Kibelbek, J., Beach. J. M., & Litman, B. J. (1990) *Biophys. J. 57*, 367a.
Lamola, A. A., Yamane, T., & Zipp, A. (1974) *Biochemistry 13*, 738-745.
Litman, B. J., Kalisky, O., & Ottolenghi, M. (1981) *Biochemistry 20*, 631-634.
Mathews, R. G., Hubbard, P. K., & Wald, G. (1963) *J. Gen. Physiol. 47*, 215-240.
Mitchell, D. C., Kibelbek, J., & Litman, B. J. (1990a) *Biophys. J. 56*, 74a.
Mitchell, D. C., Straume, M., Miller, J. L., & Litman, B. J. (1990b) *Biochemistry 29*, 9143-9149.
O'Brien, D. F., Costa, L. F., & Ott, R. A. (1977) *Biochemistry 16*, 1295-1303.
Ovchinnikov, Yu. A. (1982) *FEBS Lett. 148*, 179-191.
Ryba, N. J. P., Dempsey, C. E., & Watts, A. (1986) *Biochemistry 25*, 4818-4825.
Straume, M., Mitchell, D. C., Miller, J. L., & Litman, B. J. (1990) *Biochemistry 29*, 9135-9142.
Wiedmann, T. S.; Pates, R. D., Beach, J. M., Salmon, A., & Brown, M. F. (1988) *Biochemistry 27*, 6469-6474.

Mapping of Antigenic Epitopes on the α1 Subunit of the Inhibitory Glycine Receptor†

Stephan Schröder,‡ Werner Hoch, Cord-Michael Becker, Gabriele Grenningloh,§ and Heinrich Betz*
ZMBH, Universität Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, Federal Republic of Germany
Received May 1, 1990; Revised Manuscript Received August 7, 1990

ABSTRACT: The inhibitory glycine receptor (GlyR) is a ligand-gated chloride channel protein that occurs in developmentally regulated isoforms in the vertebrate central nervous system. Monoclonal antibodies (mAbs) against the GlyR distinguish neonatal and adult GlyR proteins by identifying distinct α subunit variants within these receptor isoforms. Here, bacterially expressed fusion proteins of the rat GlyR α1 subunit were used to localize the major antigenic epitopes of this protein within its N-terminal 105 amino acids. Synthetic peptides allowed further fine mapping of two mAb binding domains. MAb 2b, specific for the adult α1 subunit, bound to a peptide corresponding to amino acids 1-10, whereas mAb 4a, which recognizes both neonatal and adult GlyR isoforms, reacted with a peptide representing residues 96-105 of the α1 polypeptide. These data define unique and common antigenic epitopes on GlyR α subunit variants.

The inhibitory glycine receptor (GlyR)[1] of mammalian spinal cord is a ligand-gated chloride channel composed of two types (α and β) of homologous membrane-spanning subunits (Betz & Becker, 1988; Langosch et al., 1988; Betz, 1990). Immunological, pharmacological, and molecular cloning data indicate considerable subtype diversity of the ligand-binding α subunit of this receptor. Upon affinity purification from adult mammalian spinal cord, it displays an apparent molecular weight of 48K (Pfeiffer et al., 1982; Graham et al., 1985; Becker et al., 1986). In newborn rodents, a neonatal receptor species predominates which differs from the adult GlyR in binding affinity for the selective antagonist strychnine and apparent M_r (49K) of its α subunit (Becker et al., 1988). Recently, complementary and genomic DNAs encoding four variants of the α subunit have been isolated from rodents and man and shown to represent transcripts of distinct genes (Grenningloh et al., 1987, 1990a; J. Kuhse, V. Schmieden, and H. Betz, submitted for publication; B. Matzenbach, Y. Maulet, H. Betz, unpublished data). In case of the adult type α subunit [now termed α1; see Greenningloh et al. (1990a)], alternative splicing further extends GlyR α subunit heterogeneity (Malosio et al., submitted for publication).

Previous biochemical (Schmitt et al., 1987; Becker et al., 1988) and immunocytochemical (Triller et al., 1985, 1987; Altschuler et al., 1986; Araki et al., 1988; Van den Pol & Gorcs, 1988) studies of the postsynaptic GlyR complex have relied on the use of monoclonal antibodies (mAbs) for identifying specific receptor polypeptides. In particular, a quantitative dot receptor assay (DORA) has been employed to quantify α subunit levels in different brain regions and disease states (Becker et al., 1986, 1989). Moreover, differences in antigenic epitopes have been exploited to define neonatal and adult GlyR isoforms in primary cultures of spinal cord (Hoch et al., 1989) and mutant animals (C.-M. Becker and H. Betz, submitted for publication). Whereas on immunoblots the α1 subunit characteristic of the adult GlyR reacts with three mAbs (1a, 2b, and 4a) obtained by immunization with affinity-purified native receptor (Pfeiffer et al., 1984), the neonatal α subunit variant is only recognized by mAb 4a (Becker et al., 1988), suggesting variations in α subunit primary structure to underly these antigenic differences. To further define their molecular basis, we now have mapped the binding sites of different antibodies recognizing the α1 subunit of the adult rat GlyR by using bacterial fusion proteins and

† This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 317 and Leibniz Program) to C.-M.B. and H.B., Bundesministerium für Forschung und Technologie (BCT 365/1), Fonds der Chemischen Industrie, and German-Israeli Foundation.
* To whom correspondence should be addressed at the Department of Neurochemistry, Max Planck Institute for Brain Research, Deutschordenstrasse 48, D-6000 Frankfurt 60, FRG.
‡ Present address: Department for Connective Tissue Research, Max Planck Institute for Biochemistry, D-8033 Martinsried, FRG.
§ Present address: Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, CA 94720.

[1] Abbreviations: DORA, dot receptor assay; ELISA, enzyme-linked immunoassay; GlyR, inhibitory glycine receptor; mAb, monoclonal antibody; PNF, proximal N-terminal fragment; DNF, distal N-terminal fragment; ICF, intracellular fragment.

0006-2960/91/0430-42$02.50/0

synthetic peptides. Our data indicate that N-terminal sequence heterogeneity accounts for the different immunological properties of neonatal and adult GlyR isoforms.

Experimental Procedures

Materials. MAbs against purified rat GlyR (Pfeiffer et al., 1984) were produced in hybridoma culture. MAbs 1a and 4a were precipitated from the culture supernatants with 40% (w/v) $(NH_4)_2SO_4$ and dialyzed against phosphate-buffered saline. MAb 2b was affinity-purified on protein A–Sepharose (Pharmacia). A polyclonal antiserum, AS-1, raised against purified GlyR has been described previously (Pfeiffer, 1983; Hoch et al., 1989). The protein concentrations of the mAb preparations used were as follows: mAb 1a, 14 mg/mL; mAb 2b, 0.15 mg/mL; mAb 4a, 42 mg/mL.

Anti-mouse IgGs were obtained from Dianova, and anti-mouse and anti-rabbit IgGs coupled to alkaline phosphatase, from Promega. Peptide 96–105 was purchased from Multiple Peptide Systems, and peptides 1–10 and 67–81 were synthesized by Dr. R. R. Pipkorn, ZMBH. Peptides 1–10 and 67–81 contained an additional C-terminal tyrosine residue, and peptide 96–105 was amidated at the C-terminus. The expression vector pEX 34 allowing C-terminal fusions to a 12-kb MS2 polymerase fragment (Strebel et al., 1986) and polyclonal rabbit antisera against β-galactosidase and MS2 polymerase were kindly provided by Dr. E. Beck. The expression vector pEX 3 containing multiple cloning sites after a cro lac Z coding fragment covering 117 kDa of bacterial protein sequence (Stanley & Luzio, 1984) and *Escherichia coli* strain Pop 2136 were a gift of Dr. K. Stanley. [^{3}H]-Strychnine was from Amersham, fixed *Staphylococcus aureus* cells (Pansorbin) were from Calbiochem, and nitrocellulose membranes were from Schleicher and Schüll.

Cloning and Expression of GlyR Fusion Proteins. For obtaining a fragment (ICF) corresponding to the loop between putative transmembrane helices 3 and 4 of the α1 subunit, clone GR 1.6 (Grenningloh et al., 1987) was digested with *Sau*3A and *Hin*cII. The resulting DNA fragment (nucleotides 933–1181) was blunt-ended and cloned into the *Sma*I site of pEX 3.

Two fragments of the α1 subunit cDNA encompassing the proximal (PNF; nucleotides 1–336) and distal (DNF; nucleotides 430–654) parts of the N-terminal domain were generated by digestion of clone GR 1.6.1 (Grenningloh et al., 1987) with *Hae*III. Withstanding direct cloning into pEX 34, PNF was ligated into the *Sma*I site of pTZ 18 R (Pharmacia) after generation of blunt ends and introduced into *E. coli* BMH. PNF was then excised with *Eco*RI and *Pst*I and recloned into the corresponding restriction sites of pEX 34b. In addition, blunt-ended PNF was cloned into the *Sma*I site of pEX 1. DNF was ligated into the *Pst*I site of pEX 34 c, which had been blunt-ended with T4 polymerase.

The correct orientation of the fragments was confirmed by restriction analysis and sequencing or, in the case of ICF, by hybridization with a 24-mer oligonucleotide overlapping the region of ligation of lac Z and the 5′ end of the cloned fragment. Expression of fusion proteins was performed as described by Zabeau and Stanley (1982) and enrichment of fusion proteins according to Schloss et al. (1988).

Western Blotting. Enriched fusion proteins were separated on 15%, or 7–15%, SDS–polyacrylamide gels (Laemmli, 1970). Proteins were blotted onto nitrocellulose (Kyhse-Andersen, 1984) and visualized after transfer using Ponceau S (Rehm et al., 1986). For immunodetection the blots were incubated with the respective antibody diluted in TBB [50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% (w/v) Nonidet P-40, 1% (w/v) bovine serum albumin] for 2 h after blocking by a 30-min incubation with TBB. Thereafter, the blots were washed with TBS (TBB without Nonidet P-40 and BSA), TWB (TBB without BSA), and TBB for 10 min each and subsequently reacted for 30 min with anti-mouse IgG, or anti-rabbit IgG, coupled to alkaline phosphatase (both diluted 1:7500 in TBB). After three washings with TBS, TWB, and again TBS, substrate solution was added [0.33 μg/mL nitroblue tetrazolium [50 mg/mL in 70% (v/v) dimethylformamide] and 0.17 μg/mL 5-bromo-4-chloroindolyl phosphate (50 mg/mL in dimethylformamide) diluted in 0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl, and 5 mM $MgCl_2$]. Color development was allowed to proceed for 5–20 min.

Dot Receptor Assay (DORA) with Fusion Proteins. For quantitation of antibody binding, a dot assay was used (Becker et al., 1989). Fusion proteins were diluted in application buffer [0.5% (w/v) sodium desoxycholate, 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 20% (v/v) methanol] and applied to nitrocellulose by using a dot blot apparatus (Schleicher and Schüll, Minifold I). After drying and blocking of unspecific binding, the nitrocellulose sheets were reacted with antibodies and washed as described for the Western blot procedure. Then the dots were punched out into a microtiter plate and incubated with 10 mM *p*-nitrophenyl phosphate dissolved in 0.1 M Tris, pH 9.5, 0.1 M NaCl, and 5 mM $MgCl_2$, for 15–30 min. The substrate solution was transferred into another microtiter plate, and the reaction product was quantified by measuring the OD at 405 nm in an ELISA reader.

Peptide ELISA. Binding of antibodies to synthetic peptides was determined by an ELISA procedure. Peptides were dissolved in 0.1 M $NaHCO_3$, pH 9.5, at a concentration of 0.1 mg/mL and absorbed onto plastic microtiter plates by overnight incubation at 4 °C (100 μL/well). Incubation with antibodies was performed as described above for the DORA procedure. With the peptide ELISA, the enzyme reaction was allowed to proceed for 10–300 min.

Immunoprecipitation Experiments. Detergent extracts were prepared from adult rat spinal cord as described previously (Becker et al., 1988). To increase the pH value and to reduce the salt concentration, extracts were then diluted with 1 volume of 25 mM KP_i buffer, pH 9.0. To 1-mL aliquots of this diluted extract was added 1.5 μg of mAb 2b preincubated for 2 h with different amounts of peptide. After overnight incubation at 4 °C, immunocomplexes were precipitated by addition of 1 μL of anti-mouse IgG and 100 μL of a 10% suspension of fixed *S. aureus* cells (Pansorbin). Bound antibodies and antigen were collected by centrifugation at 2000*g* for 5 min. The supernatants were removed, and the pellets were washed four times with washing buffer [25 mM KP_i, pH 8.0, 0.5 M KCl, 1% (w/v) Triton X-100, 2.5 mM phosphatidylcholine, and protease inhibitors as described in Pfeiffer et al. (1982)]. Both supernatants and pellets were then tested for their [^{3}H]-strychnine binding capacity.

Strychnine Binding Assay. High-affinity binding of [^{3}H]strychnine to detergent extracts and immunocomplexes bound to Pansorbin was determined by a filtration assay (Pfeiffer & Betz, 1981). All values are the mean of triplicate determinations and are corrected for unspecific binding determined in the presence of 10 mM glycine.

Results

For an initial localization of antigenic epitopes on the α1 subunit of the rat GlyR, three different fragments of the cDNA clones GR 1-6 and 1-6-1 (Grenningloh et al., 1987) were used for construction of expression plasmids. A proximal N-terminal fragment encompassing bp 1–336 (PNF), a distal

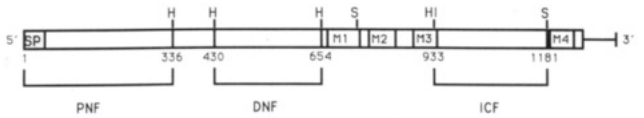

FIGURE 1: Physical map of the α1 GlyR cDNA and localization of fragments used for construction of fusion proteins. PNF, proximal N-terminal fragment; DNF, distal N-terminal fragment; ICF, intracellular fragment. Structural features of the α1 subunit: SP, part of the signal peptide encoded by GlyR clone 1.6 (Grenningloh et al., 1987); M1–M4, proposed transmembrane regions. Restriction sites (H, *Hae*III; S, *Sau*3A; HI, *Hin*cII) used for isolation of the fragments are indicated.

Table I: Molecular Weights of Fusion Proteins[a]

construct	M_r (K) calculated	observed
pEX 1, empty	117	116
pEX 1–PNF	129	139
pEX 3–ICF	126	139
pEX 34 c, empty	12	14
pEX 34 c–DNF	20	21
pEX 34 b–PNF	26	25

[a] Apparent molecular weights were determined by SDS–polyacrylamide gel electrophoresis using molecular weight markers (Bio-Rad).

N-terminal piece containing bp 430–654 (DNF), and a fragment encoding the putative intracellular domain (bp 933–1181; ICF) were subcloned into pEX vectors for production of fusion proteins (Figure 1). Expression of the resulting constructs and enrichment of the corresponding cro lac Z–PNF, cro lac Z–ICF, MS2–PNF, and MS2–DNF fusion proteins (for the apparent and calculated molecular weights see Table I) then provided antigen for Western blots and DORA antibody binding assays.

Four different antibody preparations recognizing the α1 subunit on Western blots were used for epitope mapping, i.e., mAbs GlyR 1a, 2b, and 4a (Pfeiffer et al., 1984) and a polyclonal rabbit antiserum, AS-1 (Pfeiffer, 1983; Hoch et al., 1989). All antibodies had been obtained by immunization with affinity-purified native GlyR from rat spinal cord. None of the antibodies recognized the MS2–DNF fusion protein on immunoblots after enrichment from lysates of induced bacteria (data not shown; see also Figure 3B). All, however, stained the cro lac Z–PNF and MS2–PNF proteins under the same conditions (Figure 2A and data not shown). In case of the cro lac Z–PNF fusion protein (Figure 2A), besides the 138-kDa protein an addditional major band of 129 kDa was recognized by the anti-β-galactosidase antiserum, mAb 2b and AS1 (lanes c, e, and g), but not by mAbs 1a and 4a (lanes d and f). This staining pattern indicates that the 129-kDa polypeptide is generated from the 138-kDa protein by proteolytic removal of a C-terminal fragment which contains the epitopes for mAbs 1a and 4a. Therefore, the epitopes of mAb 2b and AS1 must reside closer to the N-terminus of the α1 subunit than those of the other two GlyR antibodies.

The AS-1 antiserum also recognized the cro lac Z–ICF fusion protein (Figure 2B). None of the GlyR antibodies stained control preparations of cro lac Z (Figure 2C) or MS2 (not shown) proteins. These data suggested that the major immunogenic epitopes of the mature rat α1 subunit reside within its first 105 amino acid residues.

This conclusion was corroborated further by quantitative immunoassays using the DORA method (Becker et al., 1989). To exclude unspecific staining of the prominent fusion protein bands seen on our Western blots, all antibodies were titrated against the fusion proteins enriched by differential extraction. Parallel preparations of cro lac Z and MS2 proteins obtained from induced empty pEX 3 and pEX 34 b vectors served as controls for unspecific binding. Figure 3A–C shows that all mAbs indeed bound specifically to the PNF region. AS-1 also recognized the same fragment, but in addition displayed significant reactivity with ICF (see summarized data in Figure 3D).

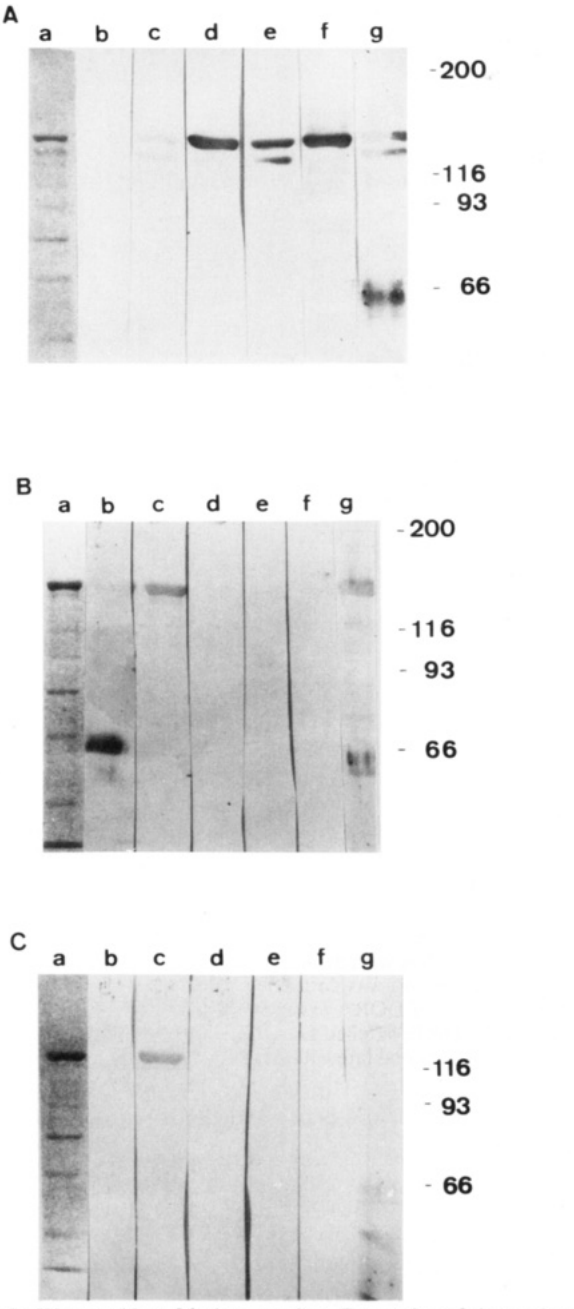

FIGURE 2: Western blot of fusion proteins. Expression of the cro lac Z–PNF fusion protein (A), the cro lac Z–ICF fusion protein (B), and cro lac Z (C) was induced in *E. coli* cultures, and enriched fusion proteins were separated on a 7–15% SDS–polyacrylamide gel. After transfer to nitrocellulose, strips of the blot were stained with Ponceau S to visualize the protein pattern (a) and subsequently reacted with the following antibodies: (b) no antibody; (c) anti-β-galactosidase; (d) mAb 1a (1:200); (e) mAb 2b (1:100); (f) mAb 4a (1:500); (g) AS 1 (1:750). Note that in (A) all antibodies stain a major band of M_r 139K. The additional band of 129K seen in (A), lanes c, e, and g, is not recognized by mAbs 1a and 4a and thus corresponds to a C-terminal degradation product of the fusion protein. The band of 65K seen in (A–C), lane g, and in (B), lane b, represents unspecific staining. The substrate reactions were incubated for the same time with all antibodies except in case of the controls without first antibody (b), where incubation times were twice as long to highlight unspecific reactivity.

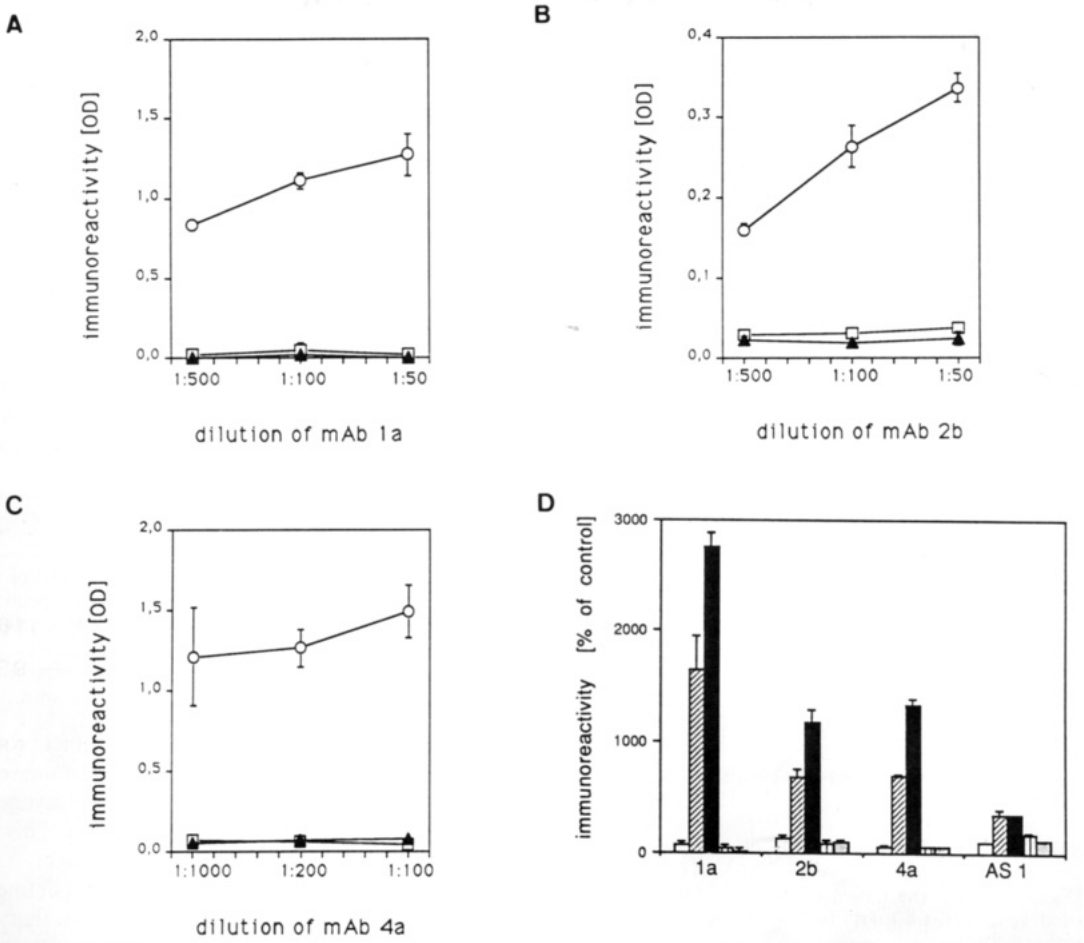

FIGURE 3: DORA with different fusion proteins. Five micrograms each of enriched cro lac Z–PNF (○), MS2–DNF (□), or cro lac Z–ICF (▲) fusion proteins was dotted onto nitrocellulose and reacted with the indicated dilutions of (A) mAb 1a, (B) mAb 2b, and (C) mAb 4a. (D) Summary of DORA experiments with different antibodies. To each dot, 1 μg each of the following fusion proteins was applied: open bars, MS2–DNF; hatched bars, MS2–PNF; black bars, cro lac Z–PNF; vertically stripped bars, cro lac Z–ICF; and dotted bars, cro lac Z. All antibodies were diluted 100-fold. Reactivities are indicated as percent of the control value obtained with MS2 protein purified from bacteria transformed with pEX 34c. All values represent the mean ±SD of two determinations.

To more precisely localize the mAb epitopes within the PNF region, three synthetic peptides corresponding to defined PNF segments were tested in a peptide ELISA: (i) peptide 1–10 corresponding to amino acids 1–10 of the mature rat α1 subunit has previously been shown to be immunogenic (Grenningloh et al., 1987); (ii) a peptide containing amino acids 67–81 [this region was chosen because it is rich in charged residues, contains two prolines and tyrosines each, and corresponds in position to the main immunogenic region of the homologous α subunit of the muscle nicotinic acetylcholine receptor (Tzartos & Lindstrom, 1980)]; and (iii) a peptide encompassing residues 96–105. Previous protease digestion experiments with primary cultures (Hoch et al., 1989) had suggested that the mAb 4a epitope resides at the C-terminal end of PNF (see Discussion). Indeed, peptide 96–105 was recognized by mAb 4a, but not by the other antibodies (Figure 4A). MAb 2b in contrast gave a positive reaction with peptide 1–10 only (Figure 4B). None of the mAbs bound to peptide 67–81 (not shown).

To confirm the conclusion obtained by peptide ELISA, immunoprecipitation experiments were in addition performed. Of the mAbs studied, only mAb 2b reacts with native GlyR and has been employed for selective immunoprecipitation of the adult receptor isoform (Becker et al., 1988). Precipitation by mAb 2b of [^{3}H]strychnine binding sites from detergent extracts of spinal cord membranes prepared from adult rats was prevented by adding the MS2–PNF fusion protein (50 μg/mL; data not shown) or peptide 1–10 (Figure 5). Also, binding of this mAb to spinal cord membranes in a membrane immunoassay (Hoch et al., 1989) was completely inhibited by 5 μg/mL peptide 1–10 (not shown). These data corroborate the N-terminal localization of the mAb 2b epitope.

Discussion

Here, antigenic epitopes recognized by different mono- and polyclonal antibodies on the α1 subunit of the rat GlyR were localized by using bacterial fusion constructs and synthetic peptides. All mAbs and serum AS-1 bound to the PNF fusion proteins, indicating that this region of the extracellular domain contains the major antigenic epitopes of the mature protein. Serum AS-1 in addition showed some reaction with sequences in the intracellular loop domain between transmembrane segments M3 and M4, a region that is highly immunogenic in other ligand-gated ion channel proteins (Ratnam et al., 1986). The epitopes of mAbs 2b and 4a could be more precisely defined by using synthetic peptides. MAb 4a recognizes a sequence between amino acids 96 and 105; mAb 2b binds to the very N-terminal 10 residues of the mature α1 subunit. In view of the comparatively high antibody concentrations required for detection of peptide immunoreactivity (Figure 4), the epitopes of both antibodies might in fact include residues and/or conformational determinants not present in our peptides. The mAb 1a epitope, although not precisely localized, must reside between residues 11 and 95 of the α1 polypeptide. A map of antibody binding sites on the rat GlyR α1 subunit deduced from our data is given in Figure 6.

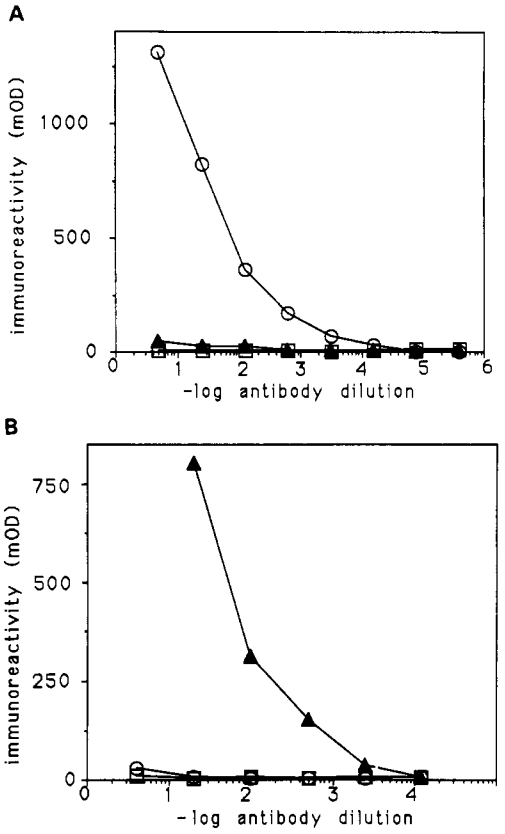

FIGURE 4: Peptide ELISA with mAbs 1a (□), 2b (▲), and 4a (○). Microtiter plates were coated with peptide 96–105 (A), or peptide 1–10 (B), and reacted with the indicated dilutions of the mAbs. Reactions were developed for 4 h (A) or 1 h (B), respectively.

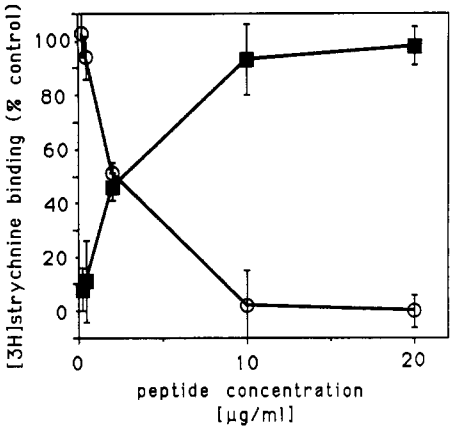

FIGURE 5: Inhibition by peptide 1–10 of GlyR immunoprecipitation with mAb 2b. GlyR was precipitated from detergent extracts of rat spinal cord by the addition of mAb 2b (1.5 μg per sample) that had been preincubated with various concentrations of peptide 1–10. The extent of receptor precipitation was monitored by determining [^{3}H]strychnine binding in the supernatant (■) and in the pellet (○). [^{3}H]Strychnine binding is indicated as percent of binding sites in a control precipitation where no peptide was added. All values represent the mean ±SD of triplicate determinations.

It is interesting to compare these epitope mapping results to previous proteolysis experiments on membrane-bound GlyR. All mAbs investigated in this study bind to the N-terminal 105 amino acids of the α1 subunit, thus defining this region as a particularly accessible and/or antigenic domain. In agreement with this conclusion, tryptic digestion readily removes 11–12 kDa from the extracellular part of GlyR α subunits (Graham et al., 1983; Hoch et al., 1989). Under these conditions, the mAb 4a epitope is preserved on the truncated membrane-bound receptor (Hoch et al., 1989). Assuming that

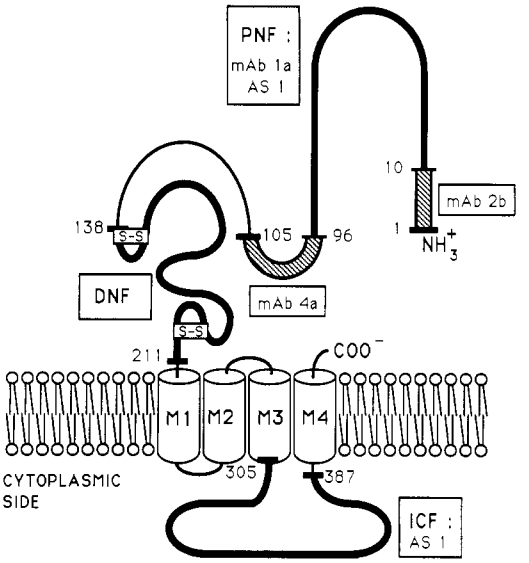

FIGURE 6: Map of antigenic epitopes on a model of the GlyR α1 subunit. The transmembrane topology of the polypeptide is assumed to correspond to that of nAChR proteins (Noda et al., 1983). Numbers correspond to amino acid positions in the mature α1 protein. Hydrophilic regions used for construction of fusion proteins are indicated by bold lines. Putative disulfide bridges are shown.

in analogy to other ligand-gated ion channel proteins (Noda et al., 1983) the loop between transmembrane segments M3 and M4 is located intracellularly, tryptic cleavage most likely occurs after lysine 95 of the α1 polypeptide. This is consistent with our assignment of the mAb 4a epitope.

Comparison of the mAb binding regions defined here with the available GlyR cDNA sequences reveals that the mAb 4a epitope is absolutely conserved in all sequenced α subunit variants (Grenningloh et al., 1987, 1990a; J. Kuhse, V. Schmieden, and H. Betz, submitted for publication). Indeed, in biochemical studies both neonatal and adult GlyR isoforms have been shown to bind this antibody (Becker et al., 1988; Hoch et al., 1989). Also, mAb 4a stains synapses not only in spinal cord but also in higher brain regions including rat and human cortex (E. Naas, H. Schröder, K. Zilles, C.-M. Becker, H. Betz, and H. Gnahn, submitted for publication). Thus, other α subunit variants may be expressed in those areas of the central nervous system. Furthermore, the 58-kDa GlyR β subunit shows only a single amino acid excange in its corresponding region (Grenningloh et al., 1990b). Interestingly, in purified GlyR preparations mAb 4a also stains the β polypeptide (Pfeiffer et al., 1984). In contrast, the N-terminal sequence recognized by mAb 2b is highly variable among different GlyR subunits, a finding that explains the selectivity of this antibody for the α1 polypeptide present in adult rat spinal cord and brain stem (Becker et al., 1988, 1989). In other words, mAbs 4a and 2b define common and unique epitopes on the α1 subunit of the rat GlyR. These antibodies therefore should be of considerable value in further classification of novel isoforms of this ligand-gated ion channel protein.

Added in Proof

The following papers cited as "submitted for publication" are now in press: Kuhse et al. (1990), Malosio et al. (1991), and Kuhse et al. (1991).

Acknowledgments

We thank I. Wolters for preparing the mAbs used in this study, Drs. E. Beck and K. Stanley for the gift of expression vectors, Dr. B. Schmitt for advice on peptide ELISAs, our

collegues for a critical reading of the manuscript, and J. Rami, I. Baro, and B. Albers for assistance during preparation of the manuscript.

References

Altschuler, R. A., Betz, H., Parakkal, M. H., Reeks, K. A., & Wenthold, R. J. (1986) *Brain Res. 369*, 316–320.

Araki, T., Yamano, M., Murakami, T., Wanaka, A., Betz, H., & Tohyama, M. (1988) *Neuroscience 25*, 613–624.

Becker, C.-M., Hermans-Borgmeyer, I., Schmitt, B., & Betz, H. (1986) *J. Neurosci. 6*, 1358–1364.

Becker, C.-M., Hoch, W., & Betz, H. (1988) *EMBO J. 7*, 3717–3726.

Becker, C.-M., Hoch, W., & Betz, H. (1989) *J. Neurochem. 53*, 125–131.

Betz, H. (1990) *Biochemistry 29*, 3591–3599.

Betz, H., & Becker, C.-M. (1988) *Neurochem. Int. 13*, 137–146.

Graham, D., Pfeiffer, F., & Betz, H. (1983) *Eur. J. Biochem. 131*, 519–525.

Graham, D., Pfeiffer, F., Simler, R., & Betz, H. (1985) *Biochemistry 24*, 990–994.

Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E. D., & Betz, H. (1987) *Nature 328*, 215–220.

Grenningloh, G., Schmieden, V., Schofield, P. R., Seeburg, P. H., Siddique, T., Mohandas, T. K., Becker, C.-M., & Betz, H. (1990a) *EMBO J. 9*, 771–776.

Grenningloh, G., Pribilla, I., Prior, P., Multhaup, G., Beyreuther, K., Taleb, O., & Betz, H. (1990b) *Neuron 4*, 963–970.

Hoch, W., Betz, H., & Becker, C.-M. (1989) *Neuron 3*, 339–348.

Kuhse, J., Schmieden, V., & Betz, H. (1990) *Neuron* (in press).

Kuhse, J., Schmieden, V., & Betz, H. (1991) *J. Biol. Chem.* (in press).

Kyhse-Anderson, J. (1984) *J. Biophys. Biochem. Methods 10*, 203–209.

Laemmli, U. (1970) *Nature 227*, 680–685.

Langosch, D., Thomas, L., & Betz, H. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7394–7398.

Malosio, M.-L., Grenningloh, G., Kuhse, J., Schmieden, V., Schmitt, B., Prior, P., & Betz, H. (1991) *J. Biol. Chem.* (in press).

Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Furutani, Y., Hirose, T., Takashima, H., Inayama, S., Miyata, T., & Numa, S. (1983) *Nature 302*, 528–532.

Pfeiffer, F. (1983) Ph.D. Thesis, Universität München.

Pfeiffer, F., & Betz, H. (1981) *Brain Res. 226*, 273–279.

Pfeiffer, F., Graham, D., & Betz, H. (1982) *J. Biol. Chem. 257*, 9389–9393.

Pfeiffer, F., Simler, R., Grenningloh, G., & Betz, H. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7224–7227.

Ratnam, M., Le Nguyen, D., Rivier, J., Sargent, P. B., & Lindstrom, J. (1986) *Biochemistry 25*, 2633–2643.

Rehm, H., Wiedenmann, B., & Betz, H. (1986) *EMBO J. 5*, 535–541.

Schloss, P., Hermans-Borgmeyer, I., Betz, H., & Gundelfinger, E. D. (1988) *EMBO J. 7*, 2889–2894.

Schmitt, B., Knaus, P., Becker, C.-M., & Betz, H. (1987) *Biochemistry 26*, 805–811.

Stanley, K. K., & Luzio, J. P. (1984) *EMBO J. 3*, 1429–1434.

Strebel, K., Becker, E., Strohmeier, K., & Schaller, H. (1986) *J. Virol. 57*, 983–991.

Triller, A., Cluzeaud, F., Pfeiffer, F., Betz, H., & Korn, H. (1985) *J. Cell Biol. 101*, 683–688.

Triller, A., Cluzeaud, F., & Korn, H. (1987) *J. Cell Biol. 104*, 947–956.

Tzartos, S., & Lindstrom, J. M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 755–759.

Van den Pol, A. N., & Gorcs, T. (1988) *J. Neurosci. 8*, 472–492.

Zabeau, M., & Stanley, K. K. (1982) *EMBO J. 1*, 1217–1224.