Henis, Y. I., & Gutman, O. (1983) *Biochim. Biophys. Acta 762*, 281–288.
Henis, Y. I., & Gutman, O. (1987) *Biochemistry 26*, 812–819.
Henis, Y. I., Gutman, O., & Loyter, A. (1985) *Exp. Cell Res. 160*, 514–526.
Hsu, M.-C., Scheid, A., & Choppin, P. W. (1979) *Virology 95*, 476–491.
Hsu, M.-C., Scheid, A., & Choppin, P. W. (1981) *J. Biol. Chem. 256*, 3557–3563.
Hsu, M.-C., Scheid, A., & Choppin, P. W. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 5862–5866.
Koppel, D. E., & Sheetz, M. P. (1981) *Nature (London) 293*, 159–161.
Koppel, D. E., Axelrod, D., Schlessinger, J., Elson, E. L., & Webb, W. W. (1976) *Biophys. J. 16*, 1315–1329.
Markwell, M. K. A., & Fox, C. F. (1980) *J. Virol. 33*, 152–166.
Markwell, M. K. A., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978) *Anal. Biochem. 87*, 206–210.
Metsikko, K., van Meer, G., & Simons, K. (1986) *EMBO J. 5*, 3429–3435.
Miura, N., Uchida, T., & Okada, Y. (1982) *Exp. Cell Res. 141*, 409–420.
Nakanishi, M., Uchida, T., Kim, J., & Okada, Y. (1982) *Exp. Cell Res. 142*, 95–101.
Nussbaum, O., Zakai, N., & Loyter, A. (1984) *Virology 138*, 185–197.
Ozawa, M., Asano, A., & Okada, Y. (1979) *Virology 99*, 197–202.
Peretz, H., Toister, Z., Laster, Y., & Loyter, A. (1974) *J. Cell Biol. 63*, 1–11.
Petersen, N. O., Felder, S., & Elson, E. L. (1984) in *Handbook of Experimental Immunology* (Weir, D. M., Herzenberg, L. A., Blackwell, C. C., & Herzenberg, L. A., Eds.) Chapter 24, pp 24.1–24.23, Blackwell Scientific Publications Ltd., Edinburgh.
Poste, G., & Pasternak, C. A. (1978) *Cell Surf. Rev. 5*, 305–367.
Richardson, C. R., Scheid, A., & Choppin, P. W. (1980) *Virology 105*, 205–222.
Sechoy, O., Philippot, J. R., & Bienvenue, A. (1987) *J. Biol. Chem. 262*, 11519–11523.
Towbin, H., Staehlin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.
Vainstein, A., Hershkovitz, M., Israel, S., Rabin, S., & Loyter, A. (1984) *Biochim. Biophys. Acta 773*, 181–188.
White, J., Kielian, M., & Helenius, A. (1983) *Q. Rev. Biophys. 16*, 151–195.
Wolf, D. E., Edidin, M., & Dragsten, P. R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2043–2045.

# Functional Reconstitution of the Glycine Receptor†

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ABSTRACT: The functional reconstitution of the chloride channel coupled glycine receptor is described. Glycine receptors were purified from the cholate extract of rat spinal cord membranes by affinity chromatography and incorporated into phospholipid vesicles by the addition of phosphatidylcholine and removal of detergent by gel filtration. The reconstituted vesicles showed the same polypeptide composition as the purified receptor (proteins of $M_r$ 48 000 and 58 000). The pharmacological characteristics of the glycine receptor were also preserved in the proteoliposomes, as demonstrated by the displacement of [$^3$H]strychnine binding by several glycinergic ligands and by photoaffinity labeling experiments. In order to observe functional responses (i.e., specific agonist-induced anion translocation), we have developed an assay based on the fluorescence quenching of an anion-sensitive entrapped probe, SPQ [6-methoxy-*N*-(3-sulfopropyl)quinolinium]. Reconstituted vesicles were loaded with the fluorescent probe during a freeze–thaw–sonication cycle in the presence of added liposomes containing cholesterol. In such a reconstituted system, glycine receptor agonists are able to increase the rate of anion influx into the vesicles. The action of agonists is blocked by the simultaneous presence of strychnine or other glycine antagonists. Our results show that the purified 48 000- and 58 000-dalton polypeptides reconstituted into phospholipid vesicles can bind ligands and promote specific ion translocation in a way similar to the glycine receptor in its native environment.

Glycine is a major inhibitory neutrotransmitter in the spinal cord and other regions of the central nervous system. The interaction of this amino acid with the glycine receptor (GlyR)[1] increases the chloride conductance of the postsynaptic membrane, thus producing a hyperpolarization (Krnjevic, 1974; Aprison & Daly, 1978). Glycine-mediated inhibitory actions are selectively antagonized by the alkaloid strychnine. The latter compound has been utilized in pharmacological, biochemical, and localization studies of the glycine receptor

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[1] Abbreviations: FTS vesicles, reconstituted vesicles subjected to a freeze–thaw–sonication cycle; GlyR, glycine receptor; SPQ, fluorescent probe 6-methoxy-*N*-(3-sulfopropyl)quinolinium.

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(Young & Snyder, 1973; Probst et al., 1986; Marvizón et al., 1986; Ruiz-Gómez et al., 1989). The use of strychnine as an affinity ligand has also allowed the purification of the receptor. Purified GlyR preparations have been reported to contain two glycosylated membrane polypeptides of relative molecular mass 48 000 and 58 000 daltons and an additional 93 000-dalton subunit which is thought to be a peripheral membrane protein associated to cytoplasmic domains of the receptor (Pfeiffer et al., 1982; Graham et al., 1985; Altschuler et al., 1986; Schmitt et al., 1987). Recent cloning of the cDNA of the strychnine binding subunit (48K) has revealed that it shares significant sequence and structural homologies with nicotinic acetylcholine receptor proteins and with the two subunits of the $GABA_A$ receptor, another chloride channel coupled receptor (Grenningloh et al., 1987; Schofield et al., 1987; Barnard et al., 1987).

The functional reconstitution of purified receptor preparations addresses the question of whether such systems contain all the necessary components to produce a physiologically relevant signal as a consequence of agonist binding. In the case of the nicotinic acetylcholine receptor, reconstituted preparations have provided experimental models in which the functional consequences of alterations in the structure or environment of receptors can be studied under controlled conditions [e.g., see Ochoa et al. (1983), Criado et al. (1984), and Huganir et al. (1986)]. In this report, the functional reconstitution of the glycine receptor purified from rat spinal cord is described for the first time. We have used purified glycine receptor polypeptides incorporated into liposomes and an adaptation of a technique recently used to monitor chloride transport in membrane vesicles and cells based on the collisional quenching by anions of a water-soluble fluorophore (SPQ) trapped in the vesicle or cell interior (Illsley & Verkman, 1987; Chen & Verkman, 1988). This experimental system could help to provide a better understanding of the molecular mechanisms involved in the function of neurotransmitter-gated chloride ion channels.

## Experimental Procedures

*Materials.* [$^3$H]Strychnine (15–30 Ci/mmol) was obtained from Amersham. L-$\alpha$-[Dipalmitoyl-1-$^{14}$C]phosphatidylcholine (107 mCi/mL) and [U-$^{14}$C]glycine (110–120 mCi/mmol) were purchased from New England Nuclear. Strychnine, glycine, $\beta$-alanine, taurine, sodium cholate, L-$\alpha$-phosphatidylcholine (type X-E), L-$\alpha$-phosphatidylcholine (type II-S), and cholesterol were obtained from Sigma. RU-5135 was donated by Roussel-Uclaf Laboratories, Paris, France. Iso-THAZ (5,6,7,8-tetrahydro-4*H*-isoxazolo[5,4-*c*]azepin-3-ol) was a gift from Dr. Krogsgaard-Larsen, The Royal Danish School of Pharmacy, Copenhagen, Denmark. 2-Aminostrychnine was synthesized from strychnine sulfate in the Organic Chemistry Department, Universidad Autónoma, Madrid. Sephadex G-50 (fine) was obtained from Pharmacia and the fluorescent probe 6-methoxy-*N*-(3-sulfopropyl)-quinolinium (SPQ) from Molecular Probes. All other materials were of the highest purity commercially available.

*Purification and Incorporation into Lipid Vesicles of the Glycine Receptor.* Glycine receptor was purified from rat spinal cord by affinity chromatography on 2-aminostrychnine–agarose as reported (Pfeiffer et al., 1982). Glycine receptors were eluted from the column with 120 mM KCl, 5 mM EDTA, 5 mM EGTA, 5 mM DTT, 2.5 mM iodoacetamide, 1 mM benzamidine, 17 milliunits/mL aprotinin, 0.1 mM phenylmethanesulfonyl fluoride, 0.1 mM benzetonium chloride, 1% sodium cholate (w/v), and 25 mM potassium phosphate buffer, pH 7.4, containing 200 mM glycine and 0.18% (w/v) L-$\alpha$-phosphatidylcholine from egg yolk. Aliquots (1000 $\mu$L) of purified receptors were mixed with 200 $\mu$L of a suspension of L-$\alpha$-phosphatidylcholine (5%, w/v) in 120 mM KCl, 1% sodium cholate (w/v), and 25 mM potassium phosphate, pH 7.4, and reconstituted by gel filtration through a Sephadex column (1.5 × 15 cm) as described (Haga et al., 1985). The column was preequilibrated and eluted with 120 mM potassium gluconate, 5 mM EDTA, 5 mM EGTA, and 25 mM potassium phosphate, pH 7.4 (reconstitution buffer). In some experiments, radiolabeled glycine or [$^{14}$C]dipalmitoylphosphatidylcholine were added to the mixture before reconstitution. Fractions (50 $\mu$L) were assayed for the presence of glycine or lipids by liquid scintillation (LKB 1219 Rackbeta) and for cholate as described (Kagawa & Racker, 1971). [$^3$H]Strychnine binding was determined essentially as previously reported in our laboratory for synaptic membrane preparations (Marvizón et al., 1986). Briefly, the reconstituted preparations (25–50 $\mu$L) were incubated at 4 °C for 10 min in a solution of 200 mM KCl and 25 mM potassium phosphate buffer, pH 7.4 (final volume 100–200 $\mu$L), containing the desired concentration of [$^3$H]strychnine (2–50 nM). Incubations were terminated by filtration (HAWP02500 Millipore filters). The filters were washed twice with ice-cold incubation buffer and dried, and the incorporated radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined in the presence of either 100 $\mu$M strychnine or 10 mM glycine. In order to create larger vesicles, the receptor-containing proteoliposomes were fused with 0.1 volume of a preparation of sonicated liposomes in a freeze–thaw–sonication cycle as described (Kasahara & Hinkle, 1977; Rosenberg et al., 1984). Liposomes consisted of crude soybean phospholipids (22.5 mg) and cholesterol (4.5 mg) premixed as chloroform solutions, evaporated to dryness under nitrogen, and resuspended in 1 mL of reconstitution buffer before sonication. Samples were electrophoresed on 10% homogeneous SDS–polyacrylamide gels as described (Laemmli, 1970). Proteolipsomes were prepared for freeze–fracture electron microscopy as described (Carrascosa et al., 1984).

*Photoaffinity Labeling of the Reconstituted Glycine Receptor.* The glycine receptor was reconstituted as described above, except that sodium chloride was used instead of potassium gluconate. This preparation was subjected to photoaffinity labeling as previously reported (Pfeiffer et al., 1982). Briefly, reconstituted preparations were incubated (30 min at 4 °C) with 40 nM [$^3$H]strychnine in the absence or presence of 100 $\mu$M strychnine or 10 mM glycine (final volume 500 $\mu$L). The samples were then placed in quartz cuvettes (1-cm path length) and illuminated for 10 min with ultraviolet light (Osram-HBO 100w/2 lamp source) at a distance of 20 cm, using a cuvette filled with chloroform as a filter to absorb radiation below 240 nm. At the end of this period, samples were freezed in liquid nitrogen, lyophilized, dissolved in SDS buffer (8% SDS, 10% glycerol, 5% $\beta$-mercaptoethanol, and 25 mM Tris-HCl, pH 6.5), electrophoresed, and subjected to fluorography (Bonner & Laskey, 1974). Dried gels were exposed to Hyperfilm-MP for 15 days at −70 °C.

*Glycine Receptor Functionality Assays.* Reconstituted vesicles were loaded with the fluorescent probe SPQ by adding the dye (10 mM final concentration) to the suspension prior to the freeze–thaw cycle. External SPQ molecules were removed by filtering the mixture through a Sephadex G-50 column (1.5 × 24 cm) equilibrated with reconstitution buffer at room temperature. The ion transport properties of the reconstituted receptor preparations (eluted in the void volume) were analyzed at 25 °C by measuring the fluorescence time

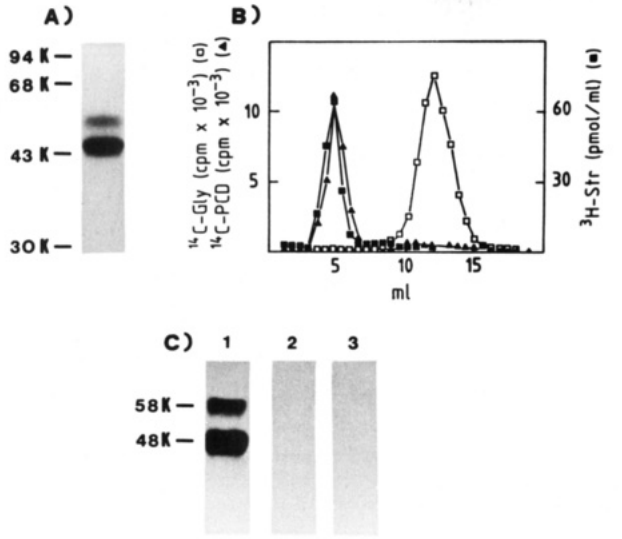


FIGURE 1: Reconstitution of purified glycine receptors into phospholipid vesicles. (A) SDS–PAGE of a purified receptor preparation (corresponding to 10 pmol of [$^3$H]strychnine binding sites) used for reconstitution studies as visualized by Coomassie-Blue staining. The position of relative molecular mass markers is shown. (B) Incorporation of the purified glycine receptor into phospholipid vesicles. The glycine receptor was specifically eluted from the affinity column with glycine and mixed with additional lipids, and the detergent was removed by gel filtration. The elution profiles for phospholipids (▲), glycine (□), and glycine receptor [$^3$H]strychnine binding activity (■) are shown. Experimental details are described under Experimental Procedures. (C) Photoaffinity labeling of the reconstituted glycine receptor. Reconstituted preparations (4 pmol of [$^3$H]strychnine binding sites) were incubated with 40 nM [$^3$H]strychnine alone (lane 1) or in the presence of 10 mM glycine (lane 2) or 100 $\mu$M strychnine (lane 3) and subjected to photoaffinity labeling, as described under Experimental Procedures.

course when the FTS proteoliposomes (100 $\mu$L) were mixed (final volume 3 mL) with a buffer containing 108 mM potassium gluconate, 12 mM NaI, and 25 mM potassium phosphate, pH 7.4, in the absence or presence of 100 $\mu$M glycine. When the effects of antagonists were tested, the reconstituted receptor was incubated in the presence of the desired drug (50 $\mu$M) for 10 min at 4 °C before addition of the quencher (NaI) and the agonist. Fluorescence was measured with a RRS 1000 Schoeffel Instrument Corp. fluorometer attached to a computer as described (Vázquez et al., 1988). The initial fluorescence ($F_i$) in the absence of iodide was set equal to 1. The initial rate of fluorescence quenching $(dF/dt)_{t=0}$ was calculated from the fluorescence time course. This parameter is related to the initial rate of anion influx into the proteoliposomes in the different experimental conditions (Illsley & Kerkman, 1987).

## Results

In the present study, we purified glycine receptors from the cholate extract of rat spinal cord membranes with an affinity gel of a specific ligand, 2-aminostrychnine. The purified protein bound 3.2 nmol of [$^3$H]strychnine/mg of protein, representing a 3000-fold purification, and displayed protein bands of apparent relative molecular mass ($M_r$) 48 000 and 58 000 when subjected to SDS–PAGE (Figure 1A). Purified glycine receptor preparations have been reported to contain an additional 93 000-dalton subunit which is thought to be a peripheral membrane protein associated with cytoplasmic domains of the receptor (Betz, 1987; Schmitt et al., 1987). However, in our hands, only a minor band of $M_r \simeq 94\,000$ was detected by silver staining in some preparations. In fact, the presence of this polypeptide has been described to be dependent on the choice of detergent and other experimental conditions (Graham et al., 1985; Schmitt et al., 1987).

Table I: Pharmacological Properties of the Glycine Receptor Reconstituted in Phospholipid Vesicles: Inhibition of [$^3$H]Strychnine Binding by Glycinergic Ligands[a]

| compound | $IC_{50}$ |
|---|---|
| agonists | |
| glycine | 75 ± 13 $\mu$M |
| $\beta$-alanine | 80 ± 7 $\mu$M |
| taurine | 179 ± 40 $\mu$M |
| antagonists | |
| Iso-THAZ | 1.7 ± 0.1 $\mu$M |
| RU-5135 | 48 ± 8 nM |

[a] Proteoliposomes were incubated with [$^3$H]strychnine (final concentration, 10 nM) as detailed under Experimental Procedures. Nonspecific binding was determined in the presence of 10 mM glycine. $IC_{50}$ values were calculated by least-squares fitting of Hill plots obtained from displacement curves performed with at least six different concentrations of inhibitor. The values are the mean ± SEM of at least three triplicate independent determinations.

The glycine receptor polypeptides eluted from the affinity column were subsequently incorporated into phospholipid vesicles (Figure 1B). A mixture of purified receptor and additional phosphatidylcholine in detergent was submitted to gel filtration to obtain detergent-free phospholipid vesicles in the void volume fraction. This method is rapid and also allows the quick removal of the glycine used for the specific elution of the receptor from the affinity column, which otherwise has to be eliminated by dialysis. A single peak of [$^3$H]strychnine binding activity coeluted with the lipids, with no apparent loss of binding sites in this step. SDS–PAGE of the reconstituted vesicles showed the same polypeptide composition of the purified receptor (data not shown). Photoaffinity labeling experiments show the incorporation of [$^3$H]strychnine to the 48K subunit and, to a lesser extent, to the 58K subunits; this incorporation is blocked by the presence of either glycine or strychnine (Figure 1C).

The reconstituted preparations exhibited pharmacological characteristics that resemble those observed for the original membranes. The binding of [$^3$H]strychnine to the GlyR proteoliposomes displayed a $K_d$ of 12 ± 1 nM (mean ± SEM of three determinations) and was displaced by the agonists glycine, $\beta$-alanine, and taurine and by the antagonists RU-5135 and Iso-THAZ (see Table I) with the expected order of potency (Marvizón et al., 1986). These results demonstrated that the binding properties of the glycine receptor had been preserved in the reconstituted vesicles.

In a following step, we have developed a fluorescence assay in order to observe functional responses, i.e., agonist-activated, antagonist-blocked anion translocation by the reconstituted glycine receptor. First, since the original reconstituted vesicles consist of small-diameter (25–30 nm average) proteoliposomes as assessed by electron microscopy (Figure 2A), the preparation was fused with additional sonicated liposomes in a freeze–thaw–sonication (FTS) cycle. This process allows the incorporation of the purified protein into larger vesicles (Figure 2B) with a more adequate internal volume to permit flux measurements (Rosenberg et al., 1984). Cholesterol, which has been reported to decrease the permeability of liposomes and to favor influx assays (Ochoa et al., 1983), was also incorporated at this point. On the other hand, we have entrapped into the FTS proteoliposomes the anion-sensitive, soluble fluorescence probe SPQ.

Figure 3A shows the time course of fluorescence quenching observed when FTS vesicles containing the receptor were mixed with an osmotically adjusted solution containing 12 mM NaI alone or in the presence of glycine or glycine plus

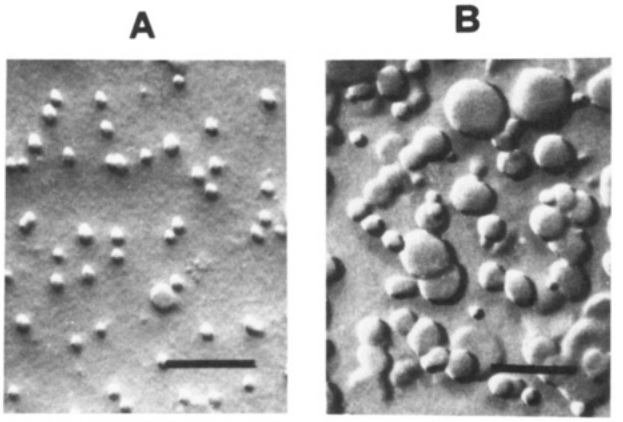


FIGURE 2: Electron microscopy of the reconstituted proteoliposomes before (A) and after (B) being subjected to a cycle of freeze–thaw–sonication. Bar, 200 nm.

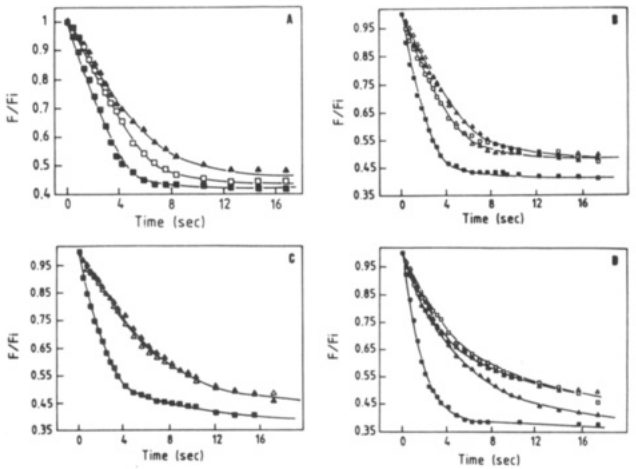


FIGURE 3: Effect of glycinergic ligands on iodide ion influx into reconstituted vesicles containing the glycine receptor. Reconstituted proteoliposomes entrapping SPQ were rapidly mixed with an osmotically adjusted external medium containing 12 mM NaI in the absence or presence of different glycinergic ligands, and the fluorescence was recorded. (A) (■) Glycine; (□) no drugs added; (▲) glycine plus strychnine. (B) (■) Glycine; (□) glycine plus RU-5135; (△) glycine plus ISO-THAZ; (▲) glycine plus strychnine. (C) (■) β-Alanine; (▲) β-alanine plus strychnine; (△) β-alanine plus ISO-THAZ. (D) (■) Taurine; (▲) taurine plus strychnine; (△) taurine plus ISO-THAZ; (□) taurine plus RU-5135. Agonist concentration was 100 μM and antagonist concentration 50 μM in all the experiments.

strychnine. Despite the relatively high decrease in fluorescence observed under basal conditions (which represents quenching of fluorescence by iodide leakage through the membrane or spontaneously opened glycine receptor channels), the agonist glycine, at physiological concentrations (100 μM), clearly enhances the rate of ion translocation into the vesicles. This effect is completely blocked by the simultaneous presence of the antagonist strychnine. Glycine or strychnine shows no effect on the time course of fluorescence quenching when FTS liposomes lacking GlyR were tested (data not shown). Figure 3B further shows that glycine promotes a more than 2-fold increase in the influx rate with respect to the situation when antagonists as RU-5135, ISO-THAZ, or strychnine are present. Other physiological agonists of the receptor, such as β-alanine (Figure 3C) or taurine (Figure 3D), also reproducibly increased to a similar extent the iodide influx rate into the reconstituted vesicles, their effect being strongly diminished by any of the three antagonists tested. Initial rates for ion translocation were estimated from the quenching curves and are shown in Table II.

Table II: Kinetic Analysis of Iodide Influx into FTS Vesicles Containing the Glycine Receptor[a]

| addition | $(dF/dt)_{t=0}$ ($s^{-1}$) |
|---|---|
| glycine | 121 ± 12 |
| glycine + strychnine | 52 ± 4 |
| glycine + RU-5135 | 54 ± 9 |
| glycine + Iso-THAZ | 59 ± 5 |
| β-alanine | 135 ± 9 |
| β-alanine + strychnine | 53 ± 9 |
| β-alanine + RU-5135 | 69 ± 10 |
| β-alanine + Iso-THAZ | 54 ± 5 |
| taurine | 140 ± 10 |
| taurine + strychnine | 56 ± 5 |
| taurine + RU-5135 | 60 ± 7 |
| taurine + ISO-THAZ | 72 ± 6 |
| none (basal influx) | 70 ± 6 |

[a] $(dF/dt)_{t=0}$ values were calculated from the initial slope of the SPQ fluorescence time course as described under Experimental Procedures and represent the mean ± SEM of 3–12 experiments performed with at least 3 different reconstituted glycine receptor preparations. Agonist concentration was 100 μM and antagonist concentration 50 μM in all the experiments.

## Discussion

Our results clearly show that the glycine receptor has been isolated and reconstituted in its active form. The polypeptide composition of the preparation used indicates that, in the absence of the 93K subunit, the 48K and 58K proteins can bind ligands and promote agonist-dependent ion translocation in a way similar to that suggested for the α and β subunits of the $GABA_A$ receptor (Schoefield et al., 1987). These results are also in agreement with the data recently obtained by Betz and collaborators using the *Xenopus* oocyte system, where the injection of mRNA produced from the 48K subunit cDNA is sufficient to lead to the expression of a glycine-gated chloride channel in the oocyte membrane (Schmieden et al., 1989). All this information, together with recent cross-linking experiments (Langosch et al., 1988), strongly indicates that the 48 000- and 58 000-dalton polypeptides form the ion channel containing core of the glycine receptor. However, a modulatory function of the 93K subunit cannot be ruled out.

The experimental model described herein could represent a relatively simple system where the functional properties of chloride channel coupled receptors could be studied. This approach is similar to that used with the acetylcholine receptor, where the transport of an external heavy-metal cation, $Tl^+$, was used to quench the fluorescence of entrapped dye (Moore & Raftery, 1980; Tomiko et al., 1986). In our experiments, iodide anion was usually employed as quencher in the external medium, since this ion is able to permeate through the glycine receptor channel (Bormann et al., 1987) and has a quenching efficiency higher than that of chloride (Krapf et al., 1988). Similar qualitative results were obtained with the latter anion (not shown).

The flux measurements reported herein present, however, some limitations, given the characteristics of the reconstituted vesicles and the time resolution of the system used. Several factors could prove critical for improving the assay and the extent of the response to the agonist. These include the relatively high permeability of proteoliposomes to iodide and chloride under basal conditions (much higher than that of $Tl^+$, for instance) (Papahadjopoulos & Watkins, 1967; Moore & Raftery, 1980); the low density of glycine receptors incorporated into the vesicles, since no receptor-enriched membranes are available as starting material as in the case of the nicotinic acetylcholine receptor; and the rapid desensitization detected upon exposure to agonists for different neurotransmitter-gated

ion channels (Huganir et al., 1986; Cash & Subarao, 1987; Krishtal et al., 1988) which could be taking place in the time scale used in these experiments. This, and whether the glycine receptor requires specific phospholipids or more cholesterol for maximum ion flux response as is the case for reconstituted preparations of the nicotinic acetylcholine receptor (Jones & McNamee, 1988), remains a subject of future investigation. In conclusion, the experimental approach described in this report may be useful in providing new insights into the molecular mechanisms of receptor gating, ion transport, and drug action in the chloride channel coupled $GABA_A$ and glycine receptor systems.

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## References

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

Aprison, M. H., & Daly, E. C. (1978) *Adv. Neurochem. 3*, 203–294.

Barnard, E. A., Darlisson, M. G., & Seeburg, P. (1987) *Trends Neurosci. 10*, 502–509.

Betz, H. (1987) *Trends Neurosci. 10*, 113–117.

Bonner, W. M., & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83–88.

Bormann, J., Hamill, O. P., & Sakman, B. J. (1987) *J. Physiol. 385*, 243–286.

Carrascosa, J. L., Lâzaro, J. M., Carrascosa, A. L., Garcîa, N., Santisteban, A., & Viñuela, E. (1984) *Virology 132*, 160–172.

Cash, D. J., & Subbarao, K. (1987) *Biochemistry 26*, 7562–7570.

Chen, P. Y., & Verkman, A. S. (1988) *Biochemistry 27*, 655–660.

Criado, M., Eibl, H., & Barrantes, F. J. (1984) *J. Biol. Chem. 259*, 9188–9198.

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

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

Haga, K., Haga, T., Ichiyama, A., Katada, T., Kurose, H., & Ui, M. (1985) *Nature 316*, 731–733.

Huganir, R. L., Delcour, A. H., Greengard, P., & Hess, G. P. (1986) *Nature 321*, 774–776.

Illsley, N. P., & Verkman, A. S. (1987) *Biochemistry 26*, 1215–1219.

Jones, O. T., & McNamee, M. G. (1988) *Biochemistry 27*, 2364–2374.

Kagawa, Y., & Racker, E. (1971) *J. Biol. Chem. 17*, 5477–5487.

Kasahara, M., & Hinkle, P. C. (1977) *J. Biol. Chem. 252*, 7384–7390.

Krapf, R., Illsley, N. P., Tseng, H. C., & Verkman, A. S. (1988) *Anal. Biochem. 169*, 142–150.

Krishtal, O. A., Osipchuk, Y. V., & Vrublevsky, S. V. (1988) *Neurosci. Lett. 84*, 271–276.

Krnjevic, K. (1974) *Physiol. Rev. 54*, 418–540.

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

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

Marvizôn, J. C. G., Vâzquez, J., Garcîa-Calvo, M., Mayor, F., Jr., Ruîz-Gômez, A., Valdivieso, F., & Benavides, J. (1986) *Mol. Pharmacol. 30*, 590–597.

Moore, H. P. H., & Raftery, M. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4509–4513.

Ochoa, E. L. N., Dalziel, A. W., & McNamee, M. G. (1983) *Biochim. Biophys. Acta 727*, 151–162.

Papahadjopoulos, D., & Watkins, J. C. (1967) *Biochim. Biophys. Acta 135*, 639–652.

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

Probst, A., Cortês, R., & Palacios, J. M. (1986) *Neuroscience 17*, 11–35.

Rosenberg, R. L., Tomiko, S. A., & Agnew, W. S. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1239–1243.

Ruiz-Gômez, A., Garcîa-Calvo, M., Vâzquez, J., Marvizôn, J. C. G., Valdivieso, F., & Mayor, F., Jr. (1989) *J. Neurochem.* (in press).

Schmieden, V., Grenningloh, G., Schofield, P. R., & Betz, H. (1989) *EMBO J. 8*, 695–700.

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

Schofield, P., Darlison, M. G., Fujitz, N., Burt, D. R., Stephenson, F. A., Rodrîguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencurse, T. A., Seeburg, P. H., & Barnard, E. A. (1987) *Nature 328*, 221–227.

Tomiko, S. A., Rosenberg, R. L., Emerick, M. C., & Agnew, W. S. (1986) *Biochemistry 25*, 2162–2174.

Vâzquez, J., Garcîa-Calvo, M., Valdivieso, F., Mayor, F., & Mayor, F., Jr. (1988) *J. Biol. Chem. 263*, 1255–1265.

Young, A. B., & Snyder, S. H. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2832–2836.