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Selectivity of Amino Acid Transmitters Acting at *N*-Methyl-D-aspartate and Amino-3-hydroxy-5-methyl-4-isoxazolepropionate Receptors

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SUMMARY

The endogenous neurotransmitter candidates L-aspartate, L-cysteine sulfinate (CSA), L-glutamate, L-homocysteate (HCA), and the endogenously occurring analogue quinolinate were compared in terms of potency, maximal activity, and selectivity for steady state activation of *N*-methyl-D-aspartate (NMDA) and non-NMDA [(*RS*)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)] types of glutamate receptors expressed in *Xenopus* oocytes injected with mRNA isolated from rat brain (minus cerebellum). Selective activation of NMDA receptors was achieved by deleting Mg^{2+} and including 3–10 μM glycine in the perfusion medium and by applying ligands in the presence of 30 μM quisqualate, which blocks the AMPA receptor and desensitizes the oocyte's own Ca^{2+}-dependent Cl^- current. Oocytes were voltage clamped, and steady state inward currents were measured in response to perfusion with agonists at known concentrations. Under the NMDA receptor-preferring condition, the potency rank order was L-glutamate (EC_{50} = 2.2 μM, 95% confidence interval = 1.4–3.6 μM) > L-aspartate (13 μM) = HCA (13 μM) > CSA (59 μM) > quinolinate (≥7200 μM). All amino acids tested evoked similar maximal currents, which were 120–159% that of NMDA itself. The Hill coefficient was greater than 1 for all agonists except L-HCA (0.6), which might reflect heterogeneity of NMDA receptors expressed. This was supported by the finding that glycine was more potent in combination with HCA than NMDA, in activating NMDA receptors. To study the activity of agonists at AMPA receptors, glycine and quisqualate were omitted and 1 mM Mg^{2+} was included to block NMDA receptors. Ca^{2+}-dependent Cl^- currents activated by L-glutamate were prevented by inclusion of 0.4 M ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid in the recording electrode. All amino acids were less potent at AMPA receptors than at NMDA receptors; the potency rank order for steady state activation of AMPA receptors was L-glutamate (EC_{50} = 11 μM, 95% confidence interval = 7.3–18 μM) > HCA (430 μM) > CSA (3300 μM). L-Aspartate and quinolinate produced little or no inward current even up to 10 mM, i.e., were inactive at forebrain AMPA receptors. The maximal currents activated by all amino acids at steady state were 5–10% that of kainate, presumably due to severe desensitization of the AMPA receptor by the natural agonists. These results are consistent with L-glutamate acting as a mixed agonist at both AMPA and NMDA synaptic receptors and L-aspartate being involved exclusively in NMDA receptor-mediated synapses.

The potential involvement of glutamate receptors in such diverse phenomena as epilepsy (1), learning (2), development of synaptic connections (3), and ischemic brain damage (4) has increased interest in the properties of excitatory synaptic receptors. The action of some endogenous compounds may also be associated with certain neurodegenerative disorders (5). The endogenous ligands for these receptors include L-glutamate, L-aspartate, and their naturally occurring analogues CSA, and HCA. These compounds, as well as quinolinate, a cyclic analogue of NMDA, are potent excitants of mammalian central neurons (6). With the exception of quinolinate, they can be released from slices of various rat brain regions by elevated K^+, in a Ca^{2+}-dependent manner (7, 8), and they are substrates for glutamate uptake (9).

Physiological studies have identified at least two ionotropic excitatory amino acid receptor subtypes that mediate synaptic transmission. Forebrain NMDA receptors are insensitive to kainate, require glycine for activation by NMDA (10), and are blocked selectively by Mg^{2+} (11) or D-APV (12). Non-NMDA (AMPA) receptors are activated by kainate, quisqualate, and AMPA, but not by NMDA, and are blocked competitively by certain quinoxalinediones such as CNQX (13). A third potential excitatory receptor, which exhibits nanomolar affinity for kainate (14), has not yet been demonstrated to have a synaptic function.

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ABBREVIATIONS: CSA, L-cysteine sulfinate; HCA, L-homocysteate; NMDA, *N*-methyl-D-aspartate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; D-APV, D-2-amino-5-phosphonovalerate; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

A key issue is the relative selectivity of the natural transmitter candidates for the two glutamate receptor families. Glutamate itself is clearly a mixed agonist active at both receptor types (15), but the selectivity of the other endogenous amino acid transmitter candidates is unclear. L-Aspartate selectively activates NMDA versus AMPA receptors in cultured hippocampal neurons (15) and in *Xenopus* oocytes injected with rat whole-brain mRNA (16). However, earlier studies on more intact preparations suggest that aspartate may also activate non-NMDA receptors in some cells (17).

The selectivity of HCA for NMDA and non-NMDA receptors is also inconclusive. Some studies have shown significant blockade of HCA-induced responses by Mg^{2+} or NMDA receptor antagonists (7, 18), whereas others report mixed activity for HCA (15, 17, 19, 20). Quinolinate, on the other hand, exhibits a strong preference for NMDA receptors in many brain regions (15, 21, 22). Finally, CSA-induced responses have not been studied as intensively but appear to be much more resistant to NMDA receptor antagonists than are responses to either HCA or quinolinate (17).

Until recently (15), there has been little attempt to compare systematically the selectivity of potential endogenous neurotransmitters for excitatory amino acid receptors. The present study was conducted to compare the selectivity, potency, and maximal activity of these amino acid neurotransmitter candidates acting at NMDA and AMPA receptors expressed in *Xenopus* oocytes injected with rat brain mRNA. Using this preparation, only L-glutamate was found to have significant activity at AMPA and NMDA receptors at micromolar concentrations. L-glutamate is, therefore, a high potency mixed agonist with low selectivity for NMDA receptors. L-Aspartate, in contrast, has the greatest selectivity for NMDA receptors. All other amino acids tested show a moderate selectivity for NMDA receptors and, with the possible exception of quinolinate, are mixed agonists. A preliminary report of some of these results has appeared (23).

Materials and Methods

The methods for RNA preparation and injection, oocyte culture, and voltage-clamp recording have been described in detail previously (24). Three separate poly(A)$^+$ RNA preparations were used in this study, each isolated from forebrain minus cerebellum of male Sprague-Dawley rats. Dose-response relationships for all agonists except L-aspartate were obtained with mRNA from 30-day old rats. L-Aspartate responses were studied using mRNA from 18 day-old rats. A previous study conducted in our laboratory showed no change in the EC_{50} for NMDA currents recorded from oocytes injected with brain mRNA from rats 1–2 days to approximately 60 days old (25). The remaining preparation of mRNA from adult rats was used to test the selectivity of block of AMPA and NMDA receptor responses by quisqualate.

Oocytes were usually voltage clamped, with two microelectrodes, at a holding potential of −60 mV at room temperature while steady state inward currents produced by the agonists were recorded. Pipettes for passing current contained 3 M CsCl and either 0.01 or 0.4 M EGTA, as specified. When antagonists were used, 2–4 min were allowed before and after agonist application for complete inhibition and recovery of agonist-induced responses. In experiments measuring the glycine potentiation of agonist-induced currents, solutions were made with triple-distilled water and with glassware baked to remove glycine. Statistical comparisons were considered significant at the $p = 0.05$ level.

Agonist purity. Agonist solutions were examined for contamination by glutamate and aspartate by phenylisothiocyanate derivatization and high performance liquid chromatographic analysis. Only L-aspartate and CSA were found to be significantly contaminated with glutamate; L-aspartate solutions contained 0.3% glutamate and CSA solutions contained 0.02% glutamate by mole fraction. Glutamate made up only 0.01% of the HCA solutions. L-Glutamate was the only agonist contaminated with aspartate (0.03%).

Materials. Cesium hydroxide, sodium chloride, and sodium sulfate were obtained from Aldrich; cesium chloride from Bethesda Research Laboratories; D-APV and NMDA from Cambridge Research Biochemicals; calcium nitrate from EM Science; calcium chloride, magnesium sulfate, and potassium chloride from Fisher Scientific; L-aspartate from Pierce Chemical Company; quisqualic acid from Research Biochemicals, Inc.; EGTA, gentamicin sulfate, HEPES, penicillin G, and sodium bicarbonate from Sigma; and 7-chlorokynurenic acid, CNQX, CSA, L-glutamate, HCA, and quinolinic acid from Tocris Neuramin.

Results

Selective activation of NMDA and AMPA receptors. Isolation of NMDA and AMPA receptor responses from each other, and from the Ca^{2+}-dependent Cl^- current present in some oocytes, was achieved by selective block of unwanted currents. AMPA receptor currents were isolated from NMDA receptor currents by perfusing oocytes with 1 mM Mg^{2+} and no added glycine. Under these conditions, even high concentrations of NMDA (300 μM) elicited no inward current (0.67 ± 0.56 nA, $n = 6$). Although AMPA receptor responses could be readily separated from NMDA receptor currents, some agonists activated a metabotropic receptor, leading to an oscillatory current produced by the oocyte's own Ca^{2+}-dependent Cl^- current (26). Metabotropic responses were suppressed by including 0.4 M EGTA in the pipette or by discarding oocytes that exhibited an oscillatory response. Agonist-induced responses under these conditions were largely due to activation of AMPA receptors, because they were unaffected by 10 μM D-APV and the degree of block by 10 μM CNQX of currents evoked by EC_{80} concentrations of L-glutamate (86 ± 7.6%), HCA (84 ± 1.7%), and kainate (96 ± 0.3%) was not statistically different (one-way analysis of variance, $p = 0.10$, $n = 7–9$).

Selective activation of NMDA receptors was achieved by applying agonists in Mg^{2+}-free medium to which 3–10 μM glycine (a near-saturating concentration) had been added. The activation of AMPA receptors was blocked by prolonged perfusion with 30 μM quisqualate (24, 27). CNQX was not used to block AMPA receptors because of its additional antagonist activity at the glycine site of the NMDA receptor (28, 29). Application of 30 μM quisqualate produced a rapidly desensitizing inward current (presumably due to activation of a metabotropic receptor) that reached a steady state level within 3–8 min, after which responses to kainate were markedly reduced. The block by quisqualate appeared to be competitive, in that the concentration-response relationship for kainate was shifted by 37-fold with no reduction in the maximum response (Fig. 1A). Block by quisqualate would appear competitive if quisqualate unbinding, recovery from desensitization, and kainate binding to the AMPA receptor are all fast relative to a 1–2-min perfusion. We confirmed that responses to very high concentrations of kainate (10–30 mM) in the presence of quisqualate were mainly due to activation of AMPA receptors rather than "breakthrough" activation of NMDA receptors, because currents induced by 30 mM kainate were not blocked by 100 μM D-APV (96 ± 26% control, $n = 3$) but were significantly reduced by 100 μM CNQX (62 ± 22%, $p = 0.021$). More importantly, the same concentration of quisqualate did not affect the re-

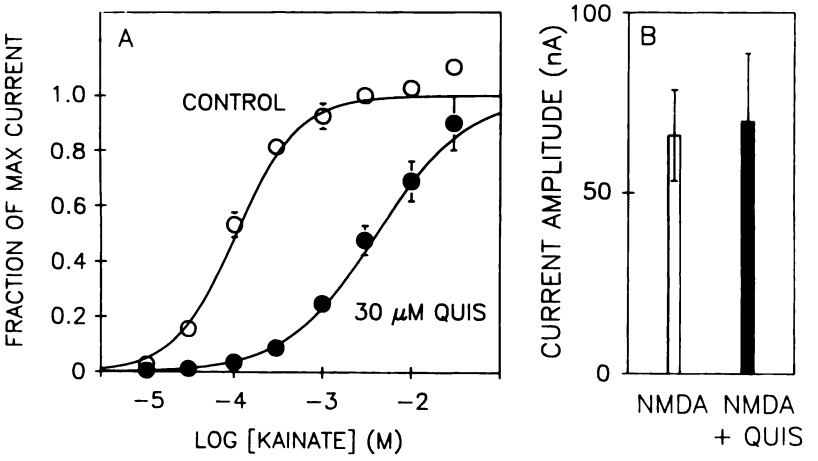

Fig. 1. Effect of quisqualate (*QUIS*) on NMDA and AMPA receptor-mediated currents. A, Kainate dose-response curve under AMPA receptor-preferring conditions (○) and in the presence of 30 μM quisqualate (●). Each *point* represents the mean ± standard error of 2–9 oocytes, in which either one or both dose-response relationships were studied. The control curve represents the fit to 38 data points pooled from nine oocytes. Only two data points were obtained at 30 mM kainate, whereas nine, nine, and three measurements were made at 1, 3, and 10 mM, respectively, making the fit less sensitive to the two points at 30 mM kainate. Quisqualate shifts the kainate dose-response curve to the right without markedly reducing the maximum current response to kainate. The kainate EC_{50} was shifted by 37-fold. B, Mean inward current ± standard error induced by 100 μM NMDA in seven oocytes held between −30 and −60 mV in the absence (□) and presence (■) of 30 μM quisqualate.

sponse to a nearly saturating concentration of NMDA. Fig. 1B demonstrates the lack of effect of 30 μM quisqualate on the mean current induced by 100 μM NMDA and 3 μM added glycine (66 ± 13 nA in the absence of quisqualate, compared with 70 ± 19 nA in its presence; $n = 7$). Similarly, there was no statistical difference between the currents produced by 300 μM L-aspartate in the presence (107 ± 16% of NMDA maximum current, $n = 9$) and absence of 30 μM quisqualate (124 ± 4%, $n = 5$). Thus, inclusion of 30 μM quisqualate in the perfusion medium greatly reduced responses only at AMPA receptors, allowing selective activation of NMDA receptors.

To verify that quisqualate also blocked AMPA receptor currents activated by the endogenous agonists, the effect of 30 μM quisqualate on EC_{80} concentrations of L-glutamate (10 μM), HCA (1 mM), and kainate (300 μM) was compared in five or six oocytes. Quisqualate reduced L-glutamate currents to 20.7 ± 11.1%, HCA currents to 7.7 ± 2.7%, and kainate responses to 12.3 ± 2.3% of their control values. The differences were not statistically significant (one-way analysis of variance, $p = 0.31$).

Activation of AMPA receptors. The activity of the agonists at AMPA receptors was compared by normalizing steady state current responses to the maximal current produced by application of 3 mM kainate. Under AMPA receptor-preferring conditions, all agonists except L-aspartate and quinolinate typically induced small amplitude, smooth, inward currents at steady state. L-Aspartate and quinolinate produced little or no inward AMPA receptor current, even up to 10 mM. The lack of L-aspartate current could not be explained by rapid and complete desensitization (compare with quisqualate), because 1 mM L-aspartate did not reduce currents elicited by 3 mM kainate (99 ± 19% of control, $n = 3$). Thus, L-aspartate does not appear to interact with forebrain AMPA receptors expressed in oocytes.

Fig. 2A shows sample responses to L-glutamate and HCA and Fig. 2B shows concentration-response relationships for all agonists at AMPA receptors. Of the agonists with activity, only L-glutamate had micromolar affinity for AMPA receptors. For each agonist, the mean EC_{50} value, Hill coefficient, and maximal response at steady-state are listed in Table 1. The maximum response of all active agonists was similar, 5–10% that of kainate. The potency rank order for the ligands acting on AMPA receptors was L-glutamate (EC_{50} = 11 μM, 95% confidence interval = 7.3–18 μM) > kainate (91 μM) > HCA (430 μM) > CSA (3300 μM) > L-aspartate = quinolinate. Aspartate

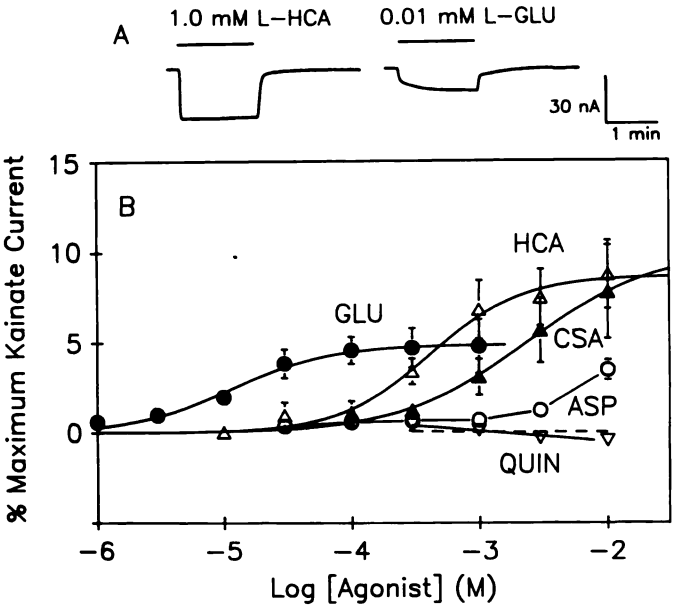

Fig. 2. Agonist-induced current responses under AMPA receptor-preferring conditions. A, Representative steady state currents induced by EC_{80} concentrations of L-glutamate (*GLU*) and HCA (with EGTA in electrode) measured at −60 mV. B, Comparison of mean dose-response curves for endogenous agonists. Steady state currents for individual oocytes were normalized to the maximum current produced by kainate (3 mM). Values for maximum current, EC_{50} and Hill slope obtained using the logistic equation were averaged from individual oocytes. Each *point* represents the mean ± standard error of three to six determinations. L-Aspartate (*ASP*) and quinolinate (*QUIN*) were unable to activate AMPA receptor current even up to 10 mM. – – –, Responses to L-aspartate after correcting for estimated responses due to contamination by L-glutamate.

solutions were contaminated with 0.3% glutamate, as detected by high performance liquid chromatography. This contamination alone can account for the observed responses of L-aspartate solutions at 3 and 10 mM. The estimated responses elicited by contaminating glutamate have been subtracted from the measured L-aspartate-induced currents, as indicated by the dashed line in Fig. 2. Therefore, neither L-aspartate nor quinolinate produced AMPA receptor current up to 10 mM. Contaminating glutamate levels were also evident in CSA and HCA solutions, although to a lesser degree (0.02% and 0.01%, respectively). This level of contamination was not great enough to account for CSA-induced or HCA-induced current at AMPA receptors.

TABLE 1

Potency and maximal activity of endogenous agonists on AMPA receptors

AMPA receptors were studied in the presence of 1 mM Mg^{2+} and no added glycine. The maximum currents (mean ± standard error) produced by the agonists at −60 mV were normalized to the maximum current produced by kainate. The EC_{50} reported for kainate was obtained using oocytes voltage clamped between −30 and −60 mV. Mean EC_{50} values are reported along with the 95% confidence intervals (numbers in parentheses). EC_{50} values for L-aspartate and quinolinate are estimated to be >10 mM, because no inward current could be elicited up to 10 mM. Hill coefficients are mean ± standard error.

Agonist	EC_{50}	Maximal activity	Hill coefficient	*n*
	μM			
Kainate	91 (72–120)	1	1.4 ± 0.06	7
L-Glutamate	11 (7.3–18)	0.05 ± 0.01	1.3 ± 0.20	6
HCA	430 (290–630)	0.10 ± 0.03	1.2 ± 0.15	6
CSA	3,300 (2,300–5,400)	0.10 ± 0.03	0.81 ± 0.15	5
L-Aspartate	>10,000			5
Quinolinate	>10,000			3

Activation of NMDA receptors. All five agonists tested were very efficacious in activating NMDA receptors. Representative currents evoked in one oocyte by approximate EC_{80} concentrations of HCA, L-glutamate, CSA, and quinolinate are depicted in Fig. 3A. Peak responses are all about the same amplitude as that produced by an EC_{95} concentration of NMDA (300 μM). At high agonist doses the peak agonist currents slowly desensitized. Leonard and Kelso (30) have demonstrated that the initial sharply rising current is often dominated by a rapidly (several seconds) desensitizing Ca^{2+}-dependent Cl^- current. This early response was highly variable in both amplitude and shape, and all measurements were made from the subsequent peak (Fig. 3A, *solid circles*). The late peak of the NMDA response is unlikely to be contaminated substantially by Ca^{2+}-dependent Cl^- current endogenous to the oocyte, because (a) its reversal potential is not affected by replacement of 50% (24) or 73%[1] of Cl^- by methanesulfonate, (b) it is not reduced by intracellular injection of EGTA (30), and (c) the peak amplitude increases rather than decreases in low external calcium,[1] presumably due to relief from slow Ca^{2+}-dependent desensitization (31, 32). D-APV (100 μM) reduced control responses to an EC_{95} concentration of NMDA by 92% and to EC_{80} concentrations of the other agonists by 89–93%, suggesting that inward current induced by each agonist under these conditions was due to activation of NMDA receptors.

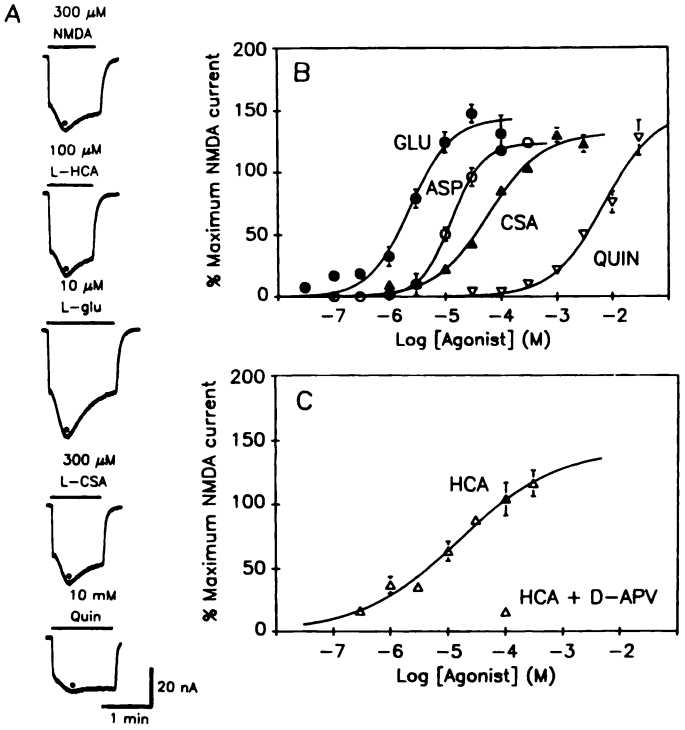

Fig. 3. Agonist-induced current responses under NMDA receptor-preferring conditions. A, Representative steady state currents induced by approximate EC_{80} concentrations of the agonists measured at −60 mV. B, Comparison of mean dose-response curves for L-glutamate (●), L-aspartate (○), CSA (▲), and quinolinate (▽). Peak amplitudes (● in A) of agonist-induced currents were normalized to the maximum current produced by NMDA (300 μM). Each *point* represents the mean ± standard error of five to seven determinations. Because the dose-response curves for CSA, L-glutamate, and HCA acting on NMDA and AMPA receptors overlap slightly, all ligands except L-aspartate were studied in the presence of 30 μM quisqualate. Responses to L-aspartate (0.3–10,000 μM) in the absence of quisqualate produced no inward current at AMPA receptors. C, Mean dose-response curve for HCA ($n = 7$) and the average block by 100 μM D-APV of currents induced by 100 μM HCA ($n = 4$). Note the shallower Hill slope of HCA, relative to the other four endogenous agonists.

The potency and maximal activation of the agonists were compared by constructing concentration-response curves for individual oocytes. Peak currents activated by each agonist were normalized to that produced by a saturating concentration of NMDA (300 μM). The slow rundown of NMDA currents (16, 33) was accounted for by bracketing test concentrations of the endogenous agonists with control applications of 300 μM NMDA. Fig. 3, B and C, shows the mean concentration-response curves for all five agonists acting at NMDA receptors. All agonists elicited larger NMDA receptor-mediated inward current than did NMDA, from ~150% of the maximum NMDA current for L-glutamate and quinolinate to 124% for L-aspartate. Also, under steady state conditions all agonists had greater activity at NMDA than at AMPA receptors.

L-Glutamate was the most potent of the agonists at NMDA receptors, having an EC_{50} of 2.2 μM (95% confidence interval = 1.4–3.6 μM), i.e., 5-fold more potent at NMDA receptors than when measured at steady state at AMPA receptors. The potency sequence of the ligands tested was L-glutamate > L-aspartate = HCA > NMDA > CSA > quinolinate. Table 2 lists the EC_{50} values and maximal activities of these agonists. A maximum response to quinolinate was not attained even at 30 mM and, therefore, only minimum values could be estimated. Except for HCA and CSA, which produced Hill slopes of 0.6 and 1.0, respectively, the Hill coefficients of the other ligands were >1.0, indicating the possibility of agonist binding at multiple sites on the NMDA receptor (15).

The dose-response curves in Figs. 2 and 3 were fit with a

[1]N. Kleckner and R. Dingledine, unpublished observations.

TABLE 2

Potency and maximal activity of endogenous agonists on NMDA receptors

NMDA receptors were studied in 0 Mg^{2+}, 3–10 μM added glycine, and the presence of 30 μM quisqualate, to suppress currents mediated by AMPA receptors. The maximum currents (mean ± standard error) produced by the agonists at −60 mV were normalized to the maximum current produced by NMDA. Mean EC_{50} values are reported along with the 95% confidence intervals (numbers in parentheses). Hill coefficients are mean ± standard error.

Agonist	EC_{50}	Maximal activity	Hill coefficient	n
	μM			
NMDA	31 (26–36)[a]	1	1.3 ± 0.06[a]	18
L-Glutamate	2.2 (1.4–3.6)	1.47 ± 0.10	1.3 ± 0.21	6
HCA	13 (7.9–21)	1.38 ± 0.16	0.60 ± 0.10	7
L-Aspartate	13 (10–18)	1.24 ± 0.04	1.7 ± 0.30	5
CSA	59 (43–81)	1.33 ± 0.08	1.0 ± 0.13	7
Quinolinate	7200[b]	1.5[b]	0.92[b]	6

[a] Values reported by Verdoorn and Dingledine (24), using identical experimental conditions.

[b] Approximate values obtained using the logistic equation, for which the maximum current was estimated as 1.5-fold that of NMDA.

single-site logistic equation, which does not assume a particular molecular model for receptor activation. We also fit the data to a two-independent binding site model for NMDA receptor activation (see Ref. 15):

$$I = I_{max} \times C^2/(1 + 2C + C^2)$$

where C is the agonist concentration divided by the microscopic equilibrium affinity constant for each identical agonist binding site. Curves generated with this model did not fit our data well except in the case of L-glutamate (microscopic K_D = 1.1 μM). Moreover, the L-aspartate dose-response curve was better fit by the logistic equation than the two-site model. This suggests that L-aspartate may bind with positive cooperativity (n = 1.7, logistic equation) or that multiple NMDA receptors were expressed in our oocytes, each with different affinity for L-aspartate but similar affinity for L-glutamate.

HCA. In contrast to all other agonists tested, the Hill coefficient calculated for HCA was very low (Fig. 3C; Table 2), significantly different from that of L-glutamate (p = 0.003) or L-aspartate (p = 0.0001). Virtually all of the HCA-induced current was blocked by 100 μM D-APV (Fig. 3C), indicating that the response was due to NMDA receptor activation. NMDA receptor activation by HCA and NMDA also differed in sensitivity to glycine. In the absence of added glycine the peak currents induced by HCA and NMDA were very low (Fig. 4), consistent with a requirement for glycine in NMDA receptor activation. Fig. 4 shows that glycine was about twice as potent (p = 0.048, n = 5 or 6) in enabling activation of the receptor by HCA (EC_{50} = 181 nM, 95% confidence interval = 120–273 nM), compared with NMDA (EC_{50} = 343 nM, 95% confidence interval = 230–512 nM). In support of these findings, we also found differential block by the glycine site antagonist 7-chlorokynurenate of NMDA receptor currents produced by approximately equieffective concentrations of HCA and NMDA. In the presence of 5 μM 7-chlorokynurenate and 3 μM added glycine, peak currents activated by 300 μM NMDA were 33 ± 7.4% of control, whereas currents activated by 100 μM HCA were 57 ± 15% of control (p = 0.05, n = 4, paired t test). These findings, taken together, indicate that significantly less glycine is necessary for activation of NMDA receptors by HCA than by NMDA.

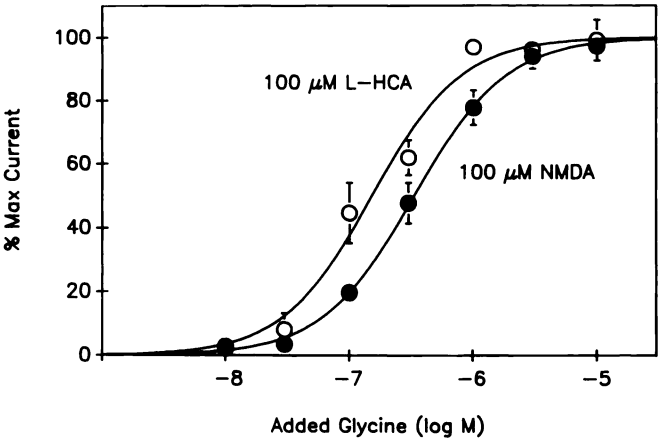

Fig. 4. Glycine dose-response curves for HCA and NMDA. Inward currents were produced by 100 μM HCA or NMDA alone and in the presence of sequentially increasing doses of added glycine. Each *point* represents the mean current ± standard error as a fraction of the maximum current produced by each agonist (n = 4 or 5). Note the greater glycine sensitivity for HCA (EC_{50} = 181 nM), compared with NMDA (343 nM).

Discussion

Selectivity of endogenous compounds for NMDA and AMPA receptors. The major populations of NMDA and AMPA receptors translated in oocytes injected with forebrain mRNA are similar in pharmacology to those receptors found in cultured hippocampal neurons. The EC_{50} values determined here are in excellent quantitative agreement with those found for cultured hippocampal neurons by Patneau and Mayer (15). In both studies, for example, neither L-aspartate nor quinolinate had detectable activity at AMPA receptors, and L-glutamate was the only endogenous agonist tested with micromolar affinity for AMPA receptors, having a steady state EC_{50} of 11 μM (Table 1) in oocytes and 19 μM in neurons. The sulfur-containing amino acids HCA and CSA had steady state EC_{50} values at AMPA receptors of 430 μM and 3300 μM in oocytes, compared with 477 μM and 3981 μM in neurons, respectively. Moreover, the potency rank order obtained for the ligands at AMPA receptors was similar to that for displacement of [^{3}H]AMPA binding to crude synaptic membranes (34).

As in the case for AMPA receptors, agonist EC_{50} values for NMDA receptors expressed in oocytes were similar to those obtained for hippocampal neurons (15). The EC_{50} values for L-glutamate were identical (2–3 μM) in both studies. Agonist potency rank orders at NMDA receptors for both studies were identical, with the exception of HCA (see below), and match that determined from the displacement by agonists of the binding of the NMDA receptor antagonist D-2-[^{3}H]amino-5-phosphonopentanoic acid to cortical synaptic membranes (35).

Agonist K_D values determined from binding (34, 35) were on average 9-fold (NMDA receptors) or 230-fold (AMPA receptors) lower than EC_{50} values measured in oocytes or neurons (15), using the logistic equation. Microscopic K_D values for NMDA receptors expressed in oocytes, calculated from a model for two independent binding sites (see Ref. 15), yielded values closer to K_D values estimated from binding data, but with the exception of L-glutamate the curve fits were not as good as those of the logistic equation.

The slow perfusion system and large diameter of oocytes precluded measurement of peak responses at AMPA receptors,

which exhibit very rapid desensitization (15, 27). Agonist-induced desensitization results in a shift to the left of the dose-response curve, distorting the EC_{50}. We have verified this with a five-site model of receptor activation (36) that incorporates a desensitized state of the bound receptor. All agonists active at AMPA receptors appeared to desensitize to the same extent, as judged by similar maximum responses (Fig. 2B). The relative potencies of these agonists are, therefore, more accurate indicators of their binding properties than the measured absolute EC_{50} values. In particular, the lack of effect on AMPA receptors of L-aspartate and quinolinate at 10 mM confirms a previous study (15). In addition, we have found that the lack of activity of L-aspartate at AMPA receptors cannot be attributed to receptor desensitization, because L-aspartate does not block kainate-induced currents. This suggests that aspartate and quinolinate are unlikely to act as neurotransmitters at AMPA receptors.

All endogenous agonists tested were full agonists at NMDA receptors, producing 1.2–1.5-fold greater maximal currents than NMDA (Fig. 3). In contrast, under AMPA receptor-selective conditions, all endogenous agonists except L-aspartate and quinolinate evoked steady state currents but with magnitudes only up to 10% of the maximum kainate-induced current (Fig. 2). It is likely that the small amplitude of the steady state maximal currents produced by the activity of endogenous agonists at AMPA receptors can be explained by receptor desensitization that is rapid relative to the perfusion rate of the system. AMPA agonists such as glutamate can prevent (cross-desensitize) responses to kainate in retinal cells (27). In addition, using a faster perfusion system Patneau and Mayer (15) showed that neuronal AMPA receptor currents fade quickly in response to glutamate, CSA, and HCA. Desensitization of non-NMDA receptors is agonist specific, however, because currents evoked by kainate do not fade, for reasons that are as yet unclear. Alternatively, responses measured at steady state may have been due to activation of nondesensitizing AMPA receptors. It is unclear whether both desensitizing and plateau responses are due to action on the same receptor, because results from identified cloned receptors point to multiple AMPA receptors that differ in kainate potency and desensitization (37, 38).

An issue not addressed here or in previous studies of non-NMDA receptors expressed natively in neurons is the molecular identity of the receptors responsible for agonist-induced current. To date, cDNAs encoding five non-NMDA glutamate receptor subunits, in two structural classes that are functional in electrophysiological assays have been isolated (37, 39, 40); several other cDNAs that encode glutamate-binding proteins have also been identified but have not yet been shown to be functional. Subunits in the GluR1-GluR4 class can coassemble with one another to form receptors with different pharmacological profiles. It is probable that responses we recorded in oocytes injected with a mixed population of rat brain mRNAs were from a heterogeneous population of heteromeric and perhaps also homomeric receptors. This is also likely to be the case for whole-cell recordings from intact neurons that express more than one subunit. A substantial contribution of homomeric GluR1 or GluR6 receptors can probably be ruled out in the present study, however, because the kainate EC_{50} values for these homomeric receptors, i.e., 30–40 μM for GluR1 (41) and 1 μM for GluR6 (40), are so different from that measured here (91 μM; Table 1).

HCA and multiple NMDA receptors. The EC_{50} of HCA acting at NMDA receptors agrees well with that calculated for cultured hippocampal neurons (15). However, the Hill coefficients are strikingly different, i.e., 0.6 in oocytes, compared with 1.3 in hippocampal neurons. One possibility for the low Hill slope measured in oocytes is that HCA may activate a heterogeneous population of NMDA receptors that exhibit slightly different affinities for HCA but similar affinities for the other agonists and NMDA. The algebraic sum of HCA dose-response curves for activation of two or more receptors would be shallow. A heterogeneous population of NMDA receptors is plausible, given the multiplicity of AMPA receptors described by recent cloning experiments (37, 39). This suggestion is supported by the observation of different glycine sensitivities for NMDA and HCA responses (Fig. 4) and by previous physiological (21, 42) and binding studies (43, 44).

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