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Properties of human brain glycine receptors expressed in *Xenopus* oocytes

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Glycine and γ-aminobutyric acid (GABA) receptors from the foetal human brain were 'transplanted' into the *Xenopus* oocyte membrane by injecting the oocytes with poly(A)$^+$-mRNA extracted from the cerebral cortex. Activation of both glycine and GABA receptors induced membrane currents carried largely by chloride ions. However, unlike the GABA-activated current, the glycine current was blocked by strychnine, and was not potentiated by barbiturate. At low doses, the glycine current increased with concentration following a 2.7th power relation, suggesting that binding of three molecules of glycine may be required to open a single membrane channel. The current induced by steady application of glycine decreased with hyperpolarization beyond about −60 mV.

Introduction

Xenopus oocytes are very useful for the study of transmitter receptors, not only because they have native receptors (Kusano *et al.* 1982) but, more importantly, because the injection of appropriate exogenous messenger RNA (mRNA) into the oocyte leads to the synthesis and incorporation into the membrane of foreign receptor–channel complexes. This 'transplantation' of receptors is particularly useful to study receptors pertaining to rather inaccessible cells, like those in the brain. For example, mRNA derived from the chick optic lobe induced the oocyte to acquire receptors to γ-aminobutyric acid (GABA) (Miledi *et al.* 1982), rat brain mRNA induced receptors to serotonin, glutamate, kainate, acetylcholine and GABA (Gundersen *et al.* 1983*a*, 1984*a*), and a mRNA derived from foetal human cerebral cortex induced receptors to kainate and serotonin (Gundersen *et al.* 1984*b*). We have now found that a different preparation of mRNA from foetal human brain induces, in addition, functional receptors for glycine and GABA.

Methods

Procedures for the extraction of poly(A)$^+$-mRNA and its injection into oocytes of *Xenopus laevis*, were as described previously (Gundersen *et al.* 1983*b*; Miledi & Sumikawa 1982). The messenger RNA was extracted from the cerebral cortex of a 15-week-old foetus, obtained after termination of pregnancy with prostaglandin. Electrophysiological recording techniques were also as used before

(Kusano *et al.* 1982; Miledi 1982). Briefly, oocytes were voltage clamped, and perfused continuously with Ringer solution (composition; NaCl, 120 mM; KCl, 2 mM; $CaCl_2$, 1.8 mM; HEPES, 5 mM, at pH 7.2) at room temperature (20–23 °C). Drugs were applied in the perfusion solution, and the resulting membrane currents were recorded.

Results

Membrane channels induced by mRNA *from human cerebral cortex*

Injection of mRNA derived from the cerebral cortex of a 15-week-old foetus into *Xenopus* oocytes caused the synthesis and incorporation into the membrane of several types of drug- and voltage-activated channels. These included voltage-activated sodium channels, and channels activated by serotonin and kainate, which we have described previously (Gundersen *et al.* 1984*b*). In addition to these, the present messenger preparation induced the formation of channels activated by glycine and, in a few ooctyes, sensitivity to GABA was also observed (figure 1).

Table 1. Drug- and voltage-operated membrane currents in *Xenopus* oocytes injected with mRNA from foetal human cerebral cortex

(Figures give peak currents in nanoamperes (mean plus standard error of mean) and number of oocytes in brackets. Control (non-injected) and injected oocytes were all from the same donor. In the case of drug activated currents, measurements were made at a clamp potential of −100 mV, and the drug concentration was 10^{-3} M. The voltage activated sodium current was measured as the peak inward current elicited by depolarization to −10 from −100 mV. Similarly, the calcium activated transient outward (T_{out}) current, and the potassium current were measured for depolarizations to 0 and +30 mV respectively (see Gundersen *et al.* 1983*b*, 1984*b* for further details). Sensitivity to serotonin was not systematically tested, but a few injected oocytes examined all gave large responses.)

	control	human cortex mRNA
glycine	1.5 s.e. 0.4 (5)	242 s.e. 105 (7)
kainate	0 (4)	10 s.e. 1.4 (4)
sodium	0 (5)	68 s.e. 26 (5)
potassium	0 (5)	0 (5)
T_{out}	16 s.e. 4 (4)	9 s.e. 2.5 (5)

Control (non-injected) oocytes showed either no response to high concentrations (1 mM) of glycine, or just detectable inward currents of 1–2 nA. In contrast, oocytes injected with human cortex mRNA gave large responses to 1 mM glycine, often with peak amplitudes of several hundred nA (table 1). The response to glycine was still present in oocytes which had been treated with collagenase to remove the follicular and other enveloping cells, which indicates that the glycine sensitivity resides in the oocyte membrane itself. Furthermore, sensitivity to glycine still developed in oocytes which were exposed to actinomycin D (50 μg ml^{-1}) to inhibit transcription of mRNA from the oocyte's own genome. Thus, the membrane channels activated by glycine almost certainly arose as a result of translation of the human messenger RNA.

Glycine and GABA *responses from injected oocytes*

Application of glycine to oocytes injected with the mRNA consistently elicited smooth inward membrane currents, and a few oocytes also showed similar currents in response to GABA (figure 1). The receptors underlying these responses were pharmacologically distinct. For example, the response to glycine was substantially reduced by 20 μM strychnine (figure 1*a–c*), while the response to GABA was not appreciably changed by this concentration of strychnine. Also, the membrane current elicited by GABA was potentiated by barbiturate, similar to the potentiation of GABA responses induced by chick brain mRNA (Smart *et al.* 1983) and

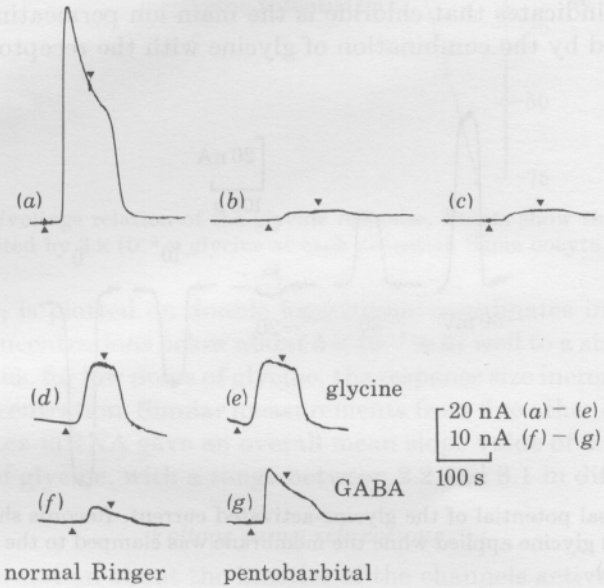

FIGURE 1. Membrane currents elicited by glycine and GABA in an oocyte injected with human cerebral cortex mRNA. Upward deflexions of the traces correspond to inward membrane currents. Drugs were applied by bath perfusion. Holding potential, −100 mV. Temperature, 23 °C.

(*a*)–(*c*) Response to glycine, and the blocking of the response by strychnine. In each frame, 10^{-3} M glycine was applied as indicated by the arrows. Traces show: (*a*) response in normal Ringer, (*b*) response after adding 20 μM strychnine, and (*c*) response 12 min after washing out the strychnine.

(*d*)–(*g*) Effect of pentobarbital on the responses elicited by 3×10^{-4} glycine (*d*), (*e*) and 10^{-4} GABA (*f*), (*g*). The records on the left (*d*) and (*f*) were obtained in normal Ringer, while those on the right (*e*) and (*g*) were obtained in the presence of 100 μM pentobarbital.

rat brain mRNA (C. B. Gundersen, R. Miledi and I. Parker, unpublished), while the glycine response changed little. Figure 1*f, g* illustrates an oocyte where the response to 10^{-4} M GABA was potentiated by a factor of about four times by 100 μM pentobarbital. The response to glycine, however, was not appreciably altered (figure 1*d, e*). We have also recently recorded similar glycine and GABA responses from oocytes injected with mRNA derived from adult rat brain, in addition to the various other receptor types previously described (Gundersen *et al.* 1983*a*, 1984*a*).

Effect of membrane potential on the glycine-activated current

The current elicited by bath application of glycine became smaller as the membrane was depolarized, and inverted direction at about −22 mV in the oocyte illustrated in figures 2 and 3. A mean value for the reversal potential, obtained from four oocytes, was -22.7 ± 0.9 mV (±s.e.m.), which corresponds to the chloride equilibrium potential in *Xenopus* oocytes (Kusano *et al.* 1982). Furthermore, the currents elicited in the same oocytes by activation of muscarinic ACh receptors and receptors to serotonin and GABA, all of which are caused by increases in chloride conductance (Kusano *et al.* 1982; Gundersen *et al.* 1983*a*; Miledi *et al.* 1982), reversed at about the same potential level as the glycine response. This indicates that chloride is the main ion permeating the membrane channels opened by the combination of glycine with the receptors.

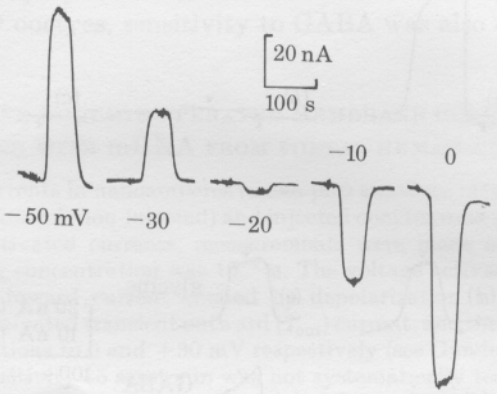

FIGURE 2. Reversal potential of the glycine-activated current. Records show currents elicited by 3×10^{-4} M glycine applied while the membrane was clamped to the potentials indicated (in millivolts).

Figure 3 shows the currents elicited by a constant dose of glycine (3×10^{-4} M) applied with the membrane potential clamped to different levels. Between about 0 and −30 mV the current/voltage relation was nearly linear, but as the potential was made more negative the current reached a maximal value at about −70 mV, and then declined with further polarization.

Concentration dependence of the glycine-activated current

In the oocyte illustrated in figure 4, responses first became detectable at a glycine concentration of about 7×10^{-5} M, and then increased in size very steeply with increasing dose. At low concentrations, a doubling in dose gave more than a fourfold increase in peak current (figure 4*a–d*). However, 2×10^{-3} M glycine gave a nearly maximal response, and a further increase to 10^{-2} M gave little additional increase (figure 4*f*, *g*). The membrane current was well maintained throughout the duration of drug application at concentrations below about 2×10^{-4} M, but with higher doses the response declined progressively more rapidly.

The relation between peak response size and glycine concentration, measured

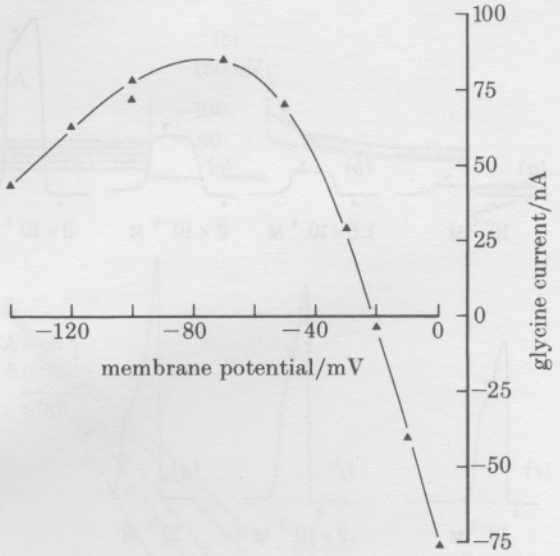

FIGURE 3. Current/voltage relation of the glycine response. Points show the peak membrane currents elicited by 3×10^{-4} M glycine at each potential. Same oocyte as figure 2.

from one oocyte, is plotted on double logarithmic coordinates in figure 4*h*. The data points at concentrations below about 5×10^{-4} M fit well to a straight line, with a slope of 2.7. Thus, for low doses of glycine, the response size increases as the 2.7th power of the concentration. Similar measurements from five other oocytes injected with human cortex mRNA gave an overall mean slope value of 2.64 ± 0.15 at low concentrations of glycine, with a range between 2.2 and 3.1 in different oocytes.

Voltage jump relaxations

To obtain information about the kinetics of the channels activated by glycine, we examined the relaxation of the clamp currents following step changes in membrane potential during steady application of glycine. This technique has been used to study acetylcholine activated channels in muscle (Neher & Sakmann 1975; Adams 1977), where the current was found to relax exponentially to a new steady value, with a time constant corresponding to the mean channel lifetime at the potential following the step.

Figure 5 illustrates the membrane currents recorded when the potential was stepped to more negative values from a holding potential of −60 mV. In the absence of glycine, the current steps were rectangular, and varied linearly with potential (figure 5*a*). However, the same potential steps applied in the presence of glycine (5×10^{-4} M) gave an initially larger current, which was followed by a slow decline (figure 5*b*). As the potential was made more negative the initial current increased, but the decline became faster and at −160 mV the current remaining at the end of the pulse was smaller than during the same pulse in the absence of glycine.

The time course of current relaxations during glycine application are plotted on semi-logarithmic coordinates in figure 5*c*. At potentials between −80 and

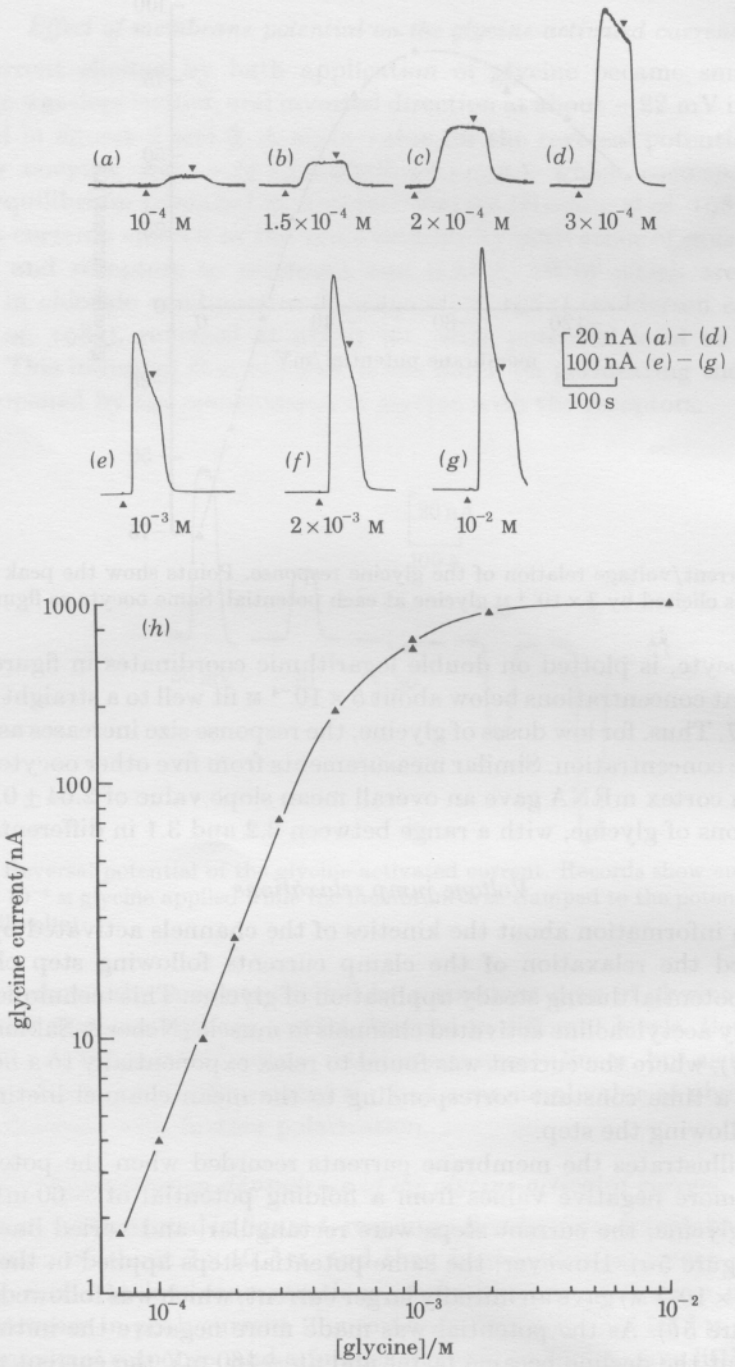

FIGURE 4. (*a*)–(*g*) Membrane currents elicited by different concentrations of glycine. All records were obtained at a potential of −100 mV. Note the change in gain between the upper and lower sets of records. (*h*) Dose–response relation for the glycine activated current, plotted on double logarithmic coordinates. All measurements were made from the same oocyte as (*a*)–(*g*), which was clamped at −100 mV.

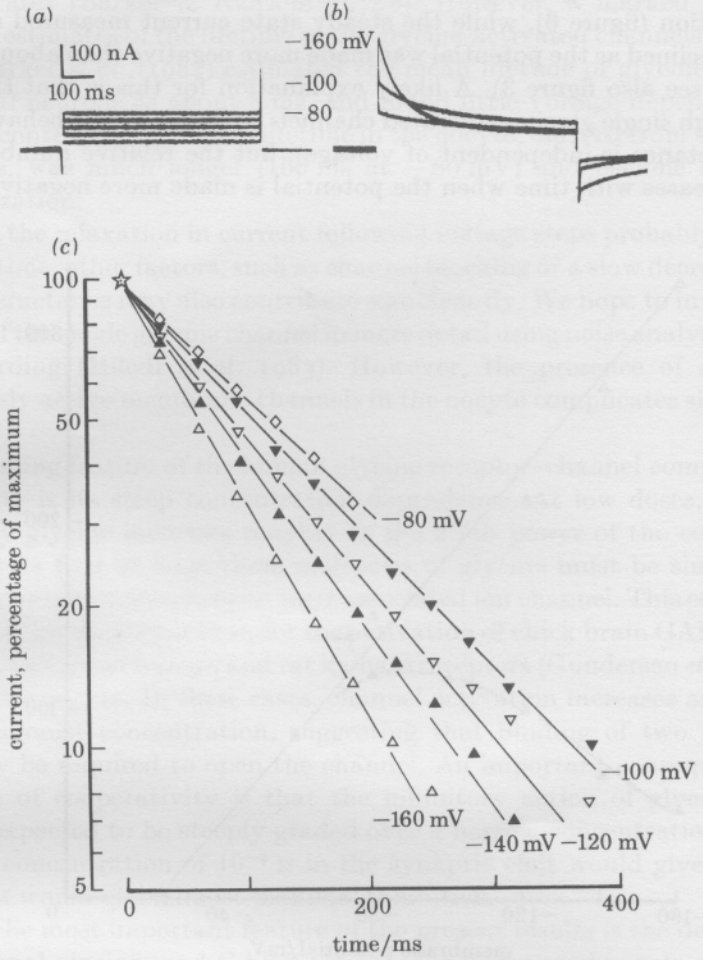

FIGURE 5. Current relaxations following voltage steps imposed during glycine application.

(*a*), (*b*) Traces show membrane currents elicited by hyperpolarizing pulses applied from a holding potential of −60 mV. Records in (*a*) were obtained in normal Ringer, at potentials of (from bottom to top) −80, −100, −120, −140 and −160 mV. Records in (*b*) were from the same oocyte, but obtained at the peak of the response elicited by 5×10^{-4} M glycine. At −60 mV the maximal glycine induced current was 87 nA. Pulse potentials are indicated in millivolts next to the traces. Temperature, 23 °C.

(*c*) Decay of the membrane current during hyperpolarizing pulses in the presence of 5×10^{-4} M glycine, plotted on semi-logarithmic coordinates. Data from the same oocyte as (*a*), (*b*). Potentials during the pulse are indicated next to the data. Holding potential was −60 mV. Decay time constants are: −80 mV, 164 ms; −100 mV, 147 ms; −120 mV, 129 ms; −140 mV, 106 ms; −160 mV, 91 ms.

−160 mV the points lie on straight lines, indicating that the current relaxations largely follow single exponential time courses. The time constants derived from the lines became shorter at more negative potentials, and for the oocyte illustrated the time constant was 164 ms at −80 mV, and 91 ms at −160 mV (temperature 23 °C).

The current at the beginning of the voltage steps in figure 5 increased linearly with polarization (figure 6), while the steady state current measured at the end of the pulse declined as the potential was made more negative than about −80 mV (figure 6 and see also figure 3). A likely explanation for this is that the current flowing through single glycine-activated channels shows an ohmic behaviour (that is, the conductance is independent of voltage), but the relative number of open channels decreases with time when the potential is made more negative.

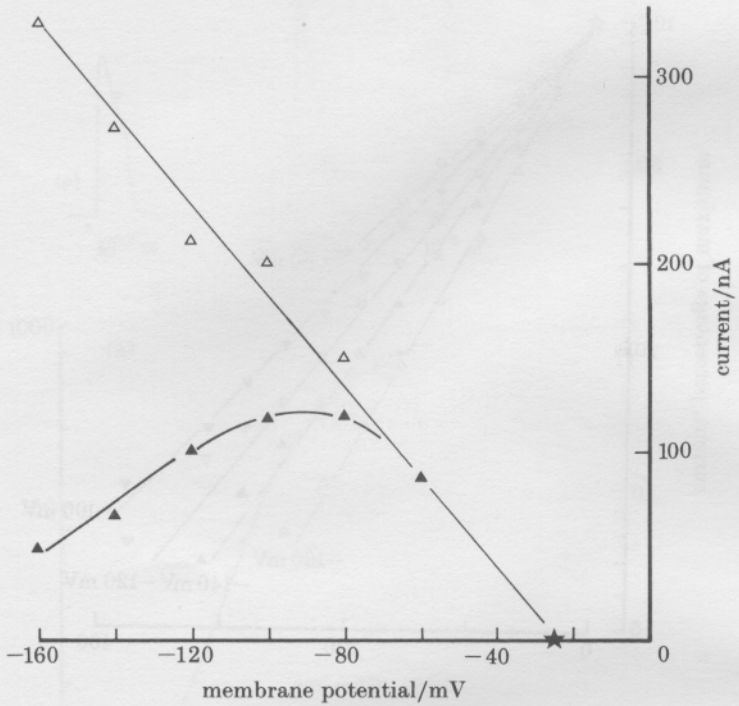

FIGURE 6. Current/voltage relations of the instantaneous (△) and steady state (▲) current activated by glycine. Data from the same oocyte as figure 6, using a glycine concentration of 5×10^{-4} M. Steady state current was measured at the end of 3 s duration pulses. Instantaneous current was measured at the onset of the pulses. In both cases, correction was made for the passive leakage current of the oocyte. The reversal potential (star) was directly measured, as in figure 2.

DISCUSSION

The properties of 'human brain' glycine- and GABA-activated channels transplanted into the *Xenopus* oocyte membrane show many similarities with those of (native) glycine channels in the neurons of the rat and cat central nervous system. For example, in both neurons and oocytes glycine- and GABA-activated currents are carried largely by chloride ions (see, for example, Barker & Ransom 1978*a*) – although, of course, the responses in the neurons are inhibitory, rather than depolarizing as in the oocyte, because of the more negative equilibrium potential for chloride. Also, the neuronal glycine channels are blocked selectively by

strychnine (Barker *et al.* 1983), while the GABA channels are selectively potentiated by barbiturates (Barker & Ransom 1978*b*). However, a marked difference is apparent in estimates of the lifetime of the glycine-activated channels. Using noise analysis, Barker *et al.* (1982) estimated the mean lifetime of glycine channels in mouse spinal neurons as about 8 ms, and found little voltage dependence of the lifetime. In contrast, the channel lifetime in the oocyte, derived from voltage jump experiments, was much longer (160 ms at −80 mV) and became shorter with hyperpolarization.

Although the relaxation in current following voltage steps probably reflects the channel lifetime, other factors, such as channel blocking or a slow decrease in single channel conductance may also contribute significantly. We hope to investigate the properties of the single glycine channel in more detail using noise analysis and patch clamp recording (Miledi *et al.* 1983). However, the presence of a variety of spontaneously active membrane channels in the oocyte complicates single channel recording.

An interesting feature of the human glycine receptor–channel complex induced in the oocyte is its steep concentration dependence. At low doses, the current activated by glycine increases roughly as the 2.7th power of the concentration, which suggests that at least three molecules of glycine must be simultaneously bound to the receptor sites to open their associated ion channel. This concentration dependence is much steeper than for the activation of chick brain GABA receptors (Miledi *et al.* 1982) and human and rat kainate receptors (Gundersen *et al.* 1984*a*, *b*) induced in the oocyte. In these cases, channel activation increases as the square, or less, of agonist concentration, suggesting that binding of two molecules of agonist may be required to open the channel. An important consequence of this high degree of cooperativity is that the inhibitory action of glycine on brain neurons is expected to be steeply graded over a narrow concentration range. For example, a concentration of 10^{-4} M in the synaptic cleft would give little effect, while 10^{-3} M would give almost maximal inhibition.

Perhaps the most important feature of the present results is the demonstration that functional glycine- and GABA-activated membrane channels can be transplanted from the human brain into the *Xenopus* oocyte. The use of this simplified system should greatly facilitate the study of these receptor–channel complexes, and their modulation by convulsant and depressant drugs.

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