effects on rhodopsin suggests that their action is not via disruption of α-βγ interaction.

The methods described here for probing receptor–G protein interaction could be used to explore sites involved in other protein-protein interactions. Similar studies will be useful to probe the specificity and cross talk of G protein interactions with other receptors. The 40 COOH-terminal amino acids are quite conserved among the family of guanine nucleotide binding proteins and are involved in binding to receptors. Monoclonal antibody 4A can cross-react with and block the activation of the α subunits of stimulatory and inhibitory G proteins, α_s (*17*), α_i (*17*), and α_k (*18*). Therefore, synthetic peptides to homologous regions of α_i and α_s may have similar blocking effects. Such peptides could potentially be useful as tools to block selectively specific receptor-mediated cellular activities.

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Requirement for Glycine in Activation of NMDA-Receptors Expressed in *Xenopus* Oocytes

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Receptors for *N*-methyl-D-aspartate (NMDA) are involved in many plastic and pathological processes in the brain. Glycine has been reported to potentiate NMDA responses in neurons and in *Xenopus* oocytes injected with rat brain messenger RNA. Glycine is now shown to be absolutely required for activation of NMDA receptors in oocytes. In voltage-clamped oocytes, neither perfusion nor rapid pressure application of NMDA onto messenger RNA–injected oocytes caused a distinct ionic current without added glycine. When glycine was added, however, NMDA evoked large inward currents. The concentration of glycine required to produce a half-maximal response was 670 nanomolar, and the glycine dose-response curve extrapolated to zero in the absence of glycine. Several analogs of glycine could substitute for glycine, among which D-serine and D-alanine were the most effective. The observation that D–amino acids are effective will be important in developing drugs targeted at the glycine site.

STRYCHNINE-INSENSITIVE GLYCINE binding sites that co-localize with NMDA binding sites in the brain (*1*) are now thought to be responsible for the potentiation of NMDA receptor responses by glycine. This effect of glycine was first observed in mouse brain neurons (*2*) and has also been observed in *Xenopus* oocytes induced to express NMDA receptors by injection of rat brain mRNA (*3*). The potentiation is specific for the NMDA receptor because no effect was seen on kainate or quisqualate currents in neurons (*2*). Glycine also enhanced the glutamate- or NMDA-dependent binding of the open channel blockers [^{3}H]*N*-(1-[2-thienyl]cyclohexyl)-3,4-piperadine ([^{3}H]TCP) and [^{3}H]MK-801 to the NMDA receptor (*4–7*). The potentiation of the NMDA receptor response by glycine has been likened to the potentiation of the γ-aminobutyric acid A ($GABA_A$) receptor by benzodiazepines (*2, 4, 5*). However, the $GABA_A$ receptor, when cloned and expressed in *Xenopus* oocytes, is still functional in the absence of benzodiazepines (*8*). We now present evidence that, in contrast to the benzodiazepine-GABA receptor relation, glycine is required for NMDA receptor activation in oocytes injected with rat brain mRNA.

Xenopus oocytes were voltage-clamped with one or two microelectrodes, and drugs were applied by perfusion or pressure ejection in medium similar to that used for culturing the oocytes (*9*). Messenger RNA extracted from rat brain encodes NMDA receptors that contain both the known regulatory components of the native neuronal receptor—voltage-dependent block by Mg^{2+} and potentiation by glycine (*3*). At a holding potential of −60 mV in nominally Mg^{2+}-free medium, application of 300 μ*M* NMDA elicited a large inward current in the presence of 3 μ*M* glycine (66 ± 13 nA,

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$n = 10$; error limits are ± SEM throughout). This concentration of NMDA elicits a 95% maximal response (*10*). In the same cells in the absence of added glycine, NMDA elicited negligible inward current (0.6 ± 0.3 nA) (Fig. 1A). Application of a much higher concentration of NMDA (3 m*M*) in the absence of added glycine resulted in a small inward current that was not reduced by 100 μ*M* D-2-amino-5-phosphonovaleric acid (D-APV) or 1 m*M* Mg^{2+}. This current, therefore, was not due to activation of NMDA receptors. We were concerned that the relatively slow rise time of the current when NMDA was applied by perfusion (10 to 90% rise time of 37 ± 5 s, $n = 10$) might mask a rapid, perhaps desensitizing, phase of the response. Therefore, brief pressure ejection of NMDA was used in the presence and absence of 30 μ*M* glycine. Application of NMDA in this manner caused no detectable current flow (0.04 ± 0.02 nA, $n = 9$) in the absence of glycine, whereas, with 30 μ*M* glycine in the perfusion solution, NMDA pulses caused a brief inward current of 5.1 ± 0.7 nA (Fig. 1B). In two cells a large, rapidly fading current was observed after application of NMDA in the presence of glycine, but this current was not seen during previous exposure of the cell to NMDA in the absence of glycine. This component may be the early peak of the two-component response seen in some cells during perfusion of NMDA in the presence of glycine or its analogs (Fig. 2). No current was observed during perfusion of NMDA and glycine in uninjected oocytes.

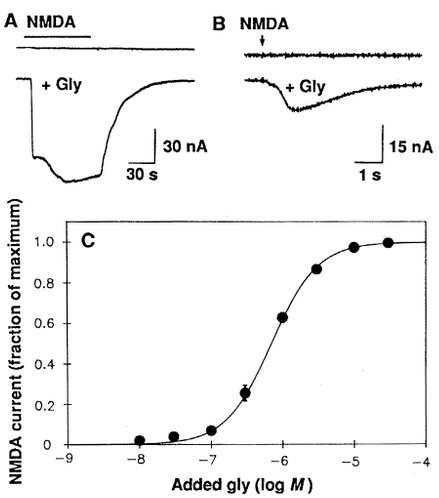

Fig. 1. Glycine permits NMDA receptor activation. (**A**) Inward currents produced in an mRNA-injected oocyte by perfusion with 300 μ*M* NMDA in the absence of added glycine (top trace) and in the presence of 3 μ*M* added glycine (bottom trace). NMDA was added to the perfusion medium during the time indicated by the horizontal bar. This concentration of NMDA elicits a current that is 95% of the maximum in 3 μ*M* glycine (*10*). The oocyte was voltage clamped at −60 mV, and its resting potential and input resistance were −64 mV and 0.9 megohm, respectively. (**B**) Inward currents induced by brief pressure application of 10 m*M* NMDA in the absence (top trace) and presence (bottom trace) of 30 μ*M* glycine. At the time indicated by the arrow, a 30-ms pulse of NMDA was ejected from a glass microelectrode (tip diameter, approximately 20 μm) at 15 psi. The cell was voltage-clamped at −60 mV and had a resting membrane potential and input resistance of −63 mV and 0.9 megohm, respectively. This oocyte responded to 100 μ*M* NMDA and 30 μ*M* glycine applied by perfusion with a 30-nA inward current. (**C**) A concentration-response curve to glycine was generated by applying 100 μ*M* NMDA in the presence of sequentially increasing concentrations of added glycine, with a washout period between NMDA applications. Each point represents the mean current, as a fraction of the maximum, from five cells. If not shown, the SEM is smaller than the symbol size. The curve is the least squares fit of the data to the logistic equation (*11*), assuming the response to NMDA in the absence of glycine to be zero. The actual current produced by 100 μ*M* NMDA in the absence of added glycine was only 1.2 ± 0.5% of maximum in these five cells.

Concentration-response curves for glycine were constructed by measuring the peak inward current elicited by 100 μ*M* NMDA in the presence of sequentially increasing concentrations of glycine in the perfusion medium. The dose-response curve (Fig. 1C) is the least squares fit to the logistic equation (*11*) and indicates a single component response with a half-maximally effective concentration of 670 n*M* (95% confidence interval, 570 to 800 n*M*) and a Hill slope of 1.35. When the minimum NMDA current was allowed to "float" in the logistic equation (*11*), the calculated NMDA current in the absence of glycine (1.1 ± 1.1 nA, $n = 5$) was not significantly different from zero ($P > 0.15$). The NMDA-induced currents that were observed previously in the absence of added glycine (*3*) were probably due to small amounts of glycine in our solutions. The effect of glycine studied here was not reduced by 10 μ*M* strychnine, in agreement with previous reports (*2, 4, 5, 7*).

Because no response to NMDA was observed in oocytes in the absence of added glycine, we could use the oocyte system as a sensitive assay for compounds that interact with the glycine site. We tested 24 analogs of glycine, including other amino acids, for their effectiveness at enabling NMDA receptor activation and found that D-serine was nearly as effective as glycine (90 ± 5.9%) when compared at 3 μ*M*, and D-alanine was also quite effective (62 ± 3.8%) (Fig. 2). The L-isomers of alanine and serine, by contrast, were much less effective (12 ± 4.3% and 7.6 ± 2.7%, respectively) and β-alanine and several other analogs were nearly ineffective (less than 5% of the glycine effect). None of the compounds reduced the current induced by NMDA plus glycine. These results complement previous reports that D-serine is an effective glycine analog at potentiating the NMDA-induced Ca^{2+} influx in cultured neurons (*4*) and at enhancing [^{3}H]TCP and [^{3}H]MK-801 binding to rat brain membranes (*4–6*).

Our results demonstrate that the NMDA receptor–channel complex requires two different agonists for activation. We propose the term "coagonist" to describe the role of glycine at NMDA receptors. This proposal raises several issues. First, glycine, like other agonists, may simply induce a conformational change in the receptor complex that is essential for channel opening or glutamate binding. Other receptor-channel complexes (for example, nicotinic and $GABA_A$) are thought to require the binding of two or more identical agonist molecules for activation (*12*). Perhaps one of the agonist binding sites in the NMDA receptor has been modified to change its specificity to accept glycine. If so, the fundamental molecular architecture of the NMDA receptor need not be different than other, better character-

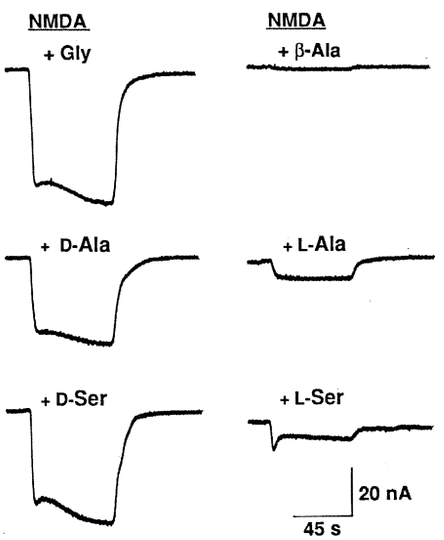

Fig. 2. Selected amino acids mimic the effect of glycine on NMDA receptor activation. Amino acids were applied by perfusion at a fixed concentration (3 μ*M*) to an mRNA-injected oocyte that was voltage clamped to −70 mV with one microelectrode. During the time indicated by the horizontal bar, the perfusion solution was changed to include the amino acid plus 100 μ*M* NMDA. The effectiveness of each amino acid at promoting receptor activation by NMDA in this oocyte, as a percentage of the glycine response, is as follows: β-alanine, 1.9%; D-alanine, 65.0%; L-alanine, 15.2%; D-serine, 84.8%; and L-serine, 11.3%. The oocyte had a membrane potential of −65 mV and an input resistance of 0.8 megohm. In other cells, each glycine agonist shown (0.1 to 10 m*M*) elicited currents that were 80 to 98% of the maximum current in the presence of glycine ($n = 3$), suggesting that the weaker analogs are not partial agonists at the glycine site. However, caution is warranted because contamination of either L-alanine or β-alanine with 0.1% glycine could account for these effects.

ized, receptor-channel complexes. The observation that the Hill coefficients for both glycine (Fig. 1C) and NMDA (*10*) are close to 1 is consistent with this interpretation. However, it is not clear yet whether the glycine and glutamate binding sites reside on the same macromolecule, or whether glycine and glutamate influence binding of each other. An alternative mechanism of action of glycine would more closely resemble that of certain enzyme cofactors. In some enzyme systems, for example, a cofactor is required for activation of the enzyme [for example, adenosine 3′,5′-monophosphate (cAMP) for protein kinase A], for binding the substrate in the active site (Mg^{2+} for adenosine triphosphatase), or for creation of the active site (pyridoxal phosphate for transaminases). Regardless of which mechanism of action of glycine proves to be true, it must be able to account for the obligatory nature of glycine for NMDA receptor activation, which is clearly dissimilar to the allosteric modulation of the $GABA_A$ receptor by benzodiazepines.

Second, both glycine and the endogenous agonist, glutamate, are present in the cerebrospinal fluid in micromolar concentrations (*13*). Their presence suggests that the NMDA receptor may be tonically activated, that sensitivity to glycine may be lower in situ, or that some mechanism for "buffering" the glycine and glutamate concentrations at glutamatergic synapses is present. Glutamate uptake systems are at least partially responsible for reducing interstitial glutamate concentration, and uptake systems for glycine have also been described (*14*). If glycine concentration is low in the vicinity of NMDA receptors, a glycinergic synapse could activate NMDA receptors. The receptor could formally be called a "glycine receptor," since both glycine and glutamate are required.

Third, although differences in membrane composition or post-translational processing in oocytes compared to neurons might account for the obligatory nature of glycine in this system, the concentration-response relation for glycine in cultured neurons (*15*) appears very similar to the one presented here. Therefore, our data are likely to represent properties of the native neuronal NMDA receptor.

Finally, because glycine appears to be necessary for NMDA receptor activation, a glycine antagonist or partial agonist might be effective at blocking the pathologies that are associated with intense activation of the NMDA receptor (*16*). More structure-activity studies will facilitate the development of new ligands for the glycine site. Small D–amino acids are effective agonists at the glycine site, which provides a basis for design of additional effective compounds. The oocyte translation system is suitable for these structure-activity studies because it allows precise regulation of drug concentration at the receptor.

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11. The dose-response curve (Fig. 1C) is the least squares fit to the logistic equation

$$I_{exp} = \frac{I_{max}}{1 + (EC_{50}/\text{glycine concentration})^n}$$

where n is the Hill slope exponent, I_{exp} is the expected current, and I_{max} is the maximum current. The current expected after perfusion of NMDA in the absence of glycine (I_{min}) was calculated by fitting the data to the equation

$$I_{exp} = \frac{I_{max} - I_{min}}{1 + (EC_{50}/\text{glycine concentration})^n} + I_{min}$$

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Legless, a Novel Mutation Found in PHT1-1 Transgenic Mice

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In this report it is shown that the PHT1-1 line of transgenic mice exhibited a pattern of developmental abnormalities when the mice were homozygous for the transgene insertion. Hindlimbs were uniformly truncated at the distal end of the femur, resulting in a "legless" appearance. Forelimbs lacked anterior structures including digits and the radius. The brains had many defects, particularly in the anterior structures of the cerebrum, including the olfactory lobes. Craniofacial malformations in the form of facial clefts also commonly occurred. Furthermore, heterozygotes of this line, with only one copy of the DNA insertion, and other transgenic lines carrying the same DNA construct appeared normal, suggesting that in the PHT1-1 line a gene significant in mammalian development has been disrupted.

IN THE PROCESS OF GENERATING transgenic mice the inserted foreign DNA will sometimes integrate into a host chromosome in a manner that alters an endogenous gene, resulting in a mutation. The potential power of this procedure, referred to as insertional mutagenesis, derives (i) from the production of a mutant mouse,

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