Purification and Characterization of the Glycine Receptor of Pig Spinal Cord†

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ABSTRACT: A large-scale purification procedure was developed to isolate the glycine receptor of pig spinal cord by affinity chromatography on aminostrychnine agarose. After an overall purification of about 10000-fold, the glycine receptor preparations contained three major polypeptides of M_r 48000, 58000, and 93000. Photoaffinity labeling with [^{3}H]strychnine showed that the [^{3}H]strychnine binding site is associated with the M_r 48000 and, to a much lesser extent, the M_r 58000 polypeptides. [^{3}H]Strychnine binding to the purified receptor exhibited a dissociation constant K_D of 13.8 nM and was inhibited by the agonists glycine, taurine, and β-alanine. Gel filtration and sucrose gradient centrifugation gave a Stokes radius of 7.1 nm and an apparent sedimentation coefficient of 9.6 S. Peptide mapping of the [^{3}H]strychnine-labeled M_r 48000 polypeptides of purified pig and rat glycine receptor preparations showed that the strychnine binding region of this receptor subunit is highly conserved between these species. Also, three out of six monoclonal antibodies against the glycine receptor of rat spinal cord significantly cross-reacted with their corresponding polypeptides of the pig glycine receptor. These results show that the glycine receptor of pig spinal cord is very similar to the well-characterized rat receptor protein and can be purified in quantities sufficient for protein chemical analysis.

Glycine is a major inhibitory neurotransmitter in mammalian spinal cord and other regions of the vertebrate central nervous system (Aprison & Daly, 1978). The binding of this amino acid to the postsynaptic glycine receptor (GlyR)[1] produces an inhibitory effect upon neuronal firing that is mediated by an increase in chloride conductance (Snyder & Bennett, 1976). This effect of glycine is antagonized by the alkaloid strychnine.

High-affinity binding of [^{3}H]strychnine to rat spinal cord membranes has been demonstrated (Young & Snyder, 1973, 1974a). This binding of [^{3}H]strychnine is inhibited by glycine and other glycinergic ligands and is thus thought to occur at the GlyR. Also, it has been suggested that strychnine and glycine bind to different sites of the GlyR and that the binding site for strychnine is associated with the chloride ion channel of this receptor (Young & Snyder, 1974b).

We have recently reported the solubilization (Pfeiffer & Betz, 1981) and affinity purification (Pfeiffer et al., 1982) of the GlyR from rat spinal cord membranes. The purified receptor was shown to have a molecular weight of 246000 with three component polypeptides of M_r 48000, 58000 and 93000. Furthermore, in photoaffinity-labeling experiments [^{3}H]-strychnine could be covalently attached to a polypeptide of M_r 48000 in both membrane-bound and purified GlyR preparations from rat spinal cord (Graham et al., 1981; Pfeiffer et al., 1982). This labeling by [^{3}H]strychnine could be prevented by glycine and its agonists but not by GABA or other neurotransmitter substances (Graham et al., 1983). We have therefore suggested that the antagonistic binding site of the GlyR is closely associated with the GlyR polypeptide of M_r 48000.

One limitation to the further biochemical characterization of the GlyR is the relatively small amount of material that can be purified from rat spinal cord (about 5–10 pmol/rat). Because large quantities of pig spinal cord could be obtained readily from abattoirs, an attempt was made to isolate the GlyR from this source. We now report the purification and characterization of the GlyR from pig spinal cord.

Experimental Procedures

Preparation of Aminostrychnine–Agarose. 2-Aminostrychnine was coupled to Affi-Gel 10 (Bio-Rad) as described previously (Pfeiffer et al., 1982).

[^{3}H]Strychnine Binding Assay. The binding of [^{3}H]-strychnine both to membranes and to solubilized receptor preparations was determined as described previously (Pfeiffer & Betz, 1981). [^{3}H]Strychnine binding to aminostrychnine–agarose eluates containing 200 mM glycine was measured after precipitation and washing of the GlyR using 20% (w/v) polyethene glycol 8000 (Pfeiffer et al., 1982). All binding assays were performed in triplicate and corrected for nonspecific binding determined in the presence of 10 mM glycine.

Preparation of Membranes. Spinal cords were obtained from adult pigs within 30 min of death, immediately frozen in liquid nitrogen, and stored at −70 °C. Frozen tissue (usually 250 g) was mixed with 10 volumes of 10 mM potassium phosphate buffer, pH 7.4, and homogenized 4 times for 20 s with an Ultraturrax T 45. After centrifugation for 30 min at 20000*g*, the pellet was resuspended by homogenization in 10 volumes of 25 mM potassium phosphate buffer, pH 7.4,

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; GABA, γ-aminobutyric acid; GlyR, glycine receptor; $NaDodSO_4$, sodium dodecyl sulfate.

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containing 0.6 M sucrose. The mixture was centrifuged for 90 min at 20000*g*, and the resulting pellet was resuspended by homogenization in 3 volumes of 25 mM potassium phosphate buffer, pH 7.4. After a further centrifugation for 20 min at 48000*g*, the pellet was resuspended by homogenization in 3 volumes of 25 mM potassium phosphate buffer, pH 7.4, containing 1 M KCl. All the above steps were performed at 4 °C, and all solutions contained 100 μM phenylmethanesulfonyl fluoride, 100 μM benzethonium chloride, 16 milliunits/mL aprotinin, and 0.2 mM ethylenediaminetetraacetic acid (EDTA).

Solubilization and Purification of the GlyR. Sodium cholate, dissolved as a 20% (w/v) stock solution in 25 mM potassium phosphate buffer, pH 7.4, was added to the freshly prepared membranes to a final conentration of 1% (w/v), and the mixture was stirred for 2 h. After centrifugation at 20000*g* for 1 h (a small aliquot of the mixture was also centrifuged at 150000*g* for 1 h and the supernatant used for assaying the extent of receptor solubilization), the supernatant was filtered through a sintered glass funnel. The filtrate was adsorbed batchwise to 25 mL of aminostrychnine–agarose beads by gentle rotation for 15 h, the beads were transferred to a small column (10 × 2 cm), and the column was washed with 20 volumes of 25 mM potassium phosphate buffer, pH 7.4, containing 1 M KCl, 1% (w/v) cholate, and 0.18% (w/v) phosphatidylcholine (egg yolk, Type X-E from Sigma). Receptor was eluted at 2.5 mL/h by using 200 mM glycine in the wash buffer, and the peak [^{3}H]strychnine binding fractions were pooled and dialyzed for 24 h against 1 L of 25 mM potassium phosphate buffer, pH 7.4, containing 1 M KCl, 1% (w/v) cholate, and 0.2% (w/v) phosphatidylcholine (soybean, Type 11-S, from Sigma) with two buffer changes. All the steps throughout the solubilization and purification were performed at 4 °C, and all solutions contained 100 μM phenylmethanesulfonyl fluoride, 100 μM benzethonium chloride, 1 mM benzamidine chloride, 16 milliunits/mL aprotinin, 5 mM EDTA, 5 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 5 mM dithiothreitol, and 2.5 mM iodoacetamide.

Photoaffinity Labeling. Photoaffinity labeling of the GlyR with [^{3}H]strychnine was performed as described by Graham et al. (1983). Before illumination, the samples were diluted to a final concentration of 0.17% (w/v) cholate. The final [^{3}H]strychnine concentration was 23 nM.

Protein Determination. Protein was determined by the Lowry method, as modified by ChandraRajan & Klein (1975) and Peterson (1977). Dilute protein solutions were concentrated by precipitation with trichloroacetic acid as described previously (Pfeiffer et al., 1982).

Electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate ($NaDodSO_4$) was performed by using the discontinuous gel system of Laemmli (1970). The gels were processed for silver staining or fluorography as described (Oakley et al., 1980; Graham et al., 1983). Glycosylated GlyR polypeptides were identified by incubating the gels with concanavalin A (Sigma) and horseradish peroxidase (Sigma) by using the method of Parish et al. (1977).

Gel Exclusion Chromatography. Gel exclusion chromatography on a Sepharose 6B column (1.6 × 60 cm) was performed as described previously (Pfeiffer & Betz, 1981), except that the buffer contained 1% (w/v) cholate instead of 1% (w/v) Triton X-100.

Sucrose Density Gradient Centrifugation. Linear sucrose/H_2O gradients [5–20% (w/v), 12 mL] were prepared in 25 mM potassium phosphate buffer, pH 7.4, containing 1 M KCl, 1% (w/v) cholate, 0.18% (w/v) soybean phosphatidylcholine, and the protease inhibitors and additives specified under Solubilization and Purification of the GlyR. The receptor preparation (250 μL) was layered on top of the gradients and centrifuged in a Spinco SW 41 Ti rotor at 39 000 rpm for 26 h at 4 °C. Fractions of 500 μL were collected and assayed for [^{3}H]strychnine binding activity. The following marker proteins were detected by enzymatic assays: pig heart mitochondrial malic dehydrogenase (4.32 S), rabbit muscle lactic dehydrogenase (6.95 S), pig heart fumarase (9.09 S), and bovine liver catalase (11.3 S).

Table I: Purification of GlyR from 250 g of Pig Spinal Cord

purification step	[^{3}H]strychnine binding: pmol	[^{3}H]strychnine binding: pmol/mg of protein	yield[a] (%)	purification (*x*-fold)
homogenate	2884	0.084[b]	100	1.0
membranes	1380	0.30	48	3.6
cholate extract	1330	0.26	46	3.1
aminostrychnine–agarose eluate (after dialysis)	261	830	9	9881

[a] Calculated from yields of the individual purification steps. [b] Corrected for 21% inhibition of [^{3}H]strychnine binding due to endogenous glycine.

Results

Purification of the GlyR. The GlyR was purified from pig spinal cord by a modification to our previously described purification of the rat receptor protein (Pfeiffer et al., 1982). In the procedure adopted here, the ionic detergent sodium cholate was used to solubilize the receptor from a crude membrane preparation. Upon solubilization (starting usually from 250 g of pig spinal cord tissue), a small aliquot (2 mL) of the extract was centrifuged for 1 h at 150000*g*. The supernatant was then assayed for [^{3}H]strychnine binding to determine the amount of receptor solubilized. The remainder of the detergent extract was centrifuged at 20000*g* for 1 h and filtered through a sintered glass funnel. The GlyR was then purified from the filtered cholate extract by affinity chromatography on aminostrychnine–agarose. By use of batchwise absorption, about 85% of the [^{3}H]strychnine binding sites were removed from the extract after 15 h (data not shown). The resin was then packed into a small column and washed thoroughly, and the receptor was fractionated from the column by using the agonist glycine. Aliquots of each fraction were precipitated and washed in the presence of polyethylene glycol to remove glycine [see Pfeiffer et al. (1982)] and assayed for [^{3}H]strychnine binding. The peak [^{3}H]strychnine binding fractions were then pooled and dialyzed to remove the agonist glycine.

The data from a typical purification experiment are shown in Table I. The preparation of crude membranes gave a 3.6-fold purification of GlyR. The recovery of [^{3}H]strychnine binding sites after solubilization was 96%. Affinity chromatography on aminostrychnine–agarose gave a >3000-fold purification with a yield of 20%. Total purification of the receptor was about 10000-fold, with a final purity of 830 pmol of [^{3}H]strychnine binding sites/mg of protein.

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis of the Purified GlyR. $NaDodSO_4$–polyacrylamide gel electrophoresis of the affinity-purified GlyR preparation of pig spinal cord revealed polypeptides of M_r 48 000, 58 000, and 93 000 upon silver staining (Figure 1, lane A). Furthermore, the same polypeptide bands were obtained with small-scale GlyR preparations from pig (Figure 1, lane B) or rat (Figure 1, lane

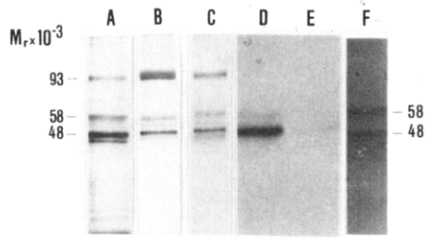

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of purified GlyR preparations. [^{3}H]Strychnine binding sites from spinal cord membranes were solubilized and purified on aminostrychnine–agarose. For lanes A–C, aliquots (0.4 pmol) of purified GlyR were electrophoresed on 10% $NaDodSO_4$–polyacrylamide gels and the gels silver stained for protein. Lane A: GlyR purified from pig spinal cord by using the procedure outlined under Experimental Procedures. Lane B: GlyR purified from pig spinal cord by using Triton X-100 as the detergent by the method of Pfeiffer et al. (1982). Lane C: GlyR purified from rat spinal cord by using Triton X-100 as the detergent by the method of Pfeiffer et al. (1982). Also, GlyR of pig spinal cord was purified and photoaffinity labeled with [^{3}H]strychnine as described under Experimental Procedures. These illuminated preparations were electrophoresed on a 10% $NaDodSO_4$–polyacrylamide gel, and the gel was subjected to fluorography. Lane D: purified GlyR (3 pmol) of pig spinal cord illuminated in the presence of [^{3}H]strychnine. Lane E: purified GlyR (3 pmol) of pig spinal cord illuminated in the presence of [^{3}H]strychnine and 1 mM glycine. For carbohydrate staining, GlyR was electrophoresed on a 10% $NaDodSO_4$–polyacrylamide gel and incubated with concanavalin A and horseradish peroxidase as described under Experimental Procedures. Lane F: purified GlyR (4 pmol) of pig spinal cord stained for carbohydrate. Lanes A–F are from four different gels aligned for comparison.

C) after affinity purification in the presence of Triton X-100 as described by Pfeiffer et al. (1982). The other polypeptides at M_r 44 000 and near to the origin of the gel seen in the cholate-solubilized preparation from pig spinal cord (Figure 1, lane A) may reflect enhanced proteolysis of the receptor due to solubilization in cholate as opposed to Triton X-100 or, alternatively, impurities arising from the scale-up in receptor preparation.

It may be noted here that upon solubilization in cholate the pig GlyR polypeptide of M_r 93 000 appeared to be particularly sensitive to proteolysis. In initial experiments, iodoacetamide was not included during cholate solubilization and purification of the pig receptor. None of the GlyR preparations resulting from these experiments contained significant amounts of the M_r 93 000 polypeptide (data not shown).

Aliquots of affinity-purified GlyR preparations were subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis, and corresponding lanes were stained for either protein or carbohydrate by using the concanavalin A/horseradish peroxidase method of Parish et al. (1977). In these experiments, the M_r 48 000 and 58 000 polypeptides were clearly labeled by the lectin and thus identified as glycopolypeptides (Figure 1, lane F). The M_r 93 000 polypeptide in contrast never showed significant concanavalin A binding and thus may not be glycosylated or contain oligosaccharides not recognized by this lectin. Identical results were obtained with GlyR preparations from rat spinal cord (data not shown).

Photoaffinity Labeling of the Purified GlyR. Photoaffinity labeling of the purified GlyR with [^{3}H]strychnine was performed by UV illumination according to Graham et al. (1981). $NaDodSO_4$–polyacrylamide gel electrophoresis and fluorography of the photoaffinity-labeled material showed that ^{3}H radioactivity was incorporated irreversibly into the polypeptide of M_r 48 000 (Figure 1, lane D). In addition, the polypeptide of M_r 58 000 was weakly labeled. Photoaffinity labeling of the purified receptor in the presence of glycine led to a dramatic reduction in the extent of labeling of both of these polypeptides (Figure 1, lane E).

Table II: Pharmacological Properties of the GlyR of Pig Spinal Cord[a]

ligand	K_D or K_I membranes	K_D or K_I affinity-purified GlyR
[^{3}H]strychnine	7.2 nM	13.8 nM
glycine	25 μM	10.7 μM
β-alanine	34 μM	9.1 μM
taurine	130 μM	32.6 μM
γ-aminobutyric acid	>1000 μM	637 μM

[a] [^{3}H]Strychnine binding to pig spinal cord membranes and to GlyR purified by affinity chromatography on aminostrychnine–agarose was performed as described under Experimental Procedures. The dissociation constant (K_D) of [^{3}H]strychnine binding to these preparations was determined by Scatchard analysis of binding data at seven [^{3}H]-strychnine concentrations between 1 and 50 nM. The experimental values in both preparations gave a straight line fit (r = −0.90 for membranes; r = −0.96 for affinity-purified glycine receptor), indicating a single class of binding sites. Inhibition constants (K_I) for γ-aminobutyric acid and the agonists glycine, β-alanine, and taurine were determined by log–probit analysis of inhibition curves as described previously (Pfeiffer & Betz, 1981). For each determination, five concentrations of displacer producing between 20 and 90% inhibition of the binding of 26–28 nM [^{3}H]strychnine were used.

Pharmacological Properties of the Pig GlyR. The pharmacological properties of the membrane-bound and the purified GlyR of pig spinal cord are summarized in Table II. Scatchard analysis of [^{3}H]strychnine binding data showed a single class of binding sites in both membranes and purified receptor preparations with dissociation constants (K_D) of 7.2 nM and 13.8 nM, respectively. The agonists glycine, β-alanine, and taurine inhibited [^{3}H]strychnine binding to both the membrane-bound and the purified pig receptor with K_I values comparable to those reported for rat GlyR (Graham et al., 1983). γ-Aminobutyric acid also was able to displace [^{3}H]-strychnine; however, millimolar concentrations were required.

Molecular Size of the Purified GlyR. The Stokes radius of the purified GlyR was 7.09 nm as determined by gel exclusion chromatography on Sepharose 6B. The recovery of purified receptor after gel chromatography was 81%. Sucrose density gradient centrifugation gave an apparent sedimentation coefficient of 9.6 S (n = 2) for the purified GlyR. The recovery of receptor after centrifugation was 59%.

Peptide Mapping of the GlyR M_r 48 000 Polypeptides from Pig and Rat Spinal Cord. Purified GlyR preparations from both pig and rat spinal cord were photoaffinity labeled with [^{3}H]strychnine as described under Experimental Procedures. Each preparation was then electrophoresed on $NaDodSO_4$–polyacrylamide gels, and the [^{3}H]strychnine-labeled polypeptide of M_r 48 000 was cut out and subjected to limited proteolysis according to the procedure of Cleveland et al. (1977). Digestion of the [^{3}H]strychnine-labeled polypeptides from both pig and rat GlyR with *Staphylococcus aureus* V8 protease produced two proteolytic fragments of identical size (M_r 28 000 and 17 000; Figure 2A). In addition, both species displayed the same pattern of radioactively labeled polypeptides of M_r 21 000, 14 000, and 7000 upon digestion of the [^{3}H]-strychnine-labeled polypeptide with papain (Figure 2B).

Immunochemical Analysis of the GlyR of Pig Spinal Cord. Recently, in our laboratory, monoclonal antibodies have been raised against the GlyR of rat spinal cord (Pfeiffer et al., 1984). These monoclonal antibodies were used in immunoblotting experiments to test for cross-reactivity against the GlyR purified from pig spinal cord. Of the six monoclonal antibodies examined, three detectably bound to pig GlyR polypeptides (Table III). Monoclonal antibody (mAb) GlyR

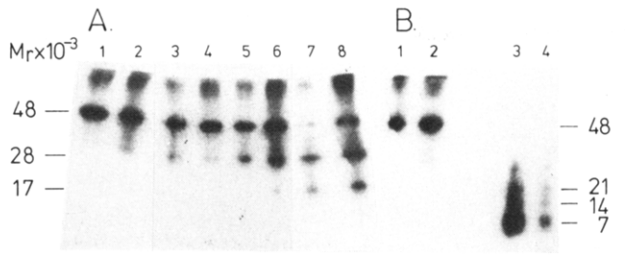

FIGURE 2: Peptide mapping of the [^{3}H]strychnine-labeled GlyR polypeptide of M_r 48 000 from pig and rat spinal cord. GlyR preparations from pig and rat spinal cord were photoaffinity labeled as described under Experimental Procedures, and multiple samples (10–30 pmol) of labeled GlyR were electrophoresed on 10% $NaDodSO_4$–polyacrylamide gels. The gels were briefly stained by using 0.1% (w/v) Coomassie Brilliant Blue (Graham et al., 1983), and the GlyR polypeptide bands of M_r 48 000 were cut out and reelectrophoresed on 12–20% $NaDodSO_4$–polyacrylamide gels with and without proteolytic digestion by using the method of Cleveland et al. (1977). Gels were stained and destained according to Steck et al. (1980) and then subjected to fluorography. (A) *Staphylococcus aureus* V8 protease (from Miles) digestions: lane 1, pig 48K polypeptide (no protease); lane 2, rat 48K polypeptide (no protease); lane 3, pig 48K polypeptide plus 10 ng of V8 protease; lane 4, rat 48K polypeptide plus 10 ng of V8 protease; lane 5, pig 48K polypeptide plus 100 ng of V8 protease; lane 6, rat 48K polypeptide plus 100 ng of V8 protease; lane 7, pig 48K polypeptide plus 1 μg of V8 protease; lane 8, rat 48K polypeptide plus 1 μg of V8 protease. (B) Papain (from Sigma) digestions: lane 1, pig 48K polypeptide (no protease); lane 2, rat 48K polypeptide (no protease); lane 3, pig 48K polypeptide plus 125 ng of papain; lane 4, rat 48K polypeptide plus 125 ng of papain.

Table III: Cross-Reactivity of Purified Pig GlyR to Monoclonal Antibodies Raised against Rat GlyR[a]

mAb	polypeptides bound	
	pig GlyR	rat GlyR[b]
GlyR1a	48K	48K
GlyR2b		48K
GlyR4a	48K + 58K	48K + 58K
GlyR5a		93K
GlyR7a	93K	93K (+48K)
GlyR9b		93K

[a] Purified GlyR of pig spinal cord was electrophoresed on $NaDodSO_4$–polyacrylamide slab gels, transferred to nitrocellulose paper, and incubated with the monoclonal antibodies listed below as described previously (Pfeiffer et al., 1984). The receptor polypeptides that bound antibody then were identified by incubation with horseradish peroxidase goat (anti-mouse IgG) antibody and subsequent staining for horseradish peroxidase activity (Graham & Karnovsky, 1966). [b] From Pfeiffer et al. (1984).

1a reacted with the polypeptide of M_r 48 000, mAb GlyR 4a with the polypeptides of 48 000 and 58 000, and mAb GlyR 7a with the polypeptide of M_r 93 000. These antibodies thus exhibited the same subunit specificity as previously found for rat GlyR (Table III).

Discussion

This report describes an extensive purification of the GlyR of pig spinal cord. In this procedure the membrane-bound receptor was first solubilized by using sodium cholate and then affinity purified on aminostrychnine–agarose. The ionic detergent, sodium cholate, was chosen for the solubilization and purification of GlyR of pig spinal cord, as opposed to the nonionic detergent, Triton X-100, used for the purification of GlyR of rat spinal cord (Pfeiffer et al., 1982). When sodium cholate was used, almost all of the [^{3}H]strychnine binding sites (96%) of pig spinal cord membranes could be solubilized. Furthermore, upon elution of the purified receptor from the aminostrychnine–agarose support using the agonist glycine, dialysis was subsequently used to remove glycine from the preparation to permit [^{3}H]strychnine binding determinations on the eluted receptor to be made. The use of sodium cholate as opposed to Triton X-100 and the elimination of our previously described wheat germ agglutinin–Sepharose chromatographic step to remove glycine from the purified receptor preparation increased 4.5-fold the recovery of GlyR from 2% [see Pfeiffer et al. (1982)] to 9%. In order to minimize possible proteolytic degradation and inactivation of the receptor, the purification of receptor (including dialysis step to remove glycine) from spinal cord tissue was completed within 48 h. Also, protease inhibitors and chelating reagents were used throughout the isolation of receptor. The final purification of the GlyR of pig spinal cord was 9881-fold, giving a [^{3}H]-strychnine binding activity of 830 pmol/mg of protein. This value is similar to that of 1425 pmol/mg of protein reported for affinity-purified GlyR of rat spinal cord (Pfeiffer et al., 1982). It is, however, considerably lower than that expected for a pure receptor protein of M_r ~250 000 (about 4 nmol/mg of protein assuming one strychnine binding site per receptor). This discrepancy is probably due to impurities (see Figure 1) and/or inactivation of the GlyR during dialysis [see also Pfeiffer et al. (1982)].

The pharmacological properties of the membrane-bound and affinity-purified GlyR of pig spinal cord were similar to those previously reported for the membrane-bound GlyR of rat spinal cord (Graham et al., 1983). The K_D of [^{3}H]strychnine binding was about 10 nM, and the agonists glycine, taurine, and β-alanine all inhibited the binding of [^{3}H]strychnine to both membranes and purified receptor preparations. Thus, as with the GlyR of rat spinal cord (Pfeiffer et al., 1982), the pharmacological characteristics of the pig receptor were conserved throughout the affinity purification procedure.

In addition to the similarity in pharmacological properties between GlyR of pig and rat spinal cord, $NaDodSO_4$–polyacrylamide gel electrophoresis of the purified GlyR of both species revealed the same polypeptide pattern. In each case, three polypeptides of M_r 48 000, 58 000, and 93 000 were identified upon silver staining. The polypeptides of M_r 48 000 and 58 000 both bound the lectin concanavalin A and thus were shown to be glycosylated. Upon UV illumination of the purified GlyR of pig spinal cord [^{3}H]strychnine was incorporated irreversibly into the polypeptide of M_r 48 000 and, to a lesser extent, into the polypeptide of M_r 58 000. The incorporation of [^{3}H]strychnine into these polypeptides could be prevented by glycine. We have previously documented that strychnine labels the GlyR polypeptide of M_r 48 000 in preparations from rat spinal cord (Graham et al., 1981; Pfeiffer et al., 1982). From the photoaffinity labeling of the purified GlyR of pig spinal cord with [^{3}H]strychnine, it now appears that the antagonistic binding site of the GlyR is not solely located in a domain of the polypeptide of M_r 48 000 but that the polypeptide of M_r 58 000 is also associated with this site.

Peptide mapping by V8 protease or papain of the GlyR photoaffinity-labeled polypeptide of M_r 48 000 from pig and rat spinal cord gave identical peptide patterns. Thus, the region of the antagonistic binding site associated with the GlyR polypeptide of M_r 48 000 appears to be highly conserved between these two species. Also, the three GlyR polypeptides of pig spinal cord were found to cross-react with at least three out of six monoclonal antibodies that have been prepared against the GlyR of rat spinal cord. All three subunits of the pig GlyR thus are immunologically related to their corresponding polypeptides of the rat receptor. The failure of the other monoclonal antibodies to bind to pig GlyR polypeptides may reflect subtle species differences in receptor structure.

Differences were also observed in the apparent molecular size properties of the purified GlyR of pig spinal cord to those of the purified GlyR of rat spinal cord. The Stokes radius of the GlyR of pig spinal cord was 7.09 nm, whereas the GlyR from rat spinal cord had a Stokes radius of 7.7 nm (Pfeiffer et al., 1982). In addition, the apparent sedimentation coefficients in sucrose gradients gave values of 9.6 and 7.7 S for pig and rat GlyR, respectively. These discrepancies in the micellar size of the two receptor preparations probably arose, however, because of the different detergents used for purification and size analysis (cholate for the pig receptor and Triton X-100 for the rat receptor) rather than species differences. Indeed, in a separate experiment, the GlyR of rat spinal cord purified by using sodium cholate as the detergent gave an apparent sedimentation coefficient in sucrose/H_2O gradients of 9.3 S (D. Graham, unpublished observation). The GlyR proteins of pig and rat thus can tentatively be concluded to have a rather similar molecular size, i.e., the previously determined M_r ~250000 (Pfeiffer et al., 1982). This molecular weight is considerably higher as calculated from the apparent molecular weights of the individual subunits. A model of the glycine receptor containing two the of M_r 48000 polypeptides and one of each of the M_r 58000 and 93000 polypeptides has recently been proposed (Betz et al., 1983; Betz, 1984).

When one strychnine binding site per GlyR molecule was assumed, pig spinal cord membranes had 0.3 pmol of GlyR/mg of protein, whereas rat spinal cord membranes had 1.95 pmol of GlyR/mg of protein. For purification purposes, however, this reduced GlyR density in pig spinal cord membranes can be compensated for, as large amounts of pig spinal cord can be obtained from abattoirs without laboratory personnel having to undertake dissection. Furthermore, although 250 g of pig spinal cord was routinely processed for purification in this report, it is possible to handle up to 2.5 kg of tissue (Einarson et al., 1982). Thus, in one purification run, GlyR from pig spinal cord may be purified in near milligram amounts (2.5 nmol). This procedure should therefore allow a detailed protein chemical analysis of this postsynaptic membrane protein.

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Registry No. GABA, 56-12-2; taurine, 107-35-7; β-alanine, 107-95-9; glycine, 56-40-6.

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