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Effects of (+)-HA-966 and 7-Chlorokynurenic Acid on the Kinetics of *N*-Methyl-D-Asparatate Receptor Agonist Responses in Rat Cultured Cortical Neurons

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SUMMARY

It has been suggested that one of the effects of glycine at the *N*-methyl-D-aspartate (NMDA) receptor complex is to reduce the amount of apparent receptor desensitization. Thus, blockade with a glycine site antagonist results in NMDA responses that show an increased amount of fade. In agreement with this, we found that antagonism of NMDA-evoked whole-cell currents by 7-chlorokynurenic acid (7-Cl-KYNA) indeed resulted in NMDA responses that displayed an increased amount of fade. However, those responses that were antagonized by (+)-HA-966 showed the opposite, i.e., less tendency to fade. On examination of these responses, it appeared that those produced in the presence of (+)-HA-966 were slower in onset and faster in offset than control responses recorded in the presence of glycine alone. Kinetic analysis of the on- and off-rates of NMDA- and glutamate-evoked NMDA receptor-mediated responses revealed that these were markedly affected by (+)-HA-966 but only slightly by 7-Cl-KYNA. The decrease of the glutamate response decay time constant and the increase of the response rise time constant produced by (+)-HA-966 indicated that it reduced the affinity of glutamate for its recognition site on the NMDA receptor by 5-fold. These results suggest that binding of (+)-HA-966 to the glycine site on the NMDA receptor complex produces an allosteric reduction in the affinity of agonists for the glutamate recognition site, whereas 7-Cl-KYNA has relatively little effect and, thus, acts more as a pure antagonist at the glycine site.

The NMDA receptor complex appears to contain, as an integral part of its structure, a site at which glycine acts as a co-agonist to facilitate channel activation (1, 2). Several compounds have been described that act as antagonists at this site and prevent NMDA receptor activation (3–7). However, there are differences in the NMDA receptor antagonist profile of some of these compounds, notably 7-Cl-KYNA and (+)-HA-966 (5, 8–10), and this has been attributed to (+)-HA-966 possessing low efficacy partial agonist activity at the glycine site (5, 11).

Concentration-jump experiments on voltage-clamped cultured neurons have led to the suggestion that one of the effects of glycine is to reduce NMDA receptor desensitization (12). It was reported that, in the presence of a low concentration of glycine, the NMDA response rose to an initial peak that faded over the course of hundreds of milliseconds to a much smaller steady state level. Increasing the concentration of glycine increased the size of the initial peak and reduced the amount of decline, so that the steady state to peak ratio was much closer to 1. Thus, addition of a glycine antagonist, such as 7-Cl-KYNA, had the same effect as lowering the glycine concentration and increased the amount of decline of the NMDA response (13). A kinetic model has been put forward to interpret these results (14), which suggests that there is a negative allosteric coupling between the glycine and glutamate recognition sites and that the binding of glutamate reduces the affinity of glycine. However, based on the results from studies on outside-out patches and small cells (15), this interpretation of the effects of glycine has been contested.

During the course of our own whole-cell voltage-clamp experiments on rat cortical neurons, we noted that, in contrast to 7-Cl-KYNA, inhibition by (+)-HA-966 resulted in a "squaring off" of NMDA responses, rather than an increase in their amount of fade. We have investigated the reasons for this difference in the effects of (+)-HA-966 and 7-Cl-KYNA on NMDA receptor-mediated responses and report here that this appears to be due, at least in part, to an alteration by (+)-HA-966 of agonist binding to the glutamate recognition site.

Experimental Procedures

Whole-cell voltage-clamp recordings were made at room temperature (22°) from rat cortical neurons in tissue culture, as described previously (16). The recording pipettes were filled with an intracellular solution of the following composition (in mM): CsF, 120; CsCl, 10; HEPES, 10;

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; 7-Cl-KYNA, 7-chlorokynurenic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,*N*′-tetraacetic acid; ACBC, 1-aminocyclobutane-1-carboxylate.

Cs-EGTA, 10; and $CaCl_2$, 0.0005; pH adjusted to 7.3 with CsOH; the pipettes had resistances of approximately 2 MΩ. The extracellular medium bathing the cells consisted of (in mM) NaCl, 149; KCl 3.25; $MgCl_2$, 2; HEPES, 10; $CaCl_2$, 2; and D-glucose, 11; to which 0.5 μM tetrodotoxin was added. Control or drug-containing solutions (modified from above by omitting $MgCl_2$ and adding 300 nM glycine) were applied to recorded cells by fast perfusion from a double-barreled pipette assembly, with each pipette of approximately 475 μm internal diameter, similar to that described by Johnson and Ascher (17). This could be moved rapidly from side to side by a stepping motor attached to a Leitz micromanipulator, and the flow of solutions from the pipettes was controlled by three-way solenoid valves and driven by gravity.

Cell currents were filtered above 3 kHz, digitized at 22 kHz using a PCM-4 VCR recorder adapter (Medical Systems Corp. Greenvale, NY), and recorded on magnetic tape for further off-line analysis. Signals were digitized using a CED 1401 laboratory interface, and exponentials were fitted using the SCAN software supplied by J. Dempster (University of Strathclyde). Results are expressed as means ± standard errors.

Results and Discussion

In agreement with the findings of Vyklicky *et al.* (13), inhibition of NMDA-induced whole-cell currents by increasing concentrations of 7-Cl-KYNA led to responses that showed a progressive increase in their amount of decline (Fig. 1). Thus, 7-Cl-KYNA was a more potent antagonist of steady state NMDA responses than peak NMDA responses. The opposite was found to be true for (+)-HA-966, the active enantiomer at the glycine site (11), where increasing concentrations resulted in NMDA responses that showed less and less tendency to fade (Fig. 1). On inspection of these responses, it appeared that those recorded in the presence of (+)-HA-966 were slower in onset and faster in offset than their equivalent controls recorded in the presence of 300 nM glycine. Therefore, we examined the effects of (+)-HA-966 and 7-Cl-KYNA on the rates of onset and decay of NMDA- and glutamate-induced responses.

By measurement of the response to sodium concentration jumps in the presence of kainate (13), the solution exchange rate time constant around the recorded cell was found to be in the range of 20–50 msec ($n = 5$). Therefore, when carrying out quantitative evaluation of the effects of (+)-HA-966 and 7-Cl-KYNA on the on- and off-rates of NMDA receptor-mediated responses, we decided also to use L-glutamate as the NMDA recognition site agonist, because its kinetics have been reported to be slower than those for NMDA (18, 19) and, thus, the results would be less affected by errors introduced by drug equilibration times.

Under the conditions of these experiments (no added magnesium and 300 nM glycine), L-glutamate activated NMDA responses with a mean pEC_{50} of 5.8 ± 0.06 ($n = 5$) and a slope factor of 1.4 ± 0.12 (see Ref. 18). Apart from the initial sigmoidal rise in the onset of responses (see below), both the rise and the decay of glutamate and NMDA responses were well described by single exponentials (Fig. 2). In the presence of 300 nM glycine, responses to approximate EC_{50} concentrations of glutamate (3 μM) and NMDA (30 μM) activated with time constants (msec) of 72 ± 6.3 ($n = 14$) and 42 ± 2.5 ($n = 17$), respectively, and decayed with time constants (msec) of 460 ± 40 ($n = 14$) and 93 ± 8.4 ($n = 17$), respectively, confirming the slow kinetics of glutamate for the NMDA receptor.

The most dramatic effect of (+)-HA-966 was on the relaxation of the glutamate-induced current after the step back into glutamate-free solution (Fig. 3B). At a concentration of 300 μM, (+)-HA-966 reduced the time constant of decay by almost

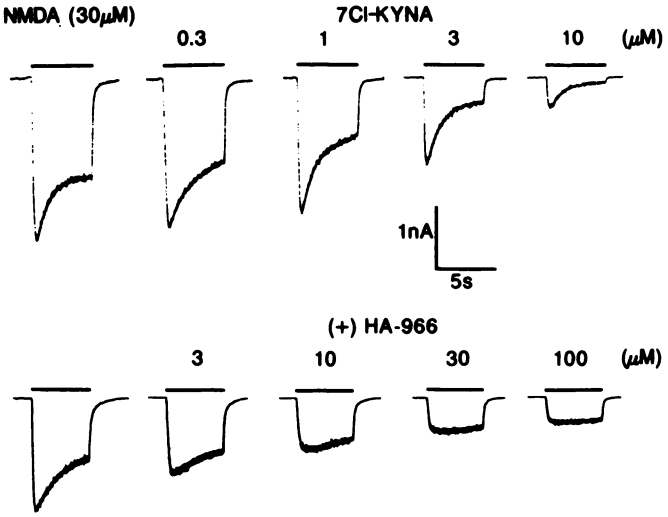

Fig. 1. Inhibition of NMDA-induced whole-cell currents by 7-Cl-KYNA and (+)-HA-966. Responses to 5-sec applications (*horizontal bars*) of NMDA (30 μM), in the presence of 300 nM glycine, were evoked at 30-sec intervals. After several consecutive control responses of constant amplitude were obtained, the effects of increasing concentrations of either 7-Cl-KYNA (*top*) or (+)-HA-966 (*bottom*) were examined. Both antagonists produced a concentration-dependent inhibition of the NMDA response. In the case of 7-Cl-KYNA, this was associated with an increase in the amount of response fade. In contrast, (+)-HA-966 had the opposite effect and caused a "squaring-off" of the NMDA responses. *Scale bar* applies to both sets of records, which were obtained from separate cells.

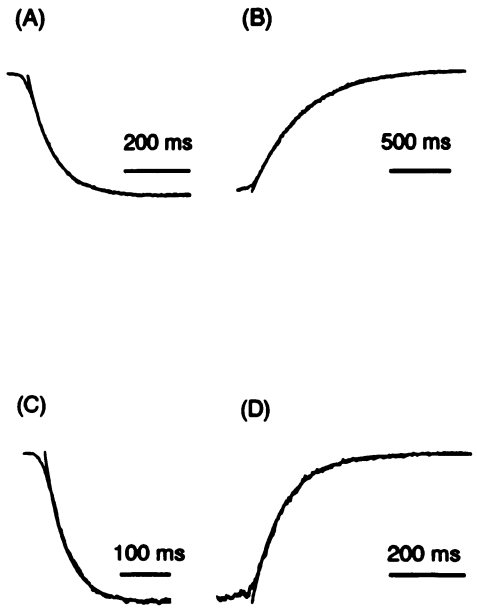

Fig. 2. Single-exponential fits for the onset and decay of glutamate- and NMDA-induced whole-cell currents. Responses were evoked by 1-sec applications of 3 μM L-glutamate (A and B) and 30 μM NMDA (C and D), in the presence of 300 nM glycine. Only the rise and decay of the responses are shown, for clarity. Digitized records of current on-rates (A and C) and relaxations (B and D) are shown, with superimposed exponential fits. Note that in all cases the experimental data are adequately described by single exponentials and that the kinetics of the NMDA response are faster than those of the L-glutamate response. The peak amplitudes of the glutamate and NMDA responses were 9 and 5.6 nA, respectively. Data were obtained from separate cells.

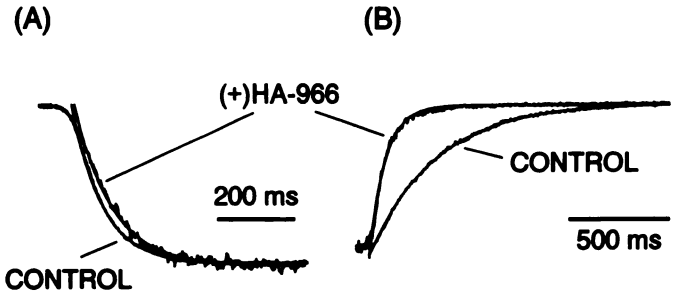

Fig. 3. Effects of (+)-HA-966 (300 μM) on the kinetics of the L-glutamate response. The responses in the absence and presence of (+)-HA-966 have been scaled to equivalent sizes, in order to facilitate comparison. The digitized experimental data are shown with the superimposed exponential fits. The peak size of the control glutamate response was 8.8 nA and was reduced to 2.4 nA in the presence of (+)-HA-966. A, (+)-HA-966 produced a slight slowing of the on-rate of the L-glutamate response. B, The rate of relaxation of the current response to L-glutamate after the step back into control perfusate was greatly increased in the presence of (+)-HA-966.

TABLE 1

Paired data for on- and off-time constants for L-glutamate (3 μM) and NMDA (30 μM) responses measured in the presence and absence of different concentrations of (+)-HA-966 and 7-Cl-KYNA

Values are means ± standard errors, with the number of cells indicated in parentheses.

	Time constant	
	On	Off
	msec	
Glutamate		
Control	78 ± 5.8 (6)	470 ± 68 (7)
+(+)-HA-966, 30 μM	97 ± 9.8	347 ± 76
Control	86 ± 6.7 (8)	476 ± 60 (8)
+(+)-HA-966, 100 μM	96 ± 6.5	246 ± 47[a]
Control	79 ± 8.8 (9)	455 ± 57 (9)
+(+)-HA-966, 300 μM	84 ± 6.9	160 ± 28[a]
Control	94 ± 5.1 (6)	500 ± 66 (6)
+7-Cl-KYNA 3 μM	98 ± 8.6	364 ± 43
Control	92 ± 8.7 (5)	481 ± 59 (5)
+7-Cl-KYNA, 10 μM	123 ± 8.0	362 ± 37
NMDA		
Control	48 ± 3.9 (4)	91 ± 7.0 (4)
+(+)-HA-966, 30 μM	67 ± 9.4	77 ± 8.2
Control	48 ± 3.0 (5)	96 ± 7.3 (5)
+(+)-HA-966, 100 μM	70 ± 8.8	77 ± 8.2
Control	47 ± 3.4 (7)	88 ± 8.1 (7)
+(+)-HA-966, 300 μM	77 ± 6.9[a]	58 ± 5.4[a]
Control	40 ± 3.3 (11)	106 ± 13.0 (11)
+7-Cl-KYNA, 3 μM	45 ± 3.7	97 ± 14.0
Control	43 ± 3.1 (9)	86 ± 7.4 (9)
+7-Cl-KYNA, 10 μM	53 ± 4.4	85 ± 7.5

[a] Statistical significance at the $p \leq 0.01$ level by paired t test.

3-fold, from 455 ± 57 to 160 ± 28 msec ($n = 9$; $p \leq 0.001$, paired t test). The effect of (±)-HA-966 on the glutamate response off-time constant was concentration dependent and became significant at a concentration of 100 μM (Table 1). The off-time constant of the NMDA response was also significantly ($p \leq 0.001$) reduced in the presence of 300 μM (±)-HA-966 (Table 1), but to a lesser extent than that of the glutamate response. This may be due to the off-rate of NMDA in the presence of (±)-HA-966 approaching the speed of solution exchange around the cell. In contrast to the effects of (±)-HA-966, 7-Cl-KYNA, at concentrations that gave similar reductions in response size, produced only a slight decrease in the off-time constants of glutamate (Fig. 4B) and NMDA (Table 1).

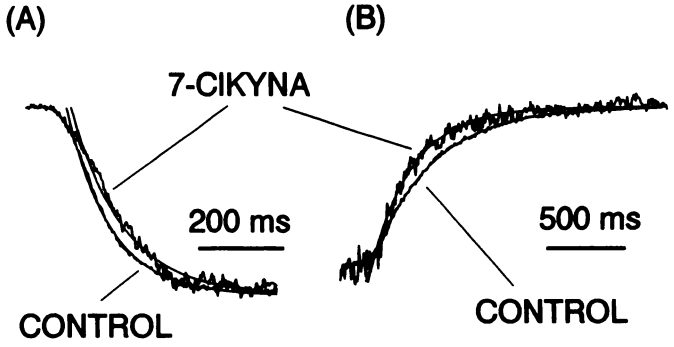

Fig. 4. Effect of 7-Cl-KYNA (10 μM) on the kinetics of the L-glutamate response. The digitized experimental data were scaled to similar sizes and are shown with superimposed exponential fits. The actual response amplitudes were 4.8 nA (control) and 0.58 nA (7-Cl-KYNA). Inhibition with 7-Cl-KYNA had only a small effect on the rates of both onset (A) and decay (B) of the L-glutamate response.

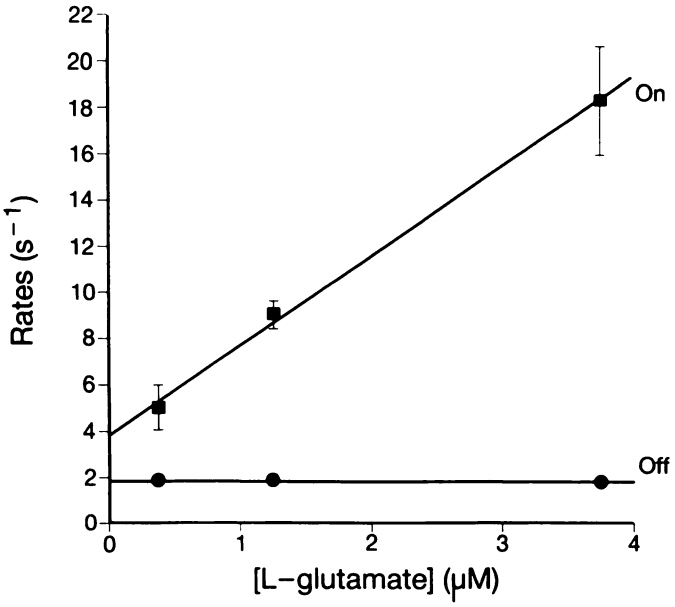

Fig. 5. Kinetic analysis of the glutamate response recorded in the presence of 300 nM glycine. Plot of the reciprocal of the on- and off-time constants versus L-glutamate concentration. The *lines* were fitted by linear regression and the *points* show the mean ± standard error, unless the error lay within the symbol size. The association rate increased with L-glutamate concentration, whereas the dissociation rate was independent of the agonist concentration.

The effect of (±)-HA-966 on the rise times of responses to NMDA and glutamate was more modest than its effect on their rate of decline (Fig. 3A) and was only significant for NMDA (Table 1). Once again, 7-Cl-KYNA failed to show any significant effects (Fig. 4A and Table 1).

As described above, the rise and particularly the decay of NMDA receptor agonist responses in the presence of glycine were well fitted by single exponentials. However, recent evidence indicates that a single binding site model for glutamate (and glycine) is an oversimplification and that the NMDA receptor complex may contain two independent binding sites for both glutamate and glycine (14, 18). Our glutamate equilibrium concentration-response curves are similar to those reported by Patneau and Mayer (18) and are well fitted by a two-independent site model (18, 20, 21). If the receptor does contain two independent sites for glutamate, then the rise of the re-

sponse to glutamate would be expected to be a sigmoidal function, with the likelihood of both sites being occupied by glutamate, and thus the receptor being activated, being the square of the probability that each independent site was occupied. After the initial sigmoidal rise of the response, such a function is reasonably well described by a single exponential. However, as previously noted (14), the initial rise of the response is compromised by the fact that drug equilibration is not instantaneous. Therefore, the initial rise of the response is affected by the time taken for the agonist to equilibrate around the cell, which also results in a sigmoidal rise of the response. Consequently, in practice it is difficult to distinguish between exponential and sigmoidal kinetics. Thus, for the purposes of providing a helpful descriptive tool to discuss the present interactions, we have used the following simple bimolecular reaction scheme.

$$A + R\text{-Gly} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} A\text{-}R\text{-Gly}$$

where R-Gly is the NMDA receptor with glycine bound and A is an NMDA recognition site agonist, such as glutamate or NMDA.

Because the results for glutamate were less affected by errors introduced by drug equilibration times, we have restricted ourselves to the analysis of these data. From the scheme above,

$$1/\tau_{on} = k_{+1} \cdot [A] + k_{-1}$$

and, as predicted from this relationship, the rate of rise of the glutamate response increased with increasing glutamate concentrations. A plot of $1/\tau_{on}$ versus glutamate concentration was linear over the range 0.375–3.75 μM and is shown in Fig. 5. From the slope of this plot the apparent association rate constant (k_{+1}) was calculated to be 3.9×10^6 M^{-1} sec^{-1} and from the zero intercept the apparent dissociation rate constant (k_{-1}) was estimated to be 3.9 sec^{-1}. This latter value was slightly higher than that obtained from the plot of $1/\tau_{off}$ (1.9 sec^{-1}), which, as expected, did not alter with changes in the glutamate concentration (Fig. 5).

In experiments in which the effect of (+)-HA-966 (300 μM) was studied, the apparent dissociation rate constant for glutamate in the presence of glycine was 2.2 sec^{-1} (calculated from $k_{-1} = 1/\tau_{off}$) and the apparent association rate constant (k_{+1}) was calculated to be 3.5×10^6 M^{-1} sec^{-1} (determined from data given in Table 1). These values give an equilibrium dissociation constant (K_D) of 0.6 μM, which is in good agreement with the EC_{50} value obtained from equilibrium concentration-response curves (see above and Ref. 18). In the presence of (+)-HA-966 (300 μM), the corresponding values for k_{-1}, k_{+1}, and K_D were calculated to be 6.3 sec^{-1}, 1.9×10^6 M^{-1} sec^{-1}, and 3.3 μM, respectively.

Thus, based on these calculations, (+)-HA-966 binding at the glycine site produced a 5-fold decrease in the affinity of glutamate for the NMDA recognition site. The fact that these effects were mediated by an action at the glycine site is demonstrated by the findings that the effect of (+)-HA-966 on NMDA responses is reversed by increases in the concentration of glycine or D-serine (11) and that the observed effects are not compatible with a competitive interaction at the NMDA recognition site.

These results indicate that, as well as competitively blocking the effect of glycine at its site on the NMDA receptor complex, (+)-HA-966 produces an allosteric reduction in affinity of agonists at the glutamate recognition site and that for glutamate this effect is largely the result of an increase in its dissociation rate constant. In comparison, 7-Cl-KYNA has much less effect on the kinetics of the glutamate response and is, thus, much more of a neutral antagonist of the glycine site.

There is evidence from receptor binding studies to support such an effect of HA-966 on the glutamate recognition site of the NMDA receptor complex. HA-966 has been shown to noncompetitively inhibit NMDA-specific L-[^{3}H]glutamate binding and to enhance the binding of competitive NMDA receptor antagonists by an increase in their affinity (9, 22), effects that can be antagonized by glycine and D-serine. A reciprocal interaction also occurs, with competitive NMDA receptor antagonists producing a reduction in the affinity of strychnine-insensitive [^{3}H]glycine binding to the NMDA receptor complex and increasing the affinity of HA-966 for this site (23, 24). In contrast, the affinity of 7-Cl-KYNA for the glycine site is not significantly changed in the presence of a competitive glutamate recognition site antagonist, nor does it allosterically affect agonist or antagonist binding to this site (9, 23). Furthermore, binding studies have also shown that glutamate enhances glycine binding (25–27) and glycine enhances glutamate binding (28, 29).

Overall, these results support the suggestion that there may be different states of the NMDA receptor complex (28); an "agonist-preferring" state, which has a higher affinity for agonists at the glycine site and the NMDA recognition site and a lower affinity for antagonists, such as HA-966 and ACBC (7), at the glycine site and ω-phosphono-α-amino acids at the glutamate recognition site, and an "antagonist-preferring" state, for which the opposite is true (9, 22, 23). Of interest, in this respect, is the finding that compounds such as HA-966 and ACBC, which have a higher affinity for the "antagonist-preferring" state and reduce agonist affinity at the glutamate recognition site, are partial agonists (5, 11, 30), whereas 7-Cl-KYNA, a full antagonist, appears not to distinguish between the different states of the receptor.

Thus, the binding studies are in complete agreement with the present results, which suggest that glutamate has a lower affinity for its recognition site when (+)-HA-966 is bound to the glycine site than when glycine itself is bound. They are, however, more difficult to reconcile with the Benveniste *et al.* (14) model of desensitization, which suggests that there is a negative allosteric interaction between agonists at the glutamate and glycine recognition sites on the NMDA receptor complex.

In conclusion, our results indicate that binding of (+)-HA-966 at the glycine site on the NMDA receptor complex produces an allosteric reduction in the affinity of agonists for the glutamate recognition site and provide functional data to support the concept of different states of the NMDA receptor. In contrast, 7-Cl-KYNA has relatively little effect on the affinity of agonists for the glutamate recognition site and, thus, acts more as a pure antagonist of the glycine recognition site.

References

1. Johnson, J. W., and P. Ascher. Glycine potentiates the NMDA response of mouse central neurones. *Nature (Lond.)* **325**:529–531 (1987).
2. Kleckner, N. W., and R. Dingledine. Requirement for glycine in activation of *N*-methyl-D-aspartic acid receptors expressed in *Xenopus* oocytes. *Science (Washington D. C.)* **241**:835–837 (1988).
3. Fletcher, E. J., and D. Lodge. Glycine reverses antagonism of *N*-methyl-D-aspartate (NMDA) by 1-hydroxy-3-aminopyrrolidone-2 (HA-966) but not by

D-2-amino-5-phosphonovalerate (D-AP5) on rat cortical slices. *Eur. J. Pharmacol.* **151**:161–162 (1988).
4. Kemp, J. A., A. C. Foster, P. D. Leeson, T. Priestley, R., Tridgett, L. L. Iversen, and G. N. Woodruff. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. USA*, **85**:6547–6550 (1988).
5. Foster, A. C., and J. A. Kemp. HA-966 antagonizes *N*-methyl-D aspartate receptors through a selective interaction with the glycine modulatory site. *J. Neurosci.* **9**:2191–2196 (1989).
6. Huettner, J. E. Indole-2-carboxylic acid: a competitive antagonist of potentiation by glycine at the NMDA receptor. *Science (Washington D. C.)* **243**:1611–1613 (1989).
7. Hood, W. F., E. T. Sun, R. P. Compton, and J. B. Monahan. 1-Aminocyclobutane-1-carboxylate (ACBC): a specific antagonist of the *N*-methyl-D-asparatate receptor coupled glycine receptor. *Eur. J. Pharmacol.* **161**:281–282 (1989).
8. Kemp, J. A., T. Priestley, and G. W. Woodruff. Differences in the *N*-methyl-D-aspartate antagonist profiles of two compounds acting at the glycine modulatory site. *Br. J. Pharmacol.* **95**:759P (1988).
9. Danysz, W., E. Fadda, J. T. Wroblewski, and E. Costa. Different modes of action of 3-amino-1-hydroxy-2-pyrrolidone (HA-966) and 7-chlorokynurenic acid in the modulation of *N*-methyl-D-aspartate-sensitive glutamate receptors. *Mol. Pharmacol.* **36**:912–916 (1989).
10. Kloog, Y., H. Lamdani-Itkin, and M. Sokolovski. The glycine site of the *N*-methyl-D-aspartate receptor channel: differences between the binding of HA-966 and of 7-chlorokynurenic acid. *J. Neurochem.* **54**:1576–1583 (1990).
11. Singh, L., A. E. Donald, A. C. Foster, P. H. Hutson, L. L. Iversen, S. D. Iversen, J. A. Kemp, P. D. Leeson, G. R. Marshall, R. J. Oles, T. Priestley, L. Thorn, M. D. Tricklebank, C. A. Vass, and B. J. Williams. Enantiomers of HA-966 display distinct central nervous system effects: (+)-HA-966 is a selective glycine/*N*-methyl-D-aspartate receptor antagonist, but (−)-HA-966 is a potent γ-butyrolactone-like sedative. *Proc. Natl. Acad. Sci. USA* **87**:347–351 (1990).
12. Mayer, M. L., L. Vyklicky, and J. D. Clements. Regulation of NMDA receptor desensitization in mouse hippocampal neurones by glycine. *Nature (Lond).* **338**:425–427 (1989).
13. Vyklicky, L., M. Benveniste, and M. L. Mayer. Modulation of *N*-methyl-D-aspartic acid receptor desensitization by glycine in mouse cultured hippocampal neurones. *J. Physiol. (Lond.)* **428**:313–331 (1990).
14. Benveniste, M., J. Clements, L. Vyklickly, and M. L. Mayer. A kinetic analysis of the modulation of *N*-methyl-D-aspartic acid receptors by glycine in mouse cultured hippocampal neurones. *J. Physiol (Lond.)* **428**: 333–357 (1990).
15. Sather, W., J. W. Johnson, G. Henderson, and P. Ascher. Glycine-insensitive desensitization of NMDA responses in cultured mouse embryonic neurones. *Neuron* **4**:725–731 (1990).
16. Priestley, T., G. N. Woodruff, and J. A. Kemp. Antagonism of responses to excitatory amino acids on rat cortical neurones by the spider toxin, argiotoxin$_{636}$. *Br. J. Pharmacol.* **97**:1315–1323 (1989).
17. Johnson, J. W., and P. Ascher. Response of neurones to fast application of *N*-methyl-D-aspartate. *Soc. Neurosci. Abstr.* **12**:58 (1986).
18. Patneau, D. K., and M. L. Mayer. Structure-activity relationships for amino acid transmitter candidates acting at *N*-methyl-D-aspartate and quisqualate receptors. *J. Neurosci.* **10**:2385–2399 (1990).
19. Lester, R. A. J., J. D. Clements, G. L. Westbrook, and C. E. Jahr. Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature (Lond.)* **346**:565–567 (1990).
20. Colquhoun, D., and D. C. Ogden. Activation of ion channels in the frog endplate by high concentrations of acetylcholine. *J. Physiol. (Lond.)* **395**:131–159 (1988).
21. Bean, B. P. ATP-activated channels in rat and bullfrog sensory neurons: concentration-dependence and kinetics. *J. Neurosci.* **10**:1–10 (1990).
22. Compton, R. P., W. F. Hood, and J. B. Monahan. Evidence for a functional coupling of the NMDA and glycine recognition sites in synaptic plasma membranes. *Eur. J. Pharmacol.* **188**:63–70 (1990).
23. Monahan, J. B., V M. Corpus, W. F. Hood, J. W. Thomas, and R. P. Compton. Characterisation of a [^{3}H]glycine recognition site as a modulatory site of the *N*-methyl-D-aspartate receptor complex. *J. Neurochem.* **53**:370–375 (1989).
24. Hood, W. F., R. P. Compton, and J. B. Monahan. *N*-methyl-D-aspartate recognition site ligands modulate activity at the coupled glycine recognition site. *J. Neurochem.* **54**:1040–1046 (1990).
25. Kessler, M., T. Terramani, G. Lynch, and M. Baudry. A glycine site associated with *N*-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. *J. Neurochem.* **52**:1319–1328 (1989).
26. Monahan, J. B., J. P. Biesterfeldt, W. F. Hood, R. P. Compton, A. A. Cordi, M. I. Vazquez, T. H. Lanthorn, and P. L. Wood. Differential modulation of the associated glycine recognition site by competitive *N*-methyl-D-aspartate receptor antagonists. *Mol. Pharmacol.* **37**:780–784 (1990).
27. Ransom, R. W., and N. L. Deschenes. Polyamines regulate glycine interaction with the *N*-methyl-D-aspartate receptor. Synapse **5**:294–298 (1990).
28. Monaghan, D. T., H. J. Olverman, L. Nguyen, J. C. Watkins, and C. W. Cotman. Two classes of *N*-methyl-D-aspartate recognition sites: differential distribution and differential regulation by glycine. *Proc. Natl. Acad. Sci. USA* **85**:9836–9840 (1988).
29. Fadda, E., W. Danysz, J. T. Wroblewski, and E. Costa. Glycine and D-serine increase the affinity of *N*-methyl-D-aspartate sensitive glutamate binding sites in rat brain synaptic membranes. *Neuropharmacology* **27**:1183–1185 (1988).
30. Kemp, J. A., T. Priestley, and G. R. Marshall. Glycine-site ligand affinity and efficacy: consequences for NMDA receptor modulation, in *Excitatory Amino Acids* (B. S. Meldrum, F. Moroni, R. P. Simon, and J. H. Woods, eds.). Raven Press, Ltd., New York, 213–222 (1991).

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