Site of G Protein Binding to Rhodopsin Mapped with Synthetic Peptides from the α Subunit

HEIDI E. HAMM,* DUSANKA DERETIC, ANATOL ARENDT, PAUL A. HARGRAVE, BERND KOENIG, KLAUS P. HOFMANN

The interaction between receptors and guanine nucleotide binding (G) proteins leads to G protein activation and subsequent regulation of effector enzymes. The molecular basis of receptor–G protein interaction has been examined by using the ability of the G protein from rods (transducin) to cause a conformational change in rhodopsin as an assay. Synthetic peptides corresponding to two regions near the carboxyl terminus of the G protein α subunit, Glu^{311}-Val^{328} and Ile^{340}-Phe^{350}, compete with G protein for interaction with rhodopsin. Amino acid substitution studies show that Cys^{321} is required for this effect. Ile^{340}-Phe^{350} and a modified peptide, acetyl-Glu^{311}-Lys^{329}-amide, mimic G protein effects on rhodopsin conformation, showing that these peptides bind to and stabilize the activated conformation of rhodopsin.

THE TRANSDUCTION OF BIOLOGICAL signals such as light, hormones, and neurotransmitters starts by a specific interaction with and subsequent activation of a receptor protein. This causes a conformational change of the receptor leading to a high-affinity interaction with a specific G protein and catalysis of guanosine triphosphate (GTP)–guanosine diphosphate (GDP) exchange on the G protein α subunit. GTP binding leads in turn to activation of effector molecules and dissociation of the receptor–G protein complex (*1, 2*). The carboxyl terminus of α is involved in interaction with receptors (*3, 4*). We have identified another region in the α subunit of the rod outer segment (ROS) G protein, transducin (G_t), Glu^{311}-Val^{328} (311–328), that is also a part of the rhodopsin binding site.

A monoclonal antibody (mAb) to the α subunit of G_t, mAb 4A, blocks G protein interaction with rhodopsin (*5, 6*) and binds to the 311–328 sequence of α (*7*). A synthetic peptide corresponding to 311–328 competitively blocked mAb 4A interaction with the G protein (Table 1). To further define this epitope, a series of smaller synthetic peptides was tested. Peptide Glu^{311}-Thr^{323} (311–323) was almost as effective as the parent peptide, whereas Tyr^{316}-Val^{328} (316–328) was 20 times less effective. Smaller peptides, Glu^{311}-Met^{319} (311–319) and Thr^{320}-Val^{328} (320–328), as well as synthetic peptides corresponding to other regions of the α subunit (Met^{1}-Ala^{23}, Asp^{8}-Ala^{23}, and Gly^{41}-Asn^{48}) and to unrelated sequences (rhodopsin Phe^{13}-Pro^{23}, Lys^{231}-Arg^{252}, and Asn^{310}-Leu^{321}) were ineffective competitors of mAb 4A binding to G_t (Table 1). Thus the eight amino acids Tyr^{316} to Thr^{323} are required for competition with mAb 4A. Amino acids 311 to 315 considerably enhance peptide competition, whereas 324 to 328 are much less important.

The effects of these peptides on G_t interaction with rhodopsin were then measured directly by a spectrophotometric assay: G_t binding to light-activated rhodopsin stabilizes the active metarhodopsin II conformation of rhodopsin (*8*) as measured by its characteristic absorbance spectrum. To measure G_t binding to metarhodopsin II, washed ROS membranes with or without extract containing G_t were placed in a cuvette in a dual-beam flash spectrophotometer (*8*) and exposed to a flash of light that bleached 4% of the rhodopsin while the difference of the absorbances at 380 nm (metarhodopsin II peak absorbance) and 417 nm (isosbestic point with metarhodopsin I) was monitored. Tight binding of G_t to metarhodopsin II shifts the metarhodopsin I–metarhodopsin II equilibrium toward metarhodopsin II (*8*), leading to the formation of more metarhodopsin II. The synthetic peptide 311–328 completely blocked the binding of G_t to rhodopsin (Fig. 1A). The smaller peptides 311–323 and 316–328 both competitively blocked binding, but their effects were partial (Fig. 1, B and C). The peptides 311–319 and 320–328 (Fig.

Table 1. Summary of competition between G protein and synthetic α subunit peptides for binding to mAb 4A or metarhodopsin II. Synthetic peptides based on the putative mAb 4A antigenic site (311–328) were used as competitors of mAb 4A binding to G protein (*24*) or G protein interaction with rhodopsin (*21*). IC_{50} and percent remaining binding at maximal inhibition were calculated according to the equation in the legend to Fig. 1. Peptide 8–23 had no ability to block mAb 4A binding; however, a non-native peptide, Cys-Tyr-8–23; could block mAb 4A binding at high concentrations. This is similar to the blockade of mAb 4A binding by this peptide in (*25*) and is interpreted as evidence that mAb 4A recognizes the NH-terminal region. This peptide, 8–23, with no extra amino acids, had no effect on mAb 4A–G protein binding. Synthetic peptides made to the GTP-binding region of α, 41–48, and nonspecific peptides made to other protein sequences (rhodopsin 13–23, 231–252, 310–321) have no effect on mAb 4A or rhodopsin binding to G protein up to a concentration of 2 m*M*. Data shown are means (n = 9) for antibody binding; SEM was within 10% of the mean. For rhodopsin binding, n = 4; SEM is shown in figures. ND, not determined.

Peptide	mAb 4A binding		Rhodopsin binding	
	IC_{50} (m*M*)	% Binding	IC_{50} (m*M*)	% Binding
311–328	0.049	0	0.380*	0
311–323	0.095	2	0.150	15
316–328	0.758*	30	0.3	<10
311–319	>2	>95	>0.7	>90
320–328	>2	>95	>0.7	>80
ac-311–329-NH_2	ND	ND	0.02	15, 83*‡
ac-305–329-NH_2	ND	ND	0.212	0
[Cys^{315}]311–322 (reduced)	0.010	0	0.079	45
[Cys^{315}]311–322 (cyclic†)	>2	>95	>0.7	86
[Phe^{316}]311–323	0.043	13	0.247	30
[Gln^{318}]311–323	0.178	0	0.272	0
[Ser^{321}]311–328	>2	>95	>0.7	>90
8–23	>2	>95	0.104	0
Cys-Tyr-8–23	0.500*	35	ND	ND
340–350	0.07	20	0.04	50, 120*‡
340–350 + ac-311–329-NH_2	ND	ND	0.001	0

*Apparent IC_{50} empirically determined when the data do not fit the competition equation. †Cyclized by an intramolecular disulfide bridge. ‡Biphasic response; the two numbers represent percent binding at minimum and maximum.

H. E. Hamm and D. Deretic, Department of Physiology and Biophysics, University of Illinois College of Medicine at Chicago, Chicago, IL 60680.
A. Arendt and P. A. Hargrave, Department of Ophthalmology and Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610.
B. Koenig and K. P. Hofmann, Institut für Biophysik und Strahlenbiologie, Albert Ludwigs Universität, D-7800 Freiburg, Federal Republic of Germany.

*To whom correspondence should be addressed.

1, D and E) as well as nonspecific peptides (rhodopsin Phe^{13}-Pro^{23}, Lys^{231}-Arg^{252}, and Asn^{310}-Leu^{321}) (not shown) have no significant effect. Adding peptides 311–319 and 320–328 together did not change this result, showing that the linear sequences alone are not enough to have an effect. Thus, the central region 316–323 is most important for blocking rhodopsin-G_t interaction, whereas the flanking regions on both the COOH- and NH_2-termini of the peptide are less important.

The ability of peptides to compete in these assays was analyzed with a computer-generated fit of the data with a Michaelis-Menten–type equation for a competitive inhibitor (Fig. 1, solid lines). The calculated concentration for 50% inhibition (IC_{50}) and percent remaining binding at maximal inhibition are shown in Table 1. Approximately 200-fold excess of peptide 311–328 (IC_{50}, 49 μM) was required for half-maximal competition with mAb 4A, and approximately 750-fold excess of peptide was required to half maximally block binding to metarhodopsin II. This result is consistent with the higher affinity of G_t for metarhodopsin II than for mAb 4A (*9, 10*). The competitive inhibition model fits the data well, which show a sigmoid curve in most cases. However, in the case of 311–328 (Fig. 1A) the competition curve has an anomalous shape and continues its downward trend at the highest peptide concentrations. This indicates that the interaction is more complex than a simple competitive inhibition.

To examine which amino acids of the sequence 311–323 are important for the blocking effect, synthetic peptides with single amino acid substitutions were tested (Fig. 2A and Table 1). Substitution of Ile^{315} by Cys resulted in a peptide that caused partial blocking, whereas substitution of Tyr^{316} by Phe had no effect. However, substitution of Cys^{321} with Ser completely eliminated the ability of this peptide to compete for binding of metarhodopsin II (Fig. 2A). It is unlikely that this conservative substitution of a hydroxyl group of Ser for a sulfhydryl group of Cys caused a large conformational change to the peptide, because both the size and charge of the residues are similar. Rather, we suspect that this Cys may be important for the light-induced binding to rhodopsin. There is known to be a critical sulfhydryl group close to the tight, light-dependent rhodopsin binding site (*11, 12*) that is not involved in dark binding of G_t to the membrane.

The structure of the competing peptides may provide some insight into the structure of this region of the α subunit that interacts with metarhodopsin II. However, the concentration of the active conformation of the peptides must be relatively small, because a 200- to 1500-fold excess of peptides is required to compete with α for binding mAb 4A or metarhodopsin II. To increase the peptide's ability to compete, peptides predicted to have a more constrained secondary structure were synthesized. A cyclic peptide without biological activity was produced by synthesizing [Cys^{315}]-311–322 and cyclizing it by introducing an intramolecular disulfide bond between Cys^{315} and Cys^{321} (Table 1). In another attempt to increase peptide potency, the NH_2- and COOH-termini were modified (acetyl-Glu^{311}-Lys^{329}-NH_2) or the parent peptide was elongated (acetyl-Glu^{305}-Lys^{329}-NH_2) to include an α helix predicted by Chou-Fasman analysis (*7*). The longer peptide, acetyl-305–329-NH_2, blocked metarhodopsin II–G_t interaction with a potency similar to that of the parent peptide (Fig. 2B and Table 1). Acetyl-311–329-NH_2 blocked G_t

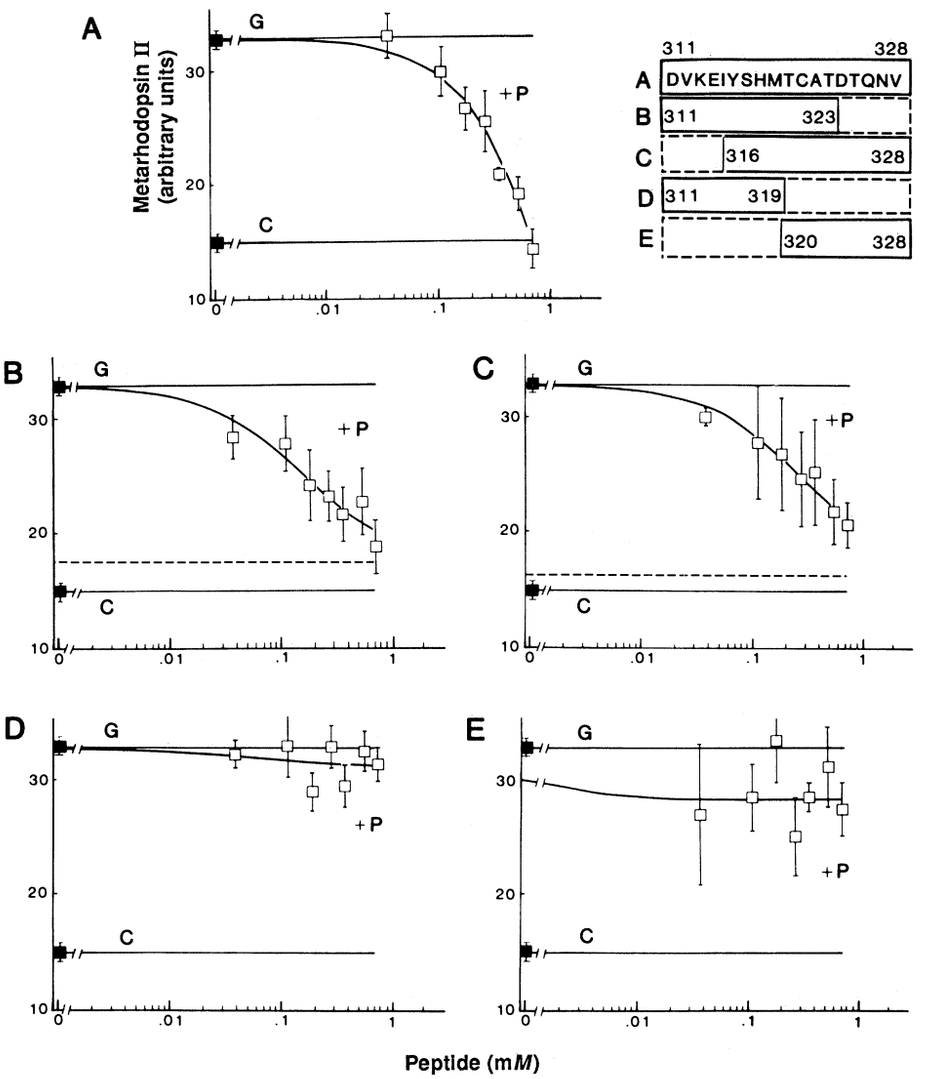

Fig. 1. Competition between synthetic α subunit peptides (P) and G protein for binding to metarhodopsin II (*21*). In the absence of G protein, light stimulation causes a control (C) amount of metarhodopsin II formation (lower ■, lower solid line in each panel). In the presence of G protein (G), tight binding of G protein to photoexcited rhodopsin stabilizes the metarhodopsin II form of rhodopsin and a larger amount of metarhodopsin II is formed (upper ■, upper solid line). The spectroscopically observed extra metarhodopsin II is a linear measure of the amount of rhodopsin–G protein complexes (*22*). (**A**) The parent peptide 311–328 completely blocked G_t interaction with metarhodopsin II, whereas (**B**) 311–323 and (**C**) 316–328 both partially blocked the interaction (dotted lines, maximal inhibition predicted by the equation given below). (**D** and **E**) The smaller peptides have no significant effect. Data shown represent mean ± SEM, $n = 4$. The inhibition of mAb 4A binding or extra metarhodopsin II formation by peptides was analyzed by the computer program DATAC (*23*) with the following equation: $[AG] = (K_P/K_G \cdot [G][A_0]) / (K_P/K_G \cdot [G] + K_P + [P])$, where A is metarhodopsin II or mAb 4A, G is substrate (G_t), and P is peptide. The concentration of A was approximately 0.25 μM in both assays. The solid curves are computer fits of this equation to the experimental data. K is given in Table 1 as the apparent IC_{50} of the peptides calculated from the equation, and the predicted maximal inhibition is referred to in Table 1 as percent binding.

interaction with metarhodopsin II at a molar excess of 100-fold over metarhodopsin II, a 20 times higher potency than 311–328. This peptide shows a biphasic response and causes an increase in metarhodopsin II at higher concentrations (Fig. 2B), suggesting that the peptide could also directly stabilize metarhodopsin II (see below). Comparison of the effects of this peptide with the two peptides of similar potency, 311–328 and acetyl-305–329-NH_2, suggests that the acetylated Asp^{311} is important in determining its higher potency.

To determine the importance of other regions of α in binding to rhodopsin and mAb 4A, we tested synthetic peptides corresponding to the NH_2- and COOH-terminal regions of α, Asp^{8}-Ala^{23} (8–23) and Ile^{340}-Phe^{350} (340–350). The NH_2-terminal peptide 8–23 had no effect on the interaction of mAb 4A with G_t in the competition enzyme-linked immunosorbent assay (ELISA) (Table 1). By contrast, 8–23 was an effective competitor of G_t binding to rhodopsin (Fig. 3A and Table 1). The COOH-terminal peptide 340–350 competitively blocked mAb 4A binding (Table 1), showing that the COOH-terminus also plays a part in the mAb 4A antigenic site. Peptide 340–350 had a biphasic effect on G_t rhodopsin binding, causing inhibition at low concentrations but causing increased binding at higher concentrations. This mixed effect is analogous to the one for the modified 311–329 peptide. It competed with G_t at even lower concentrations than acetyl-311–329-NH_2 and restored the full metarhodopsin II signal at 200 μ*M*. In addition, if this peptide was presented in combination with acetyl-311–329-NH_2, a more than additive potency of inhibition was obtained (IC_{50}, 1 μ*M*) (Table 1).

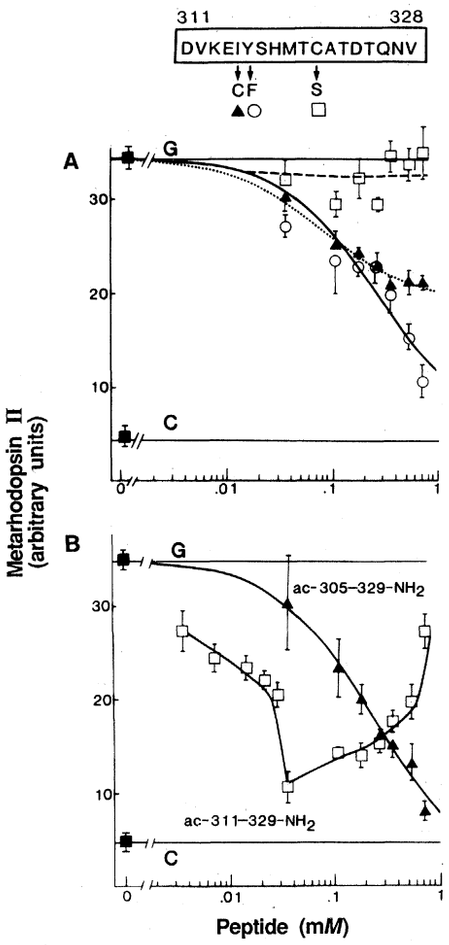

Fig. 2. (**A**) Amino acids involved in G_t interaction with rhodopsin probed by single amino acid substitutions of the parent peptide (mean ± SEM, $n = 4$). ▲, [Cys^{315}]311–323. □, [Ser^{321}]311–328. ○, [Phe^{316}]311–323. (**B**) Eliminating charged groups at the NH_2- and COOH-terminals increased the potency of the parent peptide as a competitor of metarhodopsin II–G_t interactions and appeared to mimic (at higher concentrations) the effects of G protein binding to rhodopsin (acetyl-311–329-NH_2). Elongating the parent peptide does not affect its ability to block metarhodopsin II–G_t interaction (acetyl-305–329-NH_2). ■, as in Fig. 1. Data represent the mean ± SEM, $n = 4$.

The peptides that exhibited biphasic responses, acetyl-311–329-NH_2 and 340–350, could directly stabilize metarhodopsin II in a manner similar to stabilization by G_t binding (Fig. 3B). A full metarhodopsin II signal was induced by 200 μ*M* 340–350, and 50% of the signal was induced by 700 μ*M* acetyl-311–329-NH_2 peptide. The NH_2-terminal peptide 8–23 had no direct effect on metarhodopsin II in the measurable range of concentration (Fig. 3B). The other peptides of the 311–328 series, 311–328, 311–323, 316–328, 311–319, 320–328, and [Ser^{321}]-311–328 also had no effect. Thus, it appears that the regions from amino acid 311 to 329 and from amino acid 340 to 350 of α are able to mimic G_t effects on rhodopsin if presented in an appropriate conformation.

Our data provide evidence that α sequences 311 to 329 and 340 to 350 bind to rhodopsin. Synthetic peptides from this region can block G_t binding to metarhodopsin II at low concentration (Figs. 2B and 3A) and mimic that binding at higher concentration (Fig. 3B). These two parts of α must be close to each other in the three-dimensional structure, since both regions are part of the mAb 4A binding site. Their physical proximity may be related to their synergistic action in competition for G protein binding (Table 1). There is significant sequence homology between these regions and arrestin, another protein that binds rhodopsin and functions in the turnoff of the photoreceptor excitation cascade (*13*). Peptide Lys^{313}-His^{318} is 85% identical with arrestin Lys^{211}-His^{216}, and Glu^{326}-Phe^{350} is 55% conserved with arrestin Glu^{371}-Tyr^{391} (*14*). This sequence homology may be the basis for the competitive inhibition between these proteins for binding to rhodopsin (*15*) and implies that they bind to a similar binding pocket on metarhodopsin II. Thus we suggest that Glu^{311}-Phe^{350}, the 5-kD COOH-terminal tryptic fragment, constitutes the receptor binding domain of α.

The NH_2-terminal peptide (8-23) can competitively inhibit rhodopsin-G_t interaction (Fig. 3A), but it does not mimic G_t effects on metarhodopsin II formation (Fig. 3B). This may be because the synthetic NH_2-terminal peptide can disrupt interaction between the α and βγ subunits of G_t, an interaction which is necessary for G_t interaction with rhodopsin (*16*). However, these data do not rule out the possibility of direct NH_2-terminal involvement in rhodopsin binding. The fact that acetyl-311–329-NH_2 and 340–350 both cause direct

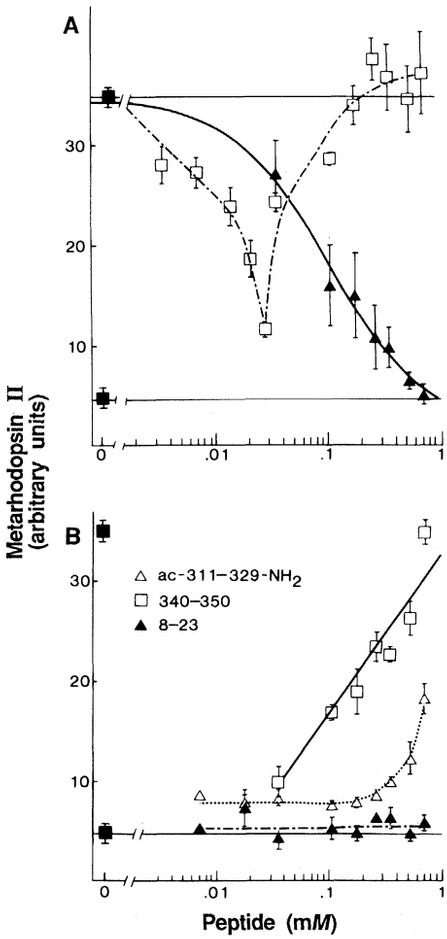

Fig. 3. Other regions of α involved in interaction with rhodopsin (mean ± SEM, $n = 4$). (**A**) Blockade of metarhodopsin II–G_t interaction. Peptide 8–23 (▲) blocked binding. Peptide 340–350 (□) had a biphasic effect, blocking binding at low concentrations and increasing it at higher concentrations. (**B**) Effect of synthetic peptides from α on rhodopsin conformation in the absence of G_t.

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.

REFERENCES AND NOTES

1. A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987).
2. M. Chabre, *Annu. Rev. Biophys. Biophys. Chem.* **14**, 331 (1985).
3. C. Van Dop *et al.*, *J. Biol. Chem.* **259**, 23 (1984).
4. H. Kurose, T. Katada, T. Amano, M. Ui, *ibid.* **258**, 4870 (1983).
5. H. E. Hamm and M. D. Bownds, *J. Gen. Physiol.* **84**, 265 (1984).
6. H. E. Hamm, D. Deretic, K. P. Hofmann, A. Schleicher, B. Kohl, *J. Biol. Chem.* **262**, 10831 (1987).
7. D. Deretic and H. Hamm, *ibid.*, p. 10839.
8. K. P. Hofmann, *Biochim. Biophys. Acta* **810**, 278 (1985); D. Emeis, H. Kühn, J. Reichert, K. P. Hofmann, *FEBS Lett.* **143**, 29 (1982); D. Emeis and K. P. Hofmann, *ibid.* **136**, 201 (1981).
9. P. W. Witt, H. E. Hamm, M. D. Bownds, *J. Gen. Physiol.* **84**, 251 (1984).
10. N. Bennett and Y. Dupont, *J. Biol. Chem.* **260**, 4156 (1985).
11. Y.-K. Ho and B. K.-K. Fung, *ibid.* **259**, 6694 (1984).
12. K. P. Hofmann and J. Reichert, *ibid.* **260**, 7990 (1985); J. Reichert and K. P. Hofmann, *FEBS Lett.* **168**, 121 (1984).
13. U. Wilden, S. W. Hall, H. Kühn, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1174 (1986).
14. G. J. Wistow, A. Katial, C. Craft, T. Shinohara, *FEBS Lett.* **196**, 23 (1986).
15. H. Kühn, S. W. Hall, U. Wilden, *ibid.* **176**, 473 (1984).
16. B. K.-K. Fung and C. R. Nash, *J. Biol. Chem.* **258**, 10503 (1983); P. A. Watkins *et al.*, *ibid.* **260**, 13478 (1985); S. E. Navon and B. K.-K. Fung, *ibid.* **262**, 15746 (1987).
17. H. E. Hamm, D. Deretic, C. A. Moore, J. S. Takahashi, M. Rasenick, *ibid.*, in press.
18. A. Yatani, H. E. Hamm, J. Codina, M. R. Mazzoni, L. Birnbaumer, A. M. Brown, *Science* **241**, 828 (1988).
19. D. S. Papermaster and W. J. Dreyer, *Biochemistry* **13**, 2438 (1974).
20. A. Arendt, D. MacKenzie, R. S. Molday, J. H. McDowell, P. A. Hargrave, in *Peptides: Structure and Function*, V. J. Hruby and D. H. Rich, Ed. (Pierce Chemical Co., Rockford, IL, 1983), p. 751.
21. Tight light-induced binding of G_t to metarhodopsin II was measured according to Hofmann (*8*). The assay of G_t binding to metarhodopsin II is based on the fact that when the temperature is ≤12.0°C and the *p*H ≥7.5 photoexcited rhodopsin exists in an equilibrium between two spectroscopically different states, metarhodopsin I [maximum absorbance (A_{max}) = 480 nm] and metarhodopsin II (A_{max} = 380 nm). Peptides and extract [0.5 mg/ml protein in isotonic saline containing 100 m*M* NaCl, 2 m*M* $MgCl_2$, 2 m*M* $CaCl_2$ 0.2 m*M* EDTA, 1 m*M* dithiothreitol (DTT), and 40 m*M* Hepes, *p*H 8.0, to give 0.1 μ*M* final G protein concentration] were added to washed bovine ROS membranes suspended in the same buffer in a cuvette (1.5 μ*M* final rhodopsin concentration) (*19*), in the dark at 5°C, *p*H 7.5. After a 2-min incubation, a flash of light bleaching 4% of the rhodopsin was presented. Metarhodopsin II was then measured spectrophotometrically (*6, 8*) by the absorbance difference at 380 nm (A_{max} of metarhodopsin II) and 417 nm (isosbestic point metarhodopsin I–metarhodopsin II) (mean ± SEM, n = 4) in the spectrophotometer described in (*8*).
22. A. Schleicher and K. P. Hofmann, unpublished data.
23. D. Bertrand and C. R. Bader, *Int. J. Bio-med. Comput.* **18**, 193 (1986).
24. Peptides were synthesized by the solid-phase Merrifield method with an Applied Protein Technologies synthesizer and purified by high-performance liquid chromatography (*20*). Synthetic peptides were used as competitors of MAb 4A binding to G protein in a competition ELISA. Synthetic peptides (0–1 m*M*) were incubated with mAb 4A (0.25 μ*M*) in 100 μl phosphate-buffered saline, *p*H 7.5, for 1 hour at room temperature then added to wells coated with G_t. After overnight incubation at room temperature, an alkaline phosphatase–linked second antibody was added to the wells, and antibody binding was detected by measurement of the fluorescent enzyme product (*9*).
25. S. Navon, and B. K.-K. Fung, *J. Biol. Chem.* **263**, 489 (1988).

26. Supported by NIH grants EY06062 and RP05369 to H.E.H., EY06225 and EY06226 to P.A.H., the Deutsche Forschungsgemeinschaft SFB 60 to K.P.H., as well as unrestricted departmental grants (H.E.H. and P.A.H.) from Research to Prevent Blindness. P.A.H. was supported by a Jules and Doris Stein Professorship from Research to Prevent Blindness. H.E.H. is the recipient of an NSF ROW Career Advancement Award. The technical assistance of I. Baumle is acknowledged.

29 March 1988; accepted 17 June 1988

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

NANCY W. KLECKNER AND RAYMOND DINGLEDINE

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,

Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7365.