys
                    360                                 370                                 380
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Fig. 2. Nucleotide and deduced amino acid sequences of the NGlyR subunit reconstructed from the sequence of pSK1140 and pSK1142. The other positive clone, pSK1141 also has this sequence without any mismatch.

mRNA. Hereafter we will refer to this protein molecule as 'neonatal glycine receptor (NGlyR)'. The NGlyR-mRNA is also detectable in the spinal cord as early as 15 days embryonic and 10 days postnatal, although the intensity is much lower (data not shown). The NGlyR-mRNA exists not only in the spinal cord but in other areas of the central nervous system of the 3-day-old rat (Fig. 3B). In particular, considerable amounts of the mRNA are present in the hippocampus and other cerebral cortical regions, where the inhibitory glycinergic system seems to function poorly in adult animals. However, this is not surprising, because Carpenter et al. [14] reported that in the embryonic and neonatal rat cerebral cortex, there is a plentiful supply of mRNA which induces synthesis of functional glycine receptors in *Xenopus* oocytes.

Fig. 4 represents comparison of amino acid sequences between NGlyR and another GlyR isoform that exists in the adult rat spinal cord (α1 or 48 kDa subunit [3,6]; for convenience referred to as adult (A) GlyR). The NGlyR exhibits 71% homology with the AGlyR. Marked differences are seen in the N-terminal region (presumably the signal peptide domain) and an intracellular loop flanked by the third and fourth transmembrane domains. In contrast, the sequences of important domains for functions, such as transmembrane regions and a proposed ligand binding site [15], are highly conserved (90% identity).

A single synthetic RNA transcribed in vitro from the NGlyR-cDNA (pSK1141), as well as from the AGlyR-cDNA, allows *Xenopus* oocytes to synthesize functional glycine receptors [6–8]. In voltage-clamped oocytes, as shown in Fig. 5, both types of the receptor generated large membrane currents in response to glycine (1 mM), but neither responded to the same concentration of GABA. The glycine-induced currents were markedly depressed in the presence of a GlyR antagonist, strychnine (200 nM). The IC_{50} values of the strychnine on the Cl- currents induced by glycine (1 mM) were 35.3 ± 1.2 nM (mean $\pm$ S.E.M., $n = 6$) for NGlyR, and 40.6 ± 0.8 nM ($n = 5$) for AGlyR, respectively. Either glycine current inverted the direction from inward to outward at about -20 mV (near E_{Cl} in oocytes), suggesting that the main carrier would be Cl^- (data not shown, see [6–8]). Thus, both types of the receptor exhibited quite similar functional properties. This seems to be reasonable because of the structural similarity of functional domains between the NGlyR and AGlyR. Nevertheless, a marked difference was observed for the action of β-alanine, a sib amino acid of

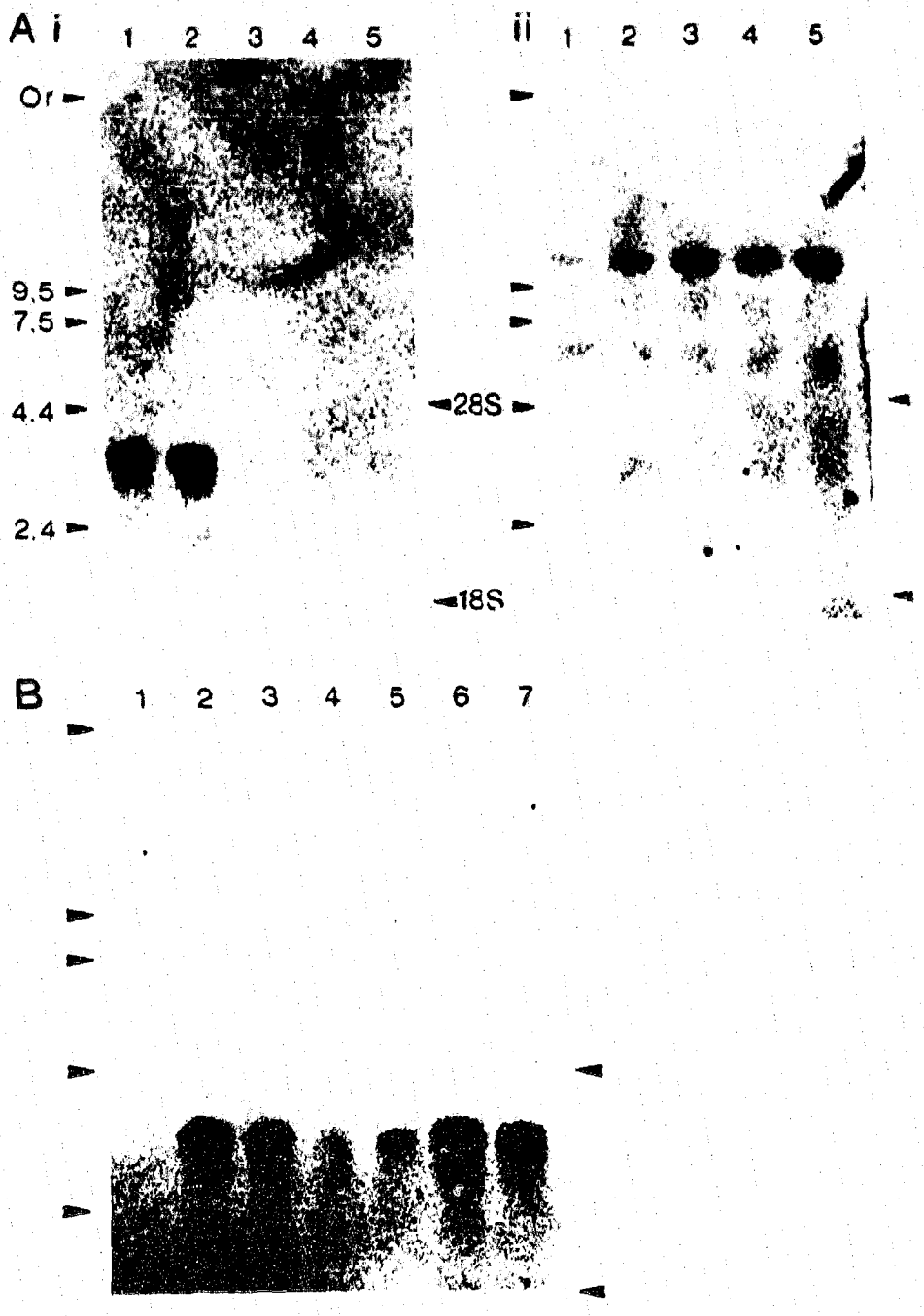

Fig. 3. Blot hybridization analysis of poly (A)$^+$ RNAs extracted from central nervous tissues of different aged rats. (Ai) The rat spinal cord RNA from day 0 (lane 1), day 3 (lane 2), day 25 (lane 3), day 50 (lane 4) and day 90 (lane 5) was blotted and hybridized with the ^{32}P-labeled pSK1141 (NGlyR-cDNA) fragment for 32 h. (Aii) The same membrane that was used in Ai was soaked in heated water (~90°C) for 10 min to strip off the cDNA probe, and rehybridized with the pGRA1 (AGlyR-cDNA) fragment (cf. [6]) for 28 h. (B) The hybridization patterns of NGlyR cDNA to the RNAs derived from the cerebral cortex (lane 2); hippocampus (lane 3); other parts of the forebrain including basal ganglia, thalamus etc. (lane 4); cerebellum (lane 5); brainstem (lane 6); and spinal cord (lane 7) of 3-day-old rats. Poly (A)$^-$ RNAs from the cerebral cortex were blotted (lane 1) as a control. The blotted RNAs were hybridized with NGlyR-cDNA (same as Ai) for 36 h. Autoradiograms were obtained after exposure for 14–17 h at −80°C with an intensifying screen. On each photograph, the arrow heads on the left indicate positions of RNA size markers (BRL), and those on the right show the positions of 28S and 18S RNAs, respectively.

glycine. On the AGlyR, β-alanine can act as a strong agonist whose potency is about one third of the glycine (32.5 ± 1.5%, $n = 8$; see Fig. 5), when compared at same concentration (1 mM). By contrast, the β-alanine-induced response was as small as 4.8% ± 0.3, ($n = 10$) of the glycine currents on the NGlyR (Fig. 5). This dif-

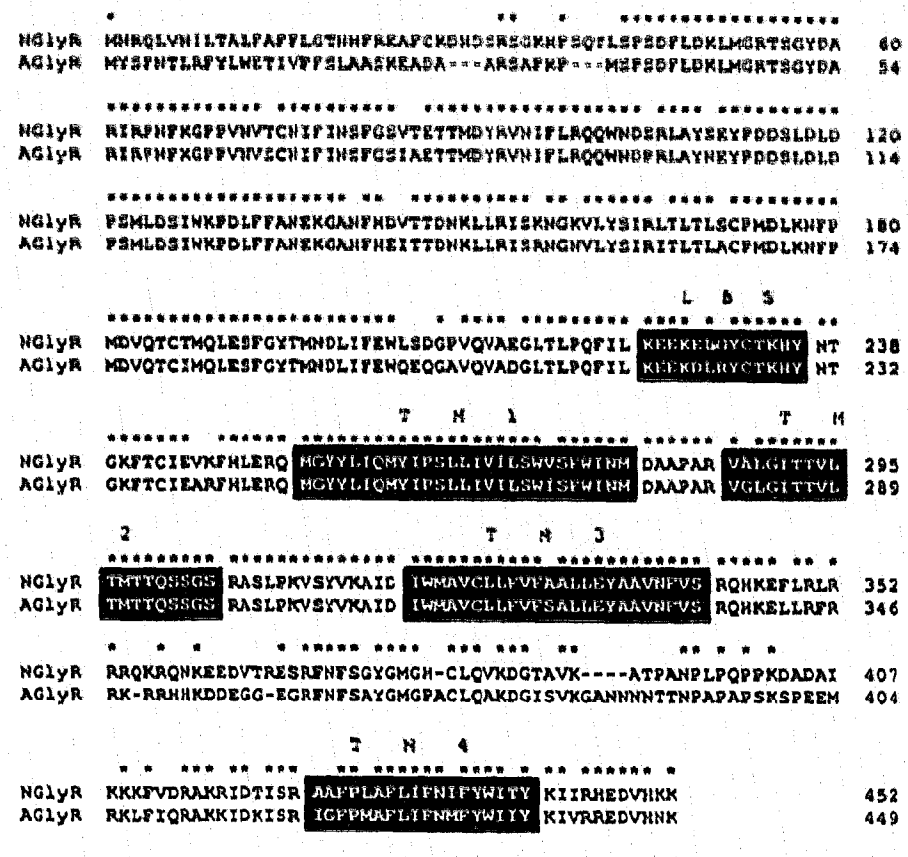

Fig. 4. Comparison of the amino acid sequences of NGlyR and AglyR. (For the nucleotide sequence of AGlyR see [6,8].) The amino acid numbers on the right are assigned from the initial methionine (M). The positions of identical amino acids are marked with asterisks. LBS = a proposed ligand binding site [15]; TM1 to TM4 = putative transmembrane domains 1 to 4.

ference may reflect structural changes in the ligand binding domain resulting from the replacement of a few amino acids (Fig. 4). It is anticipated to be able to pinpoint the site of the agonist binding domain by modifying these amino acids using the point mutation technique.

The present results confirm the existence of a heterogeneous GlyR-mRNA at the molecular level whose production is developmentally regulated. The structural similarity between the N and A types of GlyR is 71% in amino acid sequence, and 63% in nucleotide sequence, respectively. We have not yet analyzed genomic DNAs, but comparing their sequences, it seems that 2 types of mRNA are transcribed from different regions of the gene, but not produced from a single precursor RNA through an alternative splicing event. The structure of rat NGlyR exhibited a high degree of homology (93% in nucleotide sequence) to that of a human glycine receptor $\alpha 2$ subunit cloned by Grenningloh et al. [5]. Although they did not show any results of Northern blot analysis, it is conceivable that the human $\alpha 2$ receptor is of the juvenile type, because the $\alpha 2$-cDNA was isolated from libraries constructed using embryonic human cortex mRNAs [5].

The expression of functional GlyRs in *Xenopus* oocytes injected with a single mRNA transcribed from N and AGlyR-cDNA probably reflects that the receptor/ion channel is formed by assembly of a single subunit protein (Fig. 5) [5,6,7]. The homo-oligomeric receptor can also be expressed in embryonic human kidney cells transfected with the GlyR-cDNA [8]. This assembly of the receptor differs from those of $GABA_A$ and nicotinic acetylcholine receptors [16,17]; they require hetero-oligomeric assembly with 2 to 4 distinct subunits to construct essentially functional receptors in oocytes and transfected mammalian cell lines. The properties of the NGlyRs and AGlyRs constructed by the single subunit are essentially indinstinguishable from those of the GlyRs in oocytes injected with total poly(A)$^+$ mRNAs from neonatal and adult rat spinal cords, respectively [10,13]. Although a structural model of GlyR is proposed consisting of heterogeneous subunits based upon biochemical evidence [3,5,13], it appears to be no wonder that the homo-oligomeric GlyR also exists in the mammalian nervous system and has a function in neuro-transmission.

For the time being we do not know the physiological significance of the existence of the glycine receptor

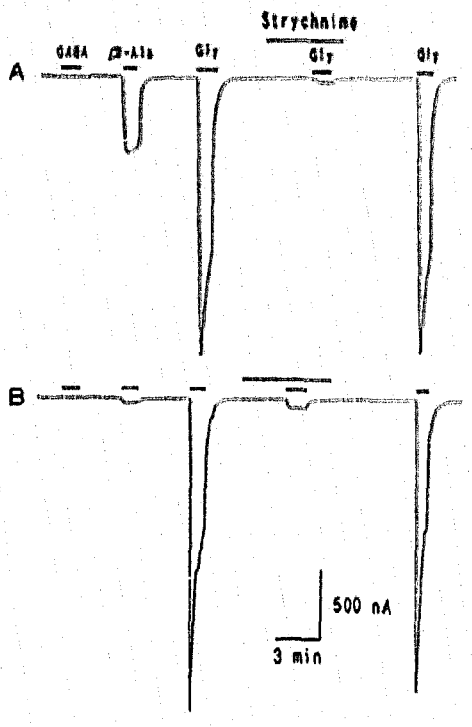

Fig. 5. Functional expression of glycine receptor/chloride channels in *Xenopus* oocytes injected with synthetic RNA of AGlyR (A) or NGlyR (B). Synthesis of the AGlyR-RNA was as previously reported [6]. The NGlyrR-RNA was transcribed in vitro from the pSK1141. The RNAs at 2.5 ng/50 nl were injected into oocytes. Amino acids (1 mM) and strychnine (200 nM) were applied by perfusion during periods indicated with horizontal bars. The traces show membrane currents when the oocytes were voltage-clamped at −70 mV. Downward deflection denotes inward current.

mRNA whose production is developmentally regulated. Schofield et al. [18] suggested that receptor subtype heterogeneity is related to neural plasticity. It may be of interest to look into the correlation between the time course of synaptogenesis in various brain regions, and that of the expression of the NGlyR-mRNA.

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REFERENCES

[1] Young, A.B. and Macdonald, R.L. (1983) in: Handbook of the Spinal Cord (Davidoff, R.A. ed.) vol. 1, Dekker, New York, pp. 1–43.
[2] Barker, J.L. (1985) in: Neurotransmitter Actions in the Vertebrate Nervous System (Rogawski, M.A. and Barker, J.L. eds.) Plenum, New York, pp. 71–100.
[3] Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E.D. and Betz, H. (1987) Nature 328, 215–220.
[4] Grenningloh, G., Schmieden, V., Schofield, P.R., Seeburg, P.H., Siddique, T., Mohandas, T.K., Becker, C.-M. and Betz, H. (1990) EMBO J. 9, 771–776.
[5] Grenningloh, G., Pribilla, I., Prior, P., Multhaup, G., Beyreuther, K., Taleb, O. and Betz, H. (1990) Neuron 4, 963–970.
[6] Akagi, H., Hirai, K. and Hishinuma, F. Neurosci. Res. (in press).
[7] Schmieden, V., Grenningloh, G., Schofield, P.R. and Betz, H. (1989) EMBO J. 8, 695–700.
[8] Sontheimer, H., Becker, C.-M., Pritchett, D.B., Schofield, P.R., Grenningloh, G., Kettenmann, H., Betz, H. and Seeburg, P.H. (1989) Neuron 2, 1491–1497.
[9] Akagi, H., Patton, D.E. and Miledi, R. (1989) Proc. Natl. Acad. Sci. USA 86, 8103–8107.
[10] Akagi, H. and Miledi, R. (1988) Science 242, 270–273.
[11] Grenningloh, G., Gundelfinger, E., Schmitt, B., Betz, H., Darlison, M.G., Barnard, E.A., Schofield, P.R. and Seeburg, P.H. (1987) Nature 330, 25–26.
[12] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[13] Akagi, H. and Miledi, R. (1988) Neurosci. Lett. 95, 262–268.
[14] Carpenter, M.K., Parker, I. and Miledi, R. (1988) Proc. R. Soc. Lond. Ser. B 234, 159–170.
[15] Betz, H., Schmitt, B., Becker, C.-M., Grenningloh, G. and Rienitz, A. (1987) in: The Molecular Biology of Receptors. (Strosberg, A.D., ed.) Ellis, Chichester, pp. 128–138.
[16] Pritchett, D.B., Sontheimer, H., Shivers, B.D., Ymer, S., Kettenman, H., Schofield, P. and Seeburg, P.H. (1989) Nature 338, 582–585.
[17] Kurosaki, T., Fukuda, K., Konno, T., Mori, Y., Tanaka, K., Mishina, M. and Numa, S. (1987) FEBS Lett. 214, 253–258.
[18] Schofield, P.R., Shivers, B.D. and Seeburg, P.H. (1990) Trends Neurosci. 13, 8–11.
[19] Kuhse, J., Schmieden, V. and Betz, H. (1990) Neuron 5, 867–873.

NOTE ADDED IN PROOF

After this paper was accepted, Kuhse, J. et al. [19] reported on cDNA cloning of a rat glycine receptor subtype, termed $\alpha 2^*$ subunit, which is expressed at developmental stage of rat spinal cord. Although a part of 5'-noncoding sequence differs considerably, the in-frame nucleotide and deduced amino acid sequences are identical to ours except for one amino acid (Gly/Glu at position 194). They showed that this substitution affects functional receptor properties, especially agonist/antagonist sensitivities. Moreover, they confirmed that a mutated receptor, by substituting Gly for Glu, markedly increases sensitivities to the drug, consistent with our results. This is the reason why the cloned receptor is named $\alpha 2^*$, and by mutual consent we rename our cloned receptor 'GlyR $\alpha 2$' for NGlyR.