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Characterization of Quisqualate Receptor Desensitization in Cultured Postnatal Rat Hippocampal Neurons

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The quisqualate class of glutamate receptors is thought to play an important role in excitatory synaptic transmission, synaptic plasticity, and neuronal death. Since desensitization is a prominent feature of the responses mediated by this class of receptors, we have characterized the rapidly desensitizing quisqualate response in cultured postnatal rat hippocampal neurons using the whole-cell patch-clamp technique. Quisqualate and its structural analogs elicit a peak current that rapidly decays to a steady-state level. In contrast, currents induced by kainate, NMDA, and their structural analogs exhibit either no decay or a much slower decay. The biophysical and pharmacological properties of the peak and steady-state quisqualate currents indicate that both are mediated by an ionotropic quisqualate receptor.

Quisqualate currents desensitized monoexponentially by ~70% with a time constant near 80 msec. Both the rate and percentage of desensitization showed slight voltage dependence and were concentration dependent, reaching maximal values at saturation. Additionally, the overlap of the dose–response curves for activation of the steady-state current and desensitization of the peak current by a conditioning dose suggests that the two processes are related. Furthermore, desensitizing quisqualate currents were observed when Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and Cl^{-} were removed from the extracellular solution or their concentrations greatly reduced. These results suggest that the decline in the response is not caused by a simple open channel block mechanism.

Despite the lack of desensitization by kainate, our observations are consistent with the hypothesis that quisqualate and kainate act at a single receptor–channel complex. Kainate and quisqualate appeared to interact competitively when applied simultaneously and noncompetitively when quisqualate was applied first. In addition, saturating doses of quisqualate and kainate gave steady-state currents of equal amplitude in neurons treated with the lectin WGA, an inhibitor of quisqualate receptor desensitization.

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Excitatory neurotransmission in the vertebrate CNS is believed to result primarily from the release of glutamate and the subsequent activation of excitatory amino acid receptors (Mayer and Westbrook, 1987; Collingridge and Lester, 1989). In addition to mediating excitatory synaptic transmission, these receptors also are thought to be involved in both synaptic plasticity and neuronal death, processes that are important during normal development and that may be involved in the pathogenesis of some CNS disorders (Choi, 1988; Collingridge and Singer, 1990; McDonald and Johnston, 1990; Meldrum and Garthwaite, 1990). These actions are mediated by three of the major classes of excitatory amino acid receptors that are defined by structural analogs of glutamate: NMDA, kainate, and quisqualate. All three receptors are linked to nonselective cationic channels (Mayer and Westbrook, 1987; Collingridge and Lester, 1989) and activate second messenger systems (Smart, 1989). In the case of quisqualate, two different receptors appear to exist—one linked to an ion channel and another linked to the phosphoinositide second messenger system (Monaghan et al., 1989; Watkins et al., 1990). In this study, we have focused on the ion channel–coupled quisqualate receptor in cultured postnatal rat hippocampal neurons.

Ionotropic quisqualate receptors are thought to mediate fast excitatory synaptic transmission in the vertebrate CNS (Mayer and Westbrook, 1987; Collingridge and Lester, 1989). The postsynaptic response evoked by activation of these receptors is increased during long-term potentiation (LTP) in the hippocampus (Kauer et al., 1988; Muller and Lynch, 1988; Muller et al., 1988; Davies et al., 1989), while it is decreased during long-term depression (LTD) in the cerebellum (Kano and Kato, 1987). These two phenomena are often considered to be physiological correlates of learning and memory (Thompson, 1986). Activation of quisqualate-gated ion channels for prolonged periods can also result in neuronal death *in vitro* and *in vivo* (Choi, 1988; McDonald and Johnston, 1990; Meldrum and Garthwaite, 1990). Thus, the ionotropic quisqualate receptor, in particular, is likely to be involved in both normal and pathological processes.

Recent studies indicate that quisqualate receptors mediate rapidly and profoundly desensitizing responses in neurons from the rodent hippocampus (Kiskin et al., 1986; Trussell et al., 1988; Mayer and Vyklicky, 1989; Tang et al., 1989; Patneau and Mayer, 1990), chick spinal cord (Vlachová et al., 1987; Trussell et al., 1988; Trussell and Fischbach, 1989; Baev et al., 1990), goldfish retina (Ishida and Neyton, 1985), stingray retina (O'Dell and Christensen, 1989a), catfish retina (O'Dell and Christensen, 1989b), rat superior colliculus (Perouansky and Grantyn, 1989), and rat dorsal root ganglion (Huettner, 1990)

as well as rat O-2A glial progenitor cells (Barres et al., 1990). These responses desensitize with time constants as fast as 3 msec and by as much as 95% of their peak amplitude (Tang et al., 1989; Trussell and Fischbach, 1989). Although the physiological role of ligand-gated ion channel desensitization is not clear, it may protect cells from the effects of repeated receptor activation, and it may serve to regulate synaptic efficacy (Ochoa et al., 1989). For example, cerebellar LTD may be a manifestation of quisqualate receptor desensitization (Ito, 1986; Kano and Kato, 1987). Given the ubiquity of rapidly desensitizing quisqualate responses and the potential physiological importance of desensitization, we have characterized the rapidly desensitizing quisqualate current in cultured postnatal rat hippocampal neurons using the whole-cell patch-clamp technique.

Some of these results have been presented previously in a preliminary form (Thio et al., 1988).

Materials and Methods

Postnatal rat hippocampal cell cultures. Hippocampal cells were cultured from 1–3 d postnatal Sprague–Dawley (Washington University Department of Psychiatry strain) rats as described previously (Trussell et al., 1988). Briefly, hippocampal slices were enzymatically digested with 1 mg/ml papain in Leibovitz's L-15 media for 20–30 min. Slices were then mechanically dissociated into single cells by gentle trituration in culture media containing 5% or 10% (v/v) horse serum, 5% or 10% (v/v) fetal calf serum, 17 mM D-glucose, 200 μM or 400 μM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. The cells were plated in culture media on collagen-coated dishes or on a glial feeder layer obtained from platings in which the neurons were permitted to die. The cells were studied after being incubated in a humidified 95% air, 5% CO_2 atmosphere at 37°C for 2–4 hr if plated on a glial feeder layer or for 3–7 d if plated on collagen.

Whole-cell electrophysiology. Voltage-clamp experiments were conducted at room temperature (22–24°C) with an EPC-7 (Adams and List Associates, Ltd., Great Neck, NY) or Axopatch-1D (Axon Instruments, Foster City, CA) amplifier using the whole-cell patch-clamp technique (Hamill et al., 1981). The cells were bathed in an extracellular solution containing (in mM) 140 NaCl, 5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, 0.001 tetrodotoxin, and 10 *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES) (pH 7.3). Any modifications to the extracellular solution are noted in the text. Drugs were dissolved in the solution bathing the cells unless otherwise noted and were applied rapidly with the pressure ejection system described previously (Trussell et al., 1988). This system permitted solution changes at the tip of an electrode located 10–20 μm away from the drug delivery pipette to occur with a time constant of 8.3 ± 1.6 msec ($n = 6$) as measured by a change in junction potential. In whole-cell recordings, the drug delivery pipette was positioned within ~2 μm of the cell soma.

Patch electrodes were pulled from borosilicate glass (World Precision Instruments, Inc., Sarasota, FL) and had resistances of 5–10 MΩ when filled with a solution containing (in mM) 140 CsCl, 4 NaCl, 0.5 $CaCl_2$, 5 ethylene glycol bis(β-aminoethyl)ether-*N,N,N',N'*-tetraacetic acid (EGTA), and 10 HEPES (pH 7.3). In some recordings, 140 CsCl was replaced with 140 Cs acetate, 120 CsCl plus 20 tetraethylammonium (TEA) Cl, 140 K-gluconate, or 70 Cs_2SO_4 plus 70 sucrose. When a lower Ca^{2+} buffering capacity was desired, the pipettes were filled with a solution composed of (in mM) 140 KCl, 4 NaCl, 0.5 EGTA, and 10 HEPES (pH 7.3). Any further modifications of the 140 mM CsCl pipette solution are given in the text, though this solution was used with the composition first listed for most experiments.

Earlier studies showed that neurons cultured for several hours to 7 d had resting membrane potentials of −50 to −60 mV and fired overshooting action potentials. Their input resistances decreased with time in culture ranging from 2250 MΩ after 2–4 hr in culture to 500 MΩ after 7 d in culture. In this study, the neurons were voltage clamped at −50 mV except as indicated. Usually, the series resistance compensation was set at 50% while the cell and pipette capacitance were maximally compensated. Current records were passed through a 1 kHz (−3 dB) 8-pole low-pass Bessel filter (Frequency Devices, Haverhill, MA) before being stored with a Gould model 220 recorder (Gould Electronics, Cleveland, OH) or digitized at 1 or 2 kHz with PCLAMP version 4.0–5.5 (Axon Instruments).

Data analysis. Data were analyzed off line using PCLAMP version 4.0–5.5, ASYSTANT version 1.0–1.1 (Asyst Software Technologies, Inc., Rochester, NY), and SIGMAPLOT version 4.0 (Jandel Scientific, Corte Madera, CA). Results are given as the mean ± standard error of the mean (SEM) (n = number of neurons) and were compared using a two-tailed t test. The Hill coefficient is reported as the slope of the line ± standard deviation obtained by fitting a plot of

$$\log \frac{\%\ \text{maximum response}}{\text{maximum response} - \%\ \text{maximum response}} \text{ versus log [drug]}$$

by linear regression.

Materials. Culture media, sera, and antibiotics were purchased from GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO) except Cs acetate (Aldrich Chemical Company, Inc., Milwaukee, WI) and some excitatory amino acid agonists and antagonists. Quisqualate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), β-*N*-oxalylamino-L-alanine (BOAA), and D-2-amino-5-phosphonovaleric acid (D-APV) were from Cambridge Research Biochemicals, Inc. (Wilmington, DE). α-Amino-3-hydroxy-4-methyl-5-isoxazolepropionic acid (4-methyl-homoibotenic acid), willardiine, 5-bromowillardiine, dihydrokainate, *trans*-(±)-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD), L-homocysteine sulphinic acid (L-HCSA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 3-((±)-2-carboxypiperazin-4-yl)propyl-l-phosphonic acid (CPP) were from Tocris Neuramin Ltd. (Essex, England).

Results

Quisqualate and its congeners evoke a rapidly decaying current

Similar to previous studies (Kiskin et al., 1986; Trussell et al., 1988; Mayer and Vyklicky, 1989; Tang et al., 1989; Patneau and Mayer, 1990), glutamate and some non-NMDA but no NMDA agonists elicited rapidly decaying currents in hippocampal neurons. The non-NMDA agonists that activated such currents included quisqualate (10–1000 μM), AMPA (1 mM), 4-methyl-homoibotenic acid (1 mM), BOAA (1 mM), willardiine (0.5–1 mM), and 5-bromowillardiine (1 mM) (Figs. 1*A*, 2*A*). All these agonists either appear to act at quisqualate receptors or are structurally similar to quisqualate (Watkins et al., 1990). Non-NMDA agonists that activated nondesensitizing currents included kainate (0.05–10 mM) as well as two structural relatives of kainate, domoate (0.1–1 mM) and dihydrokainate (1 mM) (Fig. 1*B*). *Trans*-ACPD, an agonist selective for the metabotropic quisqualate receptor (Watkins et al., 1990), did not elicit a current over the voltage range from −80 to +20 mV using the KCl pipette solution with a low Ca^{2+} buffering capacity. Although currents elicited by NMDA decayed during a sustained application, they decayed along a much slower time course than currents produced by glutamate or quisqualate (Clark et al., 1990). Other NMDA agonists include L-aspartate (1 mM), ibotenate (1 mM), D-homocysteic acid (1 mM), L-homocysteic acid (1 mM), L-HCSA (1 mM), and *threo*-β-hydroxyaspartate (1 mM) (Watkins et al., 1990), all of which did not evoke a rapidly decaying current (Fig. 1*C*).

Current–voltage relationship for the quisqualate current

The peak quisqualate current had a reversal potential of −0.6 ± 0.8 mV ($n = 18$) as determined from current–voltage (I/V) plots generated by voltage-clamping neurons at various potentials and applying 100 μM quisqualate ("steady-state I/V") (Fig. 2*A,B*). The steady-state current had a reversal potential of −1.3 ± 1.2 mV ($n = 18$) when measured from a steady-state I/V (Fig. 2*A,C*) and a reversal potential of +4.9 ± 0.9 mV ($n = 12$) when calculated from I/V plots obtained by subjecting a neuron to a

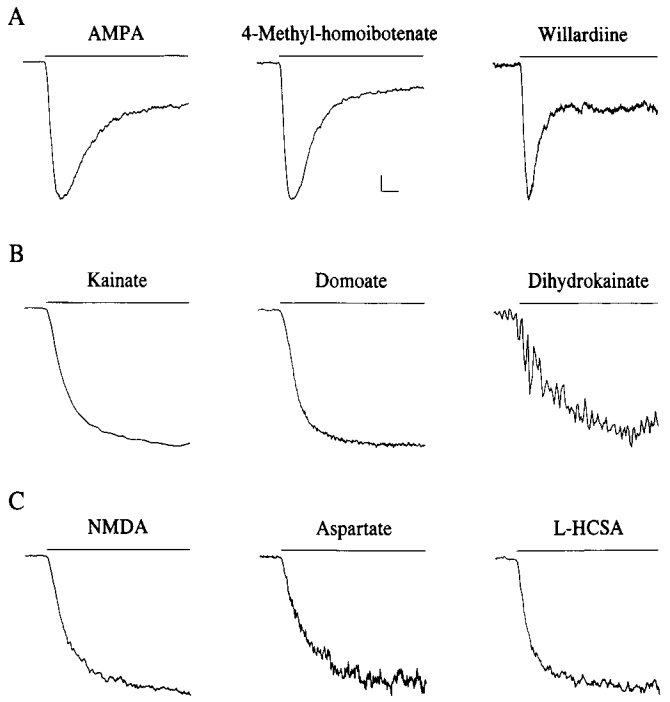

Figure 1. Excitatory amino acids related to quisqualate (*A*) elicited rapidly decaying currents while those related to kainate (*B*) and NMDA (*C*) did not: responses of neurons at a holding potential of −50 mV to 1 mM of each agonist. *Bars* above traces in all figures denote period of drug application. Vertical calibration: 130 pA (AMPA), 56 pA (4-methyl-homoibotenic acid), 33 pA (willardiine), 100 pA (kainate), 50 pA (domoate), 26 pA (dihydrokainate), 70 pA (NMDA), 26 pA (L-aspartate), 50 pA (L-HCSA). Horizontal calibration; 60 msec.

voltage step protocol during a sustained application of 100 μM quisqualate ("instantaneous *I/V*") (Fig. 2*C*). Although these experiments were performed using pipette solutions containing 145 mM Cl^-, decreasing the Cl^- concentration did not significantly alter the reversal potential of either component. When the concentration of chloride ions in the pipette was reduced from 145 to 5 mM, the reversal potential for the peak current was +2.3 ± 1.0 mV ($n = 9$) and that for the steady-state current was −2.0 ± 2.2 mV ($n = 9$). These results indicate that both components of the quisqualate response are mediated by a nonselective cationic conductance and that the decay of the current does not result from a redistribution of ions.

The decay in the quisqualate current at positive holding potentials indicates that the decay at negative holding potentials does not reflect the activation of an inward current followed by a more slowly activating outward current. The outward current would be mediated by K^+ or Cs^+ under the ionic conditions used. However, if the decay in the quisqualate response at negative holding potentials were caused by the sequential activation of an inward and an outward current, then no decay would be apparent at positive holding potentials as both currents would be outward.

The peak quisqualate current had a linear *I/V* relationship (Fig. 2*B*), while the linearity of the *I/V* relationship for the steady-state current was dependent on the protocol employed. The instantaneous *I/V* relationship for the steady-state current was nearly linear, whereas the steady-state *I/V* relationship was not (Fig. 2*C*). This observation was quantified by calculating the ratio of the absolute value of the steady-state current magnitude at +50 mV to that at −90 mV. This ratio was 1.0 ± 0.1 ($n = 15$) in the steady-state *I/V* curves. In contrast, it was 0.5 ± 0.1 ($n = 9$; two-tailed *t* test, $p < 0.01$) in the instantaneous *I/V* relationship as would be expected for a nearly linear relationship with a reversal potential near 0 mV. The nonlinearity of the steady-state *I/V* curve cannot be ascribed to the activation of NMDA receptors since similar results were obtained in the presence of 0.5 mM D,L-APV, and D-APV did not affect the steady-state current (see below). Furthermore, the *I/V* curve for NMDA currents generated by a voltage step protocol is nonlinear in the presence of extracellular Mg^{2+} in mouse spinal cord neurons because of the rapidity of the Mg^{2+} block (Mayer and Westbrook, 1985).

Concentration dependence of the quisqualate current

Both the peak and steady-state currents were evoked by quisqualate in a concentration-dependent manner (Fig. 3*A,B*). The dose–response curves were generated by normalizing the amplitudes of the peak and steady-state currents evoked by a test concentration of quisqualate to those elicited by 100 μM in a single neuron. Postnatal rat hippocampal neurons began to show a clear albeit nondecaying response to concentrations near 100 nM. A decaying current was elicited at ~10 μM, which was close to saturation for the steady-state current. The dose–response curve for the peak current, in contrast, saturated at ~300 μM. Half-maximal activation for the peak ($ED_{50(peak)}$) and steady-state ($ED_{50(ss)}$) currents occurred at 40 and 3 μM, respectively. Hill plots gave a Hill coefficient of 0.9 ± 0.04 for the peak current and 0.8 ± 0.1 for the steady-state current (Fig. 3*C*).

Activation of a non-NMDA receptor–ion channel complex produces the quisqualate current

The ability of non-NMDA but not NMDA agonists to evoke a rapidly decaying current strongly suggests that a non-NMDA rather than an NMDA receptor–channel complex is involved. Accordingly, the nonspecific excitatory amino acid antagonist (Collingridge and Lester, 1989) kynurenic acid at 1 and 10 mM reversibly reduced the peak current evoked by 100 μM quisqualate by 31 ± 6% ($n = 4$) and 69 ± 6% ($n = 5$). Neither concentration of kynurenic acid was effective at antagonizing the steady-state current produced by 100 μM quisqualate. Furthermore, 10 μM CNQX, a competitive non-NMDA antagonist (Collingridge and Lester, 1989), reversibly inhibited the peak current elicited by 100 μM quisqualate by 46 ± 5% ($n = 11$) without affecting the steady-state current (Fig. 4*A*). The steady-state current evoked by 10 μM quisqualate, however, was reversibly blocked by 53 ± 6% ($n = 19$) by 10 μM CNQX (Fig. 4*B*). Dose–response curves for the peak and steady-state currents in the presence of 10 μM CNQX were shifted to the right with no change in the maximum response as expected of a competitive antagonist (Fig. 4*C,D*). The $ED_{50(peak,CNQX)}$ and $ED_{50(ss,CNQX)}$ in the presence of 10 μM CNQX were 200 and 20 μM, respectively. The competitive NMDA antagonists D-APV and CPP did not inhibit either component of the response produced by 100 μM quisqualate (Fig. 4*E*). The peak and steady-state currents were 100 ± 6% ($n = 7$) and 100 ± 4% ($n = 7$), respectively, of control in the presence of 1 mM D-APV. The corresponding values in the presence of 1 mM CPP were 110 ± 4% ($n = 7$) and 100 ± 3% ($n = 7$). The steady-state current elicited by 10 μM quisqualate also was equal to control in the presence of 1 mM D-APV ($n = 11$).

These results together indicate that the rapidly decaying quis-

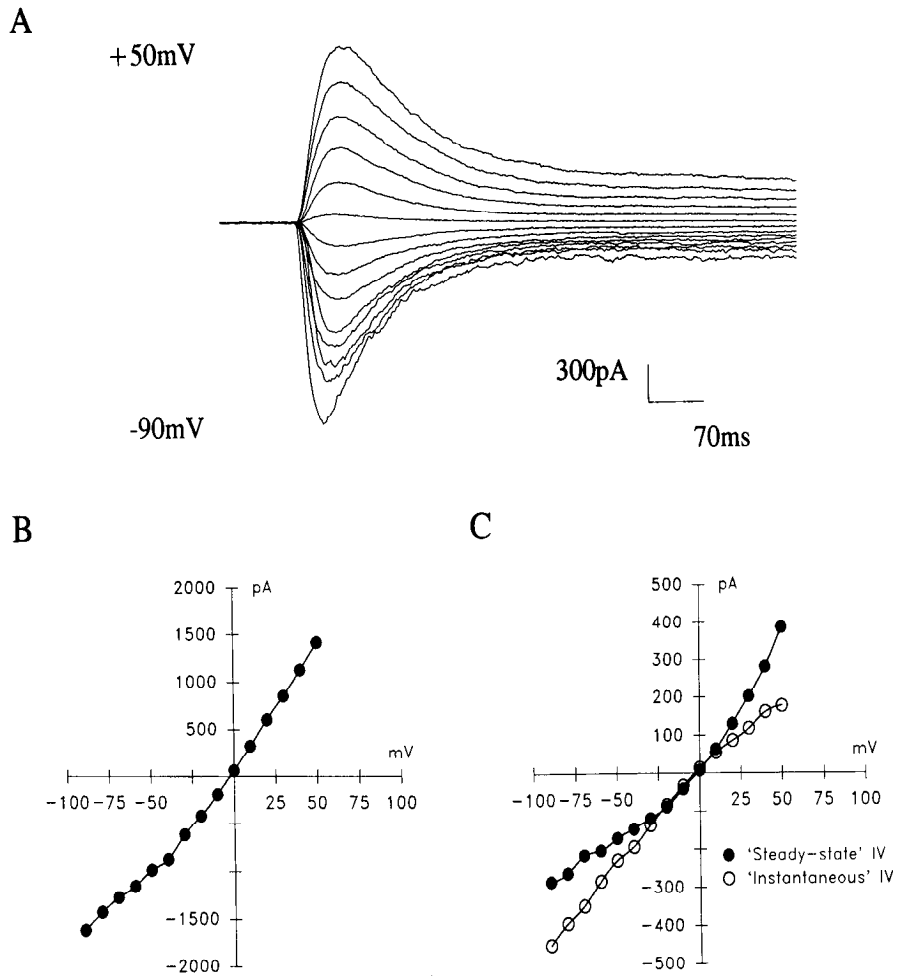

Figure 2. *I/V* relationship for the peak and steady-state quisqualate currents. *A*, Responses of a neuron to 500 msec applications of 100 μM quisqualate at voltages between −90 and +50 mV in 10 mV increments. The neuron was bathed in an extracellular solution supplemented with 200 μM $CdCl_2$ and 500 μM D,L-APV. The −80 mV trace was omitted for clarity. *B*, *I/V* relationship for the peak quisqualate current. The peak current amplitudes for the traces in *A* were plotted against voltage. *C*, "Steady-state" (*solid circles*) and "instantaneous" (*open circles*) *I/V* relationships for the steady-state current. The "steady-state *I/V*" relationship is a plot of the steady-state current amplitude versus holding potential using the data in *A*. The data for the "instantaneous *I/V*" were obtained by subjecting the neuron in *A* to a series of voltage steps before and during the steady-state current produced by a prolonged application of 100 μM quisqualate. The steps were 60 msec in duration and altered the potential from −90 to +50 mV in 10 mV increments. The steady-state current magnitude at each voltage during the voltage step protocol was determined after subtracting the control records from the records taken during the quisqualate application.

qualate current results from desensitization rather than an inadequate spatial voltage clamp, a redistribution of ions, or the sum of two opposing currents. The decline in the current does not result from an inadequate spatial voltage clamp because the decline was evident in the presence of tetrodotoxin, cesium, TEA, and cadmium. In addition, the decay was present in acutely dissociated neurons, defined as neurons cultured for 2–4 hr. Such neurons have input resistances near 2 GΩ and charging curves well described by a single exponential. The decay in the response also is not caused by enzymatic modification of the receptor–channel complex, as the response in cells dissociated nonenzymatically by mechanical trituration alone exhibited a rapidly decaying quisqualate response.

Characteristics of quisqualate receptor desensitization

When quisqualate currents desensitized, they did so along a monoexponential time course at all voltages and concentrations tested (Fig. 5*A*). The time constant of desensitization (τ_d) increased slightly with depolarization and was longer for 10–20 μM quisqualate than for concentrations above 25 μM (Fig. 5*B*,*C*). The *percent desensitization*, calculated by

$$\% \text{ desensitization} = \frac{\text{peak current} - \text{steady-state current}}{\text{peak current} - \text{baseline current}},$$

was dependent on quisqualate concentration and was slightly dependent on voltage (Fig. 5*D*). The percent desensitization reached maximal values at saturating concentrations for the peak current.

The proportion of receptors desensitized by a given concentration was estimated by measuring the extent to which a conditioning dose reduced the peak current evoked by 100 μM. Conditioning doses of ≥ 10 nM reduced the peak current induced by 100 μM quisqualate in a concentration-dependent manner (Fig. 6). The decrease in the peak current was augmented as the conditioning dose increased, with a conditioning dose of 10 μM producing a nearly maximal effect and 1 μM giving a half-maximal effect ($ED_{50(\text{ss desensitization})}$) (Fig. 6*A*,*B*). Thus, the percentage of receptors desensitized by a given concentration of quisqualate correlates with its ability to activate the steady-state current, meaning that the dose–response curves for the steady-state current and the desensitization induced by a conditioning dose overlap. Both curves have a similar ED_{50}, and both rise and saturate at comparable concentrations. However, it should be noted that conditioning doses of 10 and 50 nM desensitized ~10% of the receptors in some neurons showing no detectable macroscopic response to these concentrations (Fig. 6*C*).

Ionic requirements of quisqualate receptor desensitization

Quisqualate receptor desensitization did not require the presence of extracellular Ca^{2+}, Mg^{2+}, Na^+, or K^+ (Fig. 7). In the nominal absence of both Ca^{2+} and Mg^{2+}, τ_d and the % desen-

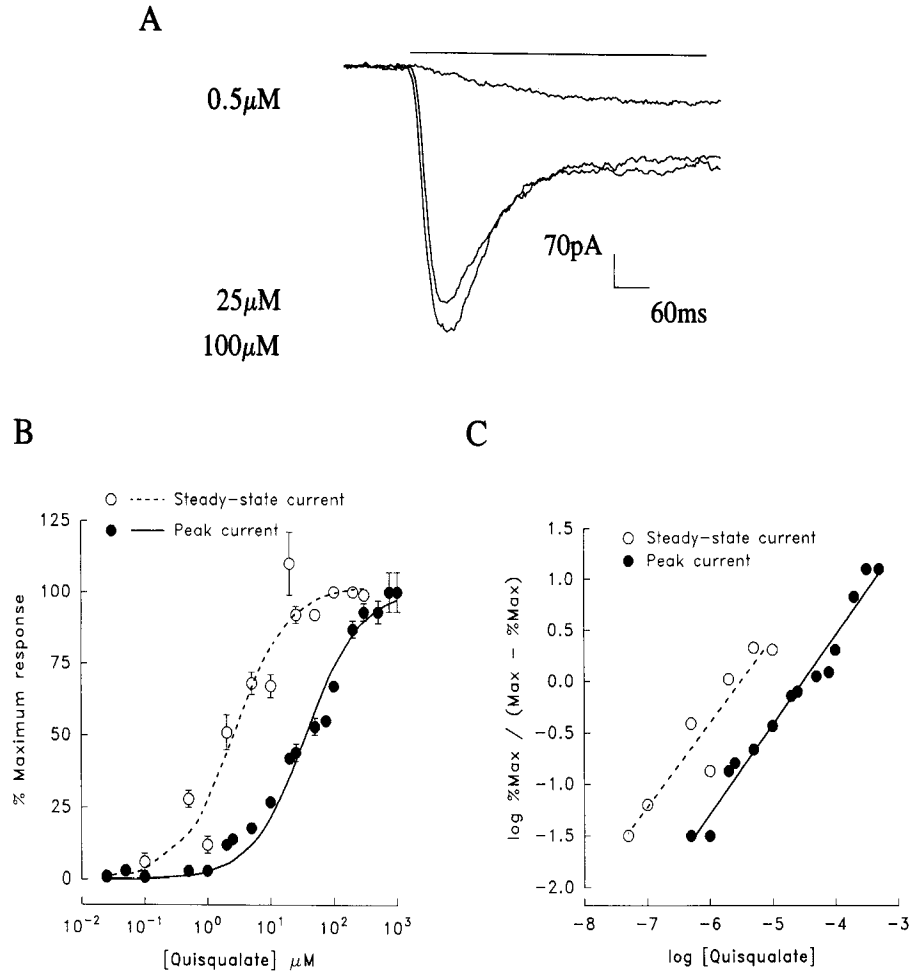

Figure 3. Concentration dependence of the peak and steady-state quisqualate currents. *A,* Responses of a neuron voltage clamped at −50 mV to 0.5, 25, and 100 μM quisqualate. *B,* Dose–response curves for the peak and steady-state quisqualate currents. Data points were obtained by normalizing the amplitude of the current elicited by a test quisqualate concentration to that elicited by 100 μM quisqualate in a single neuron at a holding potential of −50 mV. The raw data for the peak (*solid circles*) and steady-state (*open circles*) currents were visually fit to the curves shown. The visual fit for the peak current (*solid curve*) gave an $ED_{50(peak)}$ of 40 μM, while the visual fit for the steady-state current (*broken curve*) gave an $ED_{50(ss)}$ of 3 μM. *Error bars* depict ±SEM, and those not shown are smaller than the symbol (n = 5–29 for each point). *C,* Hill plots for peak and steady-state quisqualate currents obtained using the mean percent maximum response values in *B.* The raw data for the peak (*solid circles*) and steady-state (*open circles*) were fit to lines (*solid line* for peak, *broken line* for steady-state) having respective slopes of 0.9 ± 0.04 and 0.8 ± 0.1, respectively.

sitization were similar to those found in the presence of both cations when examined in the same neuron (Fig. 7*A,* Table 1). Furthermore, the dependence of these two parameters on voltage and quisqualate concentration was similar in the presence and nominal absence of Mg^{2+}, confirming that an NMDA conductance was not contributing significantly to the quisqualate current (Fig. 7*D,E*). The voltage and concentration dependence of both parameters in the nominal absence of K^+ in the extracellular solution and Cs^+ in the pipette solution was also similar to those observed under control conditions (Fig. 7*B,F,G*). In addition, a rapidly desensitizing quisqualate receptor–mediated current qualitatively similar to control currents was observed when (1) the extracellular solution contained no added Ca^{2+} and 10 mM Mg^{2+}, (2) the extracellular solution contained 10 mM Ca^{2+} and no added Mg^{2+}, (3) the only monovalent cation in the pipette and extracellular solutions was Cs^+ (Fig. 7*C*), (4) the $[Cl^-]$ in the extracellular and pipette solutions was reduced to 5 mM, and (5) the buffer in the pipette and extracellular solutions was 3-[*N*-morpholino]propanesulfonic acid (MOPS) or NaH_2PO_4 instead of HEPES.

Table 1. Effect of Ca^{2+} and Mg^{2+} on quisqualate receptor desensitization

$[Ca^{2+}]$ (mM)	$[Mg^{2+}]$ (mM)	Percent desensitization (mean ± SEM)	τ_d (msec; mean ± SEM)
0	0	67 ± 3 (5)	87 ± 4 (5)
3	0	64 ± 6 (5)	80 ± 11 (5)
3	1	67 ± 4 (5)	81 ± 10 (5)

Neurons voltage clamped at −50 mV were bathed in an extracellular solution containing no added Ca^{2+} and Mg^{2+} and were exposed to 100 μM quisqualate in the presence of different concentrations of Ca^{2+} and Mg^{2+}. The extracellular solution also contained 200 μM D,L-APV to prevent neuronal swelling that normally occurs in the absence of divalent cations (L. L. Thio, D. B. Clifford, and C. F. Zorumski, unpublished observations).

Interaction between non-NMDA analogs

Non-NMDA analogs that elicited a rapidly desensitizing current interacted with each other and with non-NMDA analogs that elicited a nondesensitizing current. The non-NMDA analogs that evoked a desensitizing current cross-desensitized the response to quisqualate. Specifically, an application of 100 μM quisqualate during the steady-state current induced by glutamate (1 mM), AMPA (1 mM), BOAA (1 mM), 4-methyl-homoibotenic acid (1 mM), willardiine (1 mM), and 5-bromowillardiine (1 mM) produced a much smaller peak current than a control application (Fig. 8*A*).

A similar relationship was observed between quisqualate and kainate. When kainate was applied during the steady-state current evoked by 100 μM quisqualate, the total current induced by quisqualate plus kainate response was reduced compared to kainate alone (Fig. 8*B*). The kainate dose–response curve ob-

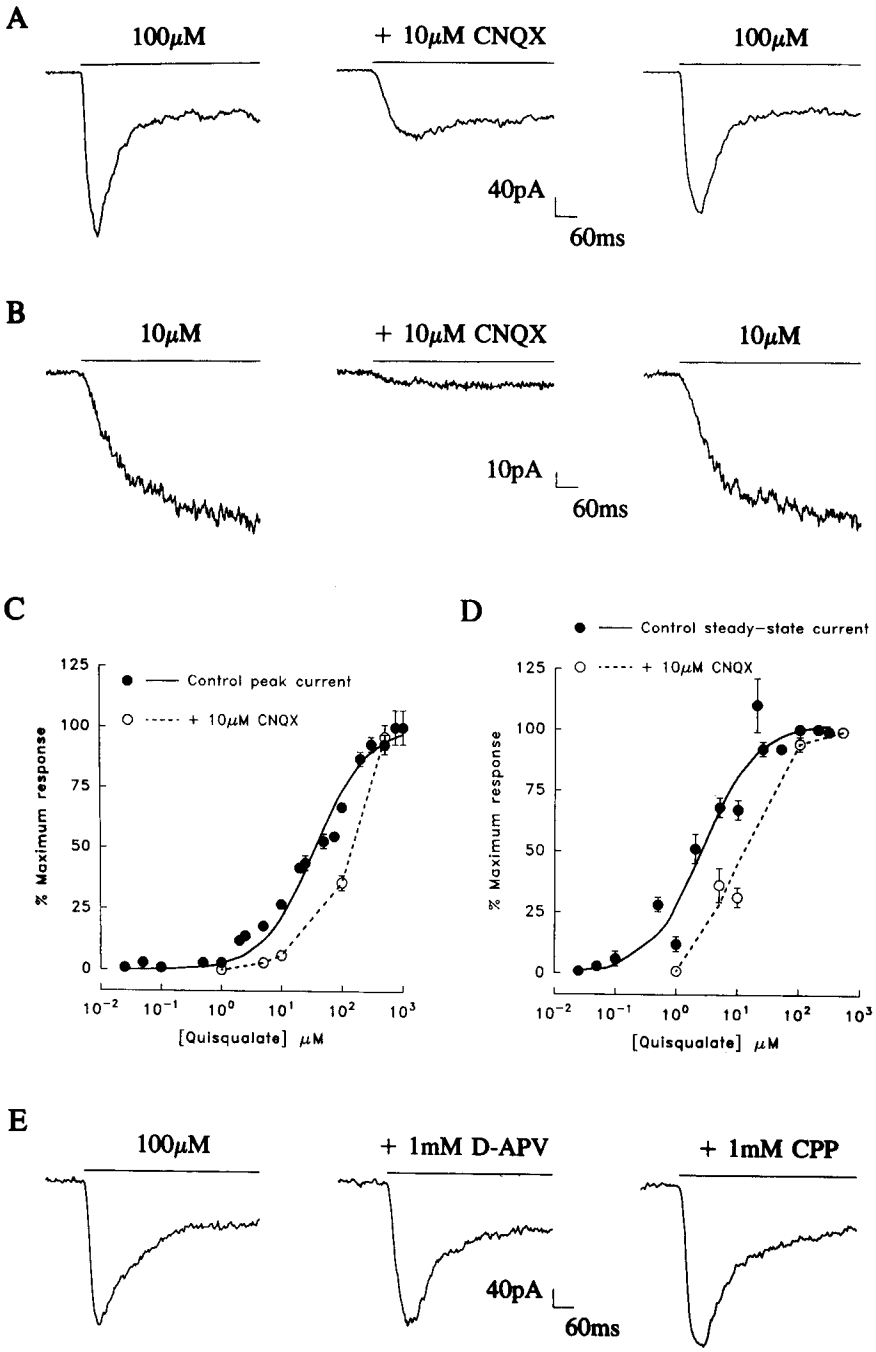

Figure 4. Sensitivity of the peak and steady-state quisqualate currents to excitatory amino acid antagonists. *A*, CNQX (10 μM) reversibly blocked the peak current induced by 100 μM quisqualate. Traces show successive responses of a neuron at a holding potential of −50 mV to 100 μM quisqualate (*left*), to 100 μM quisqualate plus 10 μM CNQX (*middle*), and to 100 μM quisqualate (*right*). *B*, CNQX (10 μM) reversibly inhibited the steady-state current evoked by 10 μM quisqualate. The protocol is analogous to that in *A*. *C* and *D*, CNQX (10 μM) shifts the dose–response curves for the peak (*C*) and steady-state (*D*) quisqualate currents to the right without altering the maximum response. Data points were obtained by expressing the amplitude of the current induced by a given concentration of quisqualate plus 10 μM CNQX as a percentage of the current induced by quisqualate alone at −50 mV. Then, the corresponding point on the control (*solid circles*) was multiplied by this value. The raw data in the presence of 10 μM CNQX (*open circles*) were then visually fit to the curves shown (*broken lines*), which gave an $ED_{50(peak,CNQX)}$ of 200 μM and an $ED_{50(ss,CNQX)}$ of 20 μM. *Error bars* represent ±SEM, and those not shown are smaller than the symbol (n = 5–19 for each point). The control data were taken from Figure 3*B*. *E*, D-APV and CPP did not antagonize the quisqualate response. Traces show responses of a neuron voltage clamped at −50 mV to 100 μM quisqualate (*left*), 100 μM quisqualate + 1 mM D-APV (*middle*), and 100 μM quisqualate + 1 mM CPP (*right*).

tained while 100 μM quisqualate induced a steady-state current revealed an interaction between the two agonists that was not competitive (Fig. 8*C*). By itself, kainate activated a nondesensitizing current in a concentration-dependent manner with an $ED_{50(kainate)}$ of 300 μM and a Hill coefficient of 1.2 ± 0.1. When the kainate dose–response curve was obtained while 100 μM quisqualate elicited a steady-state current, the maximum response attainable was reduced and the $ED_{50(kainate,pre)}$ was 300 μM. In contrast, applying kainate and 10 μM quisqualate simultaneously unveiled an apparently competitive interaction between the two agonists (Fig. 8*D,E*). The dose–response curve for kainate acquired in this manner was shifted to the right with no change in the maximum response. In this case, the $ED_{50(kainate,co)}$ was 1 mM.

The reduction in the maximum kainate response in the presence of 100 μM quisqualate may reflect the desensitization of at least some kainate receptors by quisqualate. This possibility was further examined in neurons bathed in 580 nM WGA, a lectin that blocks quisqualate receptor desensitization (Zorumski et al., 1990). In these neurons, the steady-state response to 1 mM kainate was 110 ± 9% (n = 7) of the steady-state response to 100 μM quisqualate compared to 1400 ± 240% (n = 7) in control neurons (Fig. 8*E,F*). Furthermore, the response to an application of 1 mM kainate plus 100 μM quisqualate was 110 ± 8% (n = 7) of the response to 100 μM quisqualate alone and 110 ± 6% (n = 7) of the response to 1 mM kainate alone (Fig. 8*F*). Consistent with these results, applying 1 mM kainate during the steady-state current to 100 μM quisqualate produced a total

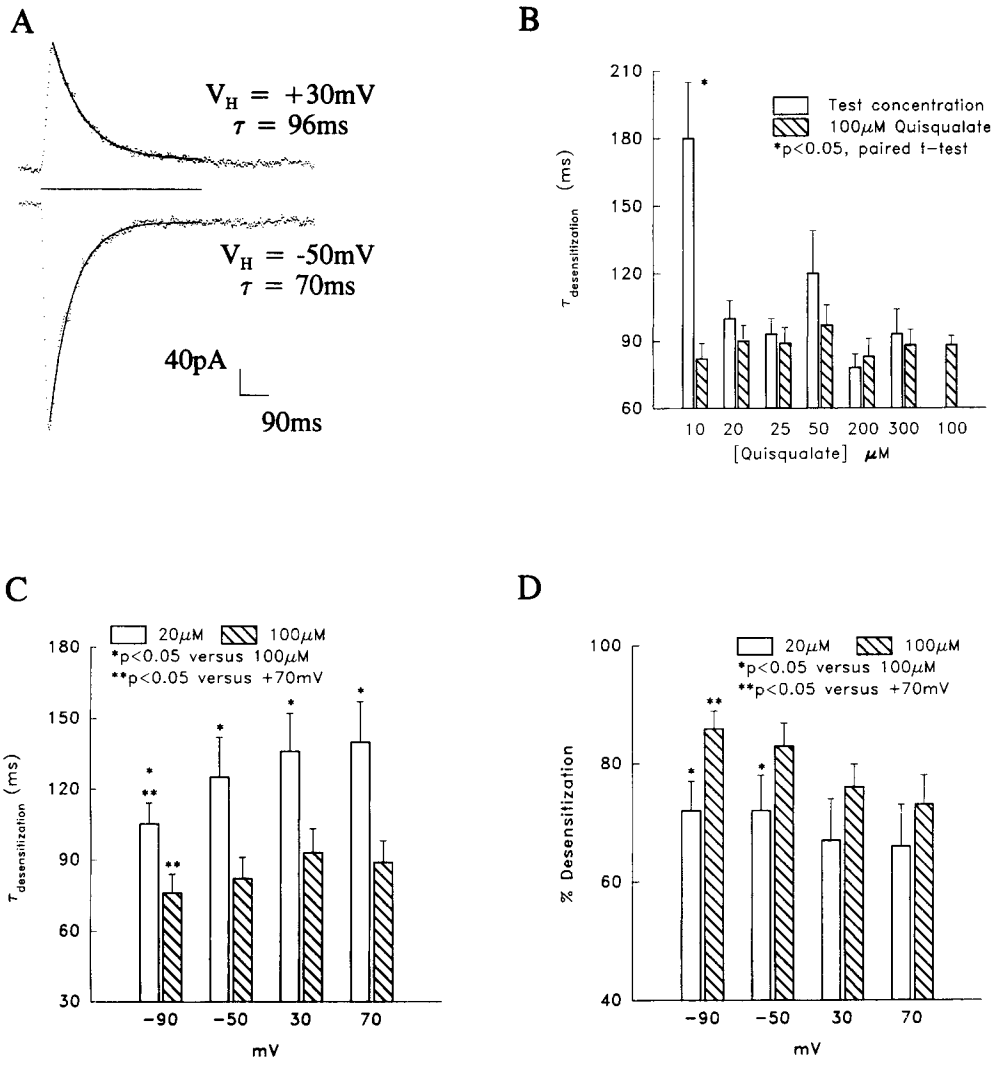

Figure 5. Concentration and voltage dependence of quisqualate receptor desensitization. *A*, Response of a neuron at holding potentials of +30 mV (*top*) and −50 mV (*bottom*) to 100 μM quisqualate. Decays of raw data (*dotted traces*) were fit to single exponential functions (*solid traces*) with time constants of 96 msec at +30 mV and 70 msec at −50 mV. *B*, Dependence of τ_d on quisqualate concentration. τ_d for 100 μM quisqualate (*hatched bars*) was compared to τ_d for a test quisqualate concentration (*open bars*) in a single neuron voltage clamped at −50 mV. τ_d for each test concentration was compared to that for 100 μM quisqualate in the same set of neurons using a two-tailed paired *t* test. Only the τ_d for 10 μM was significantly different from that of 100 μM ($p < 0.05$). The *bar* at the *far right* shows the τ_d for 100 μM in a series of 37 neurons. *C* and *D*, Dependence of τ_d (*C*) and percent desensitization (*D*) on quisqualate concentration and voltage. τ_d and the % desensitization for 20 μM quisqualate (*open bars*) were compared to τ_d and the percent desensitization for 100 μM quisqualate (*hatched bars*) in five neurons at the potentials indicated. *, Paired two-tailed *t* test compared to 100 μM quisqualate at the same potential, $p < 0.05$. **, Paired two-tailed *t* test compared to the same concentration of quisqualate at +70 mV, $p < 0.05$. *Error bars* in *B–D* represent ±SEM.

current that was 94 ± 8% (n = 7) of the current to 100 μM quisqualate alone and 96 ± 9% (n = 7) of the current to 1 mM kainate alone.

Discussion

An ionotropic quisqualate receptor mediates the rapidly desensitizing quisqualate response

Quisqualate and glutamate evoked a rapidly desensitizing current that decayed to a steady-state level in cultured postnatal rat hippocampal neurons. Both the peak and the steady-state currents were activated by quisqualate in a concentration-dependent manner, and both had a linear *I/V* relationship in the presence of extracellular Mg^{2+}. For the steady-state current, a linear *I/V* relationship was found in the presence of extracellular Mg^{2+} when determined using the "instantaneous" but not the "steady-state" method. The reason for the nonlinear "steady-state *I/V*" is unknown but does not reflect a contribution by NMDA receptors. The near 0 mV reversal potential for both currents suggests that they are mediated by a nonselective cationic conductance. Accordingly, quisqualate elicited an inward peak and steady-state current at negative holding potentials when both extracellular and intracellular K^+ (Cs^+) was replaced with Na^+ or vice versa. Both currents were inhibited by CNQX, a competitive non-NMDA antagonist, but not by D-APV and CPP, competitive NMDA antagonists. In addition, other non-NMDA analogs such as AMPA, BOAA, 4-methyl-homoibotenic acid, willardiine, and 5-bromowillardiine, which are all structurally related to quisqualate, induced similar responses capable of cross-desensitizing quisqualate responses. Of all the quisqualate analogs tested, only *trans*-ACPD, which activates the metabotropic quisqualate receptor, did not activate a current under the conditions used in this study. These results suggest that both the peak and steady-state currents are mediated by a nonselective cationic, ionotropic quisqualate receptor.

The characteristics of the quisqualate response in cultured hippocampal neurons from 1–3 d postnatal rats are most similar to those from acutely dissociated hippocampal neurons from 2–3-week-old rats (Kiskin et al., 1986, 1990). The $ED_{50(peak)}$, Hill coefficient for the peak current, τ_d, and $ED_{50(ss\ desensitization)}$ in these two preparations are comparable. Although an $ED_{50(ss)}$ was not reported in these studies, the $ED_{50(ss)}$ is 10-fold lower than the $ED_{50(peak)}$ in embryonic hippocampal neurons (Tang et al., 1989; Patneau and Mayer, 1990). Another preparation exhibiting a rapidly decaying quisqualate response that shares many characteristics with the response described here is retinal horizontal cells (O'Dell and Christensen, 1989a,b). The lower Hill coeffi-

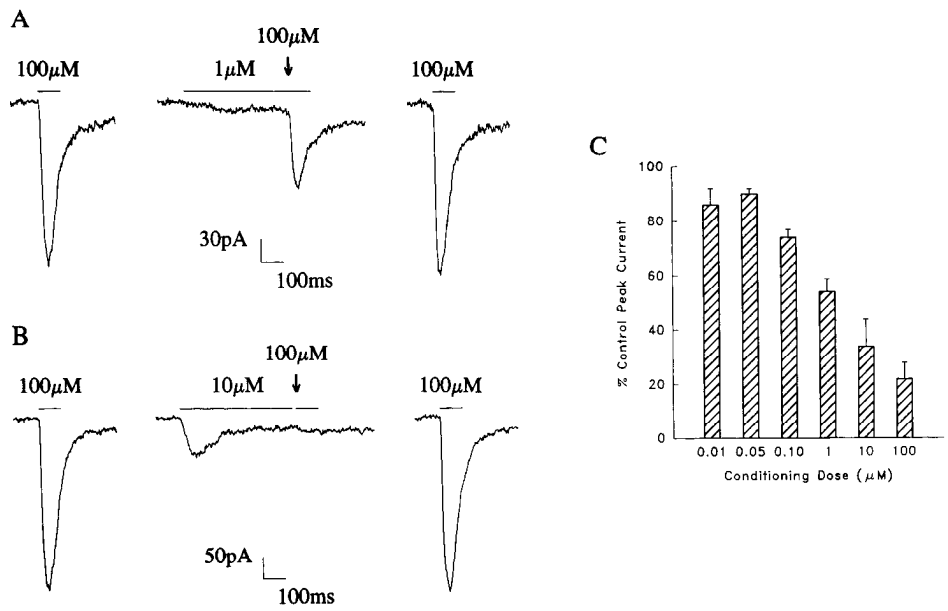

Figure 6. Desensitization of quisqualate receptors by conditioning doses of quisqualate. *A* and *B*, Response of a neuron at a holding potential of −50 mV to an application of 100 μM quisqualate (*left*), application of a conditioning dose of 1 μM (*A*) or 10 μM (*B*) quisqualate followed by a challenge to 100 μM quisqualate beginning at *arrow* (*middle*), and recovery of 100 μM quisqualate response (*right*). *C*, Effect of conditioning doses of quisqualate on the peak current evoked by 100 μM quisqualate at −50 mV. The percent control peak current was determined by applying a 100 msec test pulse of 100 μM quisqualate. After the neuron recovered, a conditioning dose was applied for 500 msec, and 25 msec later another 100 msec test pulse was applied. Although 25 msec elapsed between the end of the conditioning pulse and the test pulse, the conditioning dose should not have dissipated by much since these experiments were performed in a static bath. The peak current of the test pulse after the conditioning pulse as measured from the original baseline was then expressed as a percentage of the control peak current. Because the steady-state current induced by the conditioning dose was included in the measurement, the percent control peak current was not zero when the conditioning dose was 100 μM. However, in all cells examined, no additional current was evoked by the test pulse when a 100 μM conditioning dose was used. *Error bars* show ±SEM (n = 5–6 for each conditioning dose).

cient is the most conspicuous discrepancy between our results and some of the other studies. The Hill coefficient is ≥1.5 for the peak current in horizontal cells and is ≥1.5 for the steady-state current in embryonic mouse hippocampal neurons. The reason for these differences is unclear, but the two preparations may have different receptor–channel complexes since they are derived from different species and developmental stages. Interestingly, the quisqualate response in chick spinal cord neurons changes during embryogenesis (Baev et al., 1990).

A channel block mechanism does not cause quisqualate receptor desensitization

The decay in the quisqualate current may be caused by an ion or other substance blocking the pore of the quisqualate channel. However, a critical role for extracellular Na^+, K^+, Ca^{2+}, or Mg^{2+} in producing the decay is unlikely since the decay of the response in the presence of these ions was similar to that in their nominal absence. Another ion that remains a candidate as a blocker of quisqualate-gated channels is H^+, though pH changes in the range of 6.3–8.3 have no effect on quisqualate responses in embryonic hippocampal neurons (Tang et al., 1990; Vyklický et al., 1990).

Other potential blockers include quisqualate and glutamate (Vlachová et al., 1987), which, like ACh and some of its analogs (Adams and Sakmann, 1978; Trautmann, 1982; Sine and Steinbach, 1984), may block the channels they gate. While agonist blockade of nicotinic ACh receptor (nAChR) channels is strongly influenced by voltage, the weak voltage dependence of quisqualate receptor desensitization does not dismiss this model since the block of nAChR channels by some barbiturates is not voltage dependent (Adams, 1976). In fact, the marked decay observed at negative holding potentials would suggest a weakly voltage dependent block since quisqualate and glutamate have a net negative charge at physiological pH (Boden et al., 1986). If the decay reflects blockade of the channel by quisqualate or glutamate, then these molecules may bind to the vestibule of the channel, or a neutral portion of the molecule, such as the zwitterionic part, may be responsible for the block (Ishida and Neyton, 1985).

While the decay of the quisqualate current may reflect the blockade of the channel by quisqualate itself, several observations are inconsistent with quisqualate acting as a simple open channel blocker according to the model

$$Q + R \leftrightharpoons QR \leftrightharpoons QR_o + Q \leftrightharpoons QR_oQ,$$

where Q represents quisqualate, R represents the quisqualate receptor–channel complex, QR represents the bound but closed receptor–channel complex, QR_o represents the open receptor–channel complex, and QR_oQ represents the open but blocked receptor–channel complex. This model predicts that τ_d would decrease linearly with increasing quisqualate concentration. It also predicts that magnitude of the steady-state current would decrease with increasing quisqualate concentration assuming the steady-state current is produced by unblocked channels alone. However, both τ_d and the steady-state current remained constant at concentrations above 20 μM. Finally, although the mean burst duration of the channel would be expected to increase with increasing quisqualate concentration according to this model

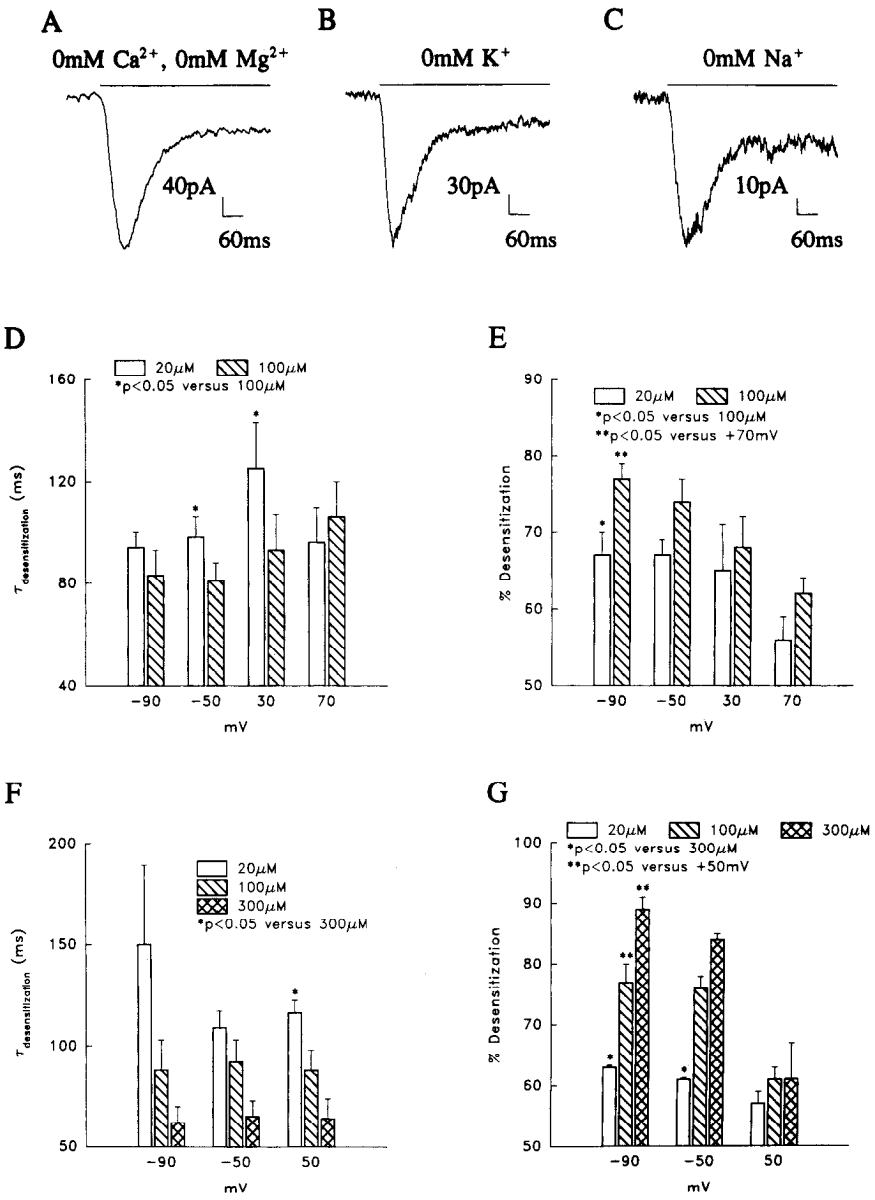

Figure 7. Quisqualate receptor desensitization did not require extracellular Ca^{2+}, Mg^{2+}, K^+, or Na^+. *A*, Response induced by 100 μM quisqualate in a neuron bathed in an extracellular solution containing no added Ca^{2+} or Mg^{2+}; 200 μM D,L-APV was added to prevent neuronal swelling. *B*, Response evoked by 100 μM quisqualate in a neuron bathed in an extracellular solution containing no added K^+. Both the KCl in the extracellular solution and the CsCl in the pipette solution were replaced with an equimolar concentration of NaCl ($[NaCl]_{out} = [NaCl]_{in} = 145$ mM). *C*, Response elicited by 100 μM quisqualate in a neuron bathed in an extracellular solution containing no added Na^+. The NaCl in the extracellular and pipette solutions was substituted with CsCl ($[CsCl]_{out} = [CsCl]_{in} = 145$ mM). Records in *A–C* were taken from neurons voltage clamped at −50 mV. *D* and *E*, Dependence of τ_d (*D*) and percent desensitization (*E*) on quisqualate concentration and voltage in the absence of Mg^{2+}. τ_d and the percent desensitization for 20 μM (*open bars*) and 100 μM (*hatched bars*) quisqualate were measured in three neurons at the indicated potentials. Neurons were bathed in an extracellular solution containing 3 mM $CaCl_2$ and no added Mg^{2+}. *, Paired two-tailed *t* test compared to 100 μM quisqualate at the same voltage, $p < 0.05$. **, Paired two-tailed *t* test compared to the same quisqualate concentration at +70 mV, $p < 0.05$. *F* and *G*, Dependence of τ_d (*F*) and the percent desensitization (*G*) on quisqualate concentration and voltage when Na^+ is the only monovalent cation present. τ_d and the percent desensitization for 20 μM (*open bars*), 100 μM (*hatched bars*), and 300 μM (*crosshatched bars*) quisqualate were measured in three neurons at the voltages listed. The composition of the extracellular and pipette solutions was as in *B*. *, Paired two-tailed *t* test compared to 300 μM quisqualate at the same potential, $p < 0.05$. **, Paired two-tailed *t* test compared to the same quisqualate concentration at +70 mV, $p < 0.05$. All *error bars* depict ±SEM.

(Neher and Steinbach, 1978), the mean burst duration showed little dependence on quisqualate concentration over the range 2.5–1000 μM (Thio et al., 1990).

Relationship between the steady-state and peak quisqualate currents

Concentrations of quisqualate near 1 μM (Fig. 6*C*) desensitized the peak current by ~50% while the $ED_{50(peak)}$ was 40 μM. Therefore, quisqualate was ~40 times more potent in desensitizing the peak current in cultured postnatal rat hippocampal neurons than it was in activating it. Similarly, both glutamate and quisqualate in rat hippocampal neurons from 2-week-old rats (Kiskin et al., 1986, 1990) and glutamate in cultured chick spinal cord neurons (Trussell and Fischbach, 1989) are 50–100 times more potent in desensitizing receptors than in activating them. However, in these latter studies, doses capable of desensitizing

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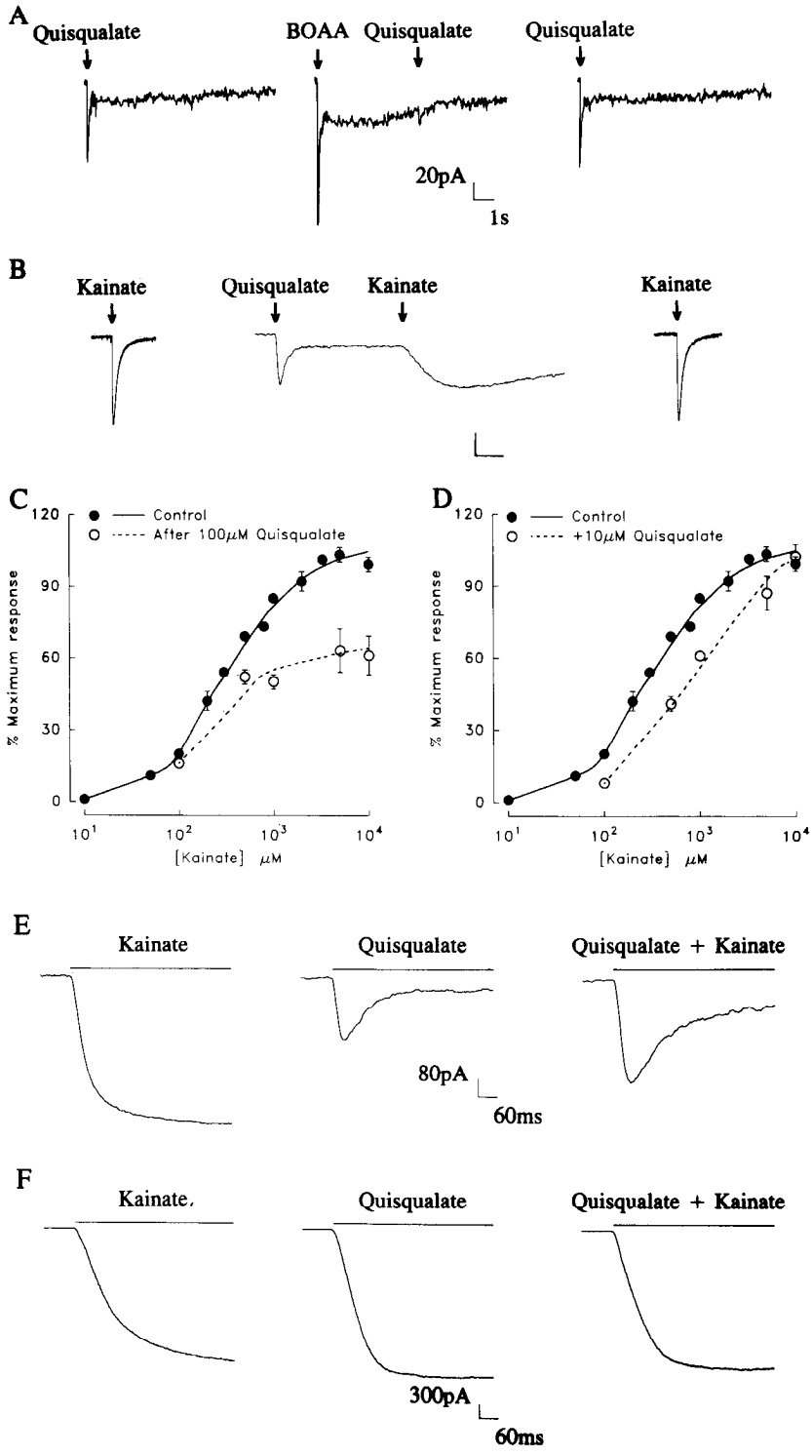
Figure 8. Interaction between non-NMDA analogs. *A*, Cross-desensitization between non-NMDA agonists: response of a neuron to 100 μM quisqualate applied at *arrow* (*left*), application of 1 mM BOAA followed by a challenge to 100 μM quisqualate (*middle*), and recovery of 100 μM quisqualate response (*right*). All agonist applications were 500 msec. *B*, Desensitization of kainate responses by quisqualate: response of a neuron to 1 mM kainate applied at *arrow* (*left*), application of 100 μM quisqualate followed by an application of 1 mM kainate (*middle*), and recovery of 1 mM kainate response (*right*). All agonist applications were 100 msec. Vertical calibration, 200 pA. Horizontal calibration: *left* and *right*, 10 sec;



middle, 400 msec. *C*, Interaction between kainate and quisqualate when quisqualate is preapplied. The control kainate dose–response curve was generated by normalizing the amplitude of the peak current induced by a test kainate concentration to that induced by 1 mM kainate in a single neuron. Raw data (*solid circles*) were fit visually (*solid curve*) to estimate the $ED_{50(kainate)}$, which was 300 μM. The kainate dose–response curve was then repeated using the paradigm in *B*. The amplitude of the current induced by a test kainate concentration during the steady-state current produced by 100 μM quisqualate was measured from the original baseline and expressed as a percentage of the current induced by the test concentration alone. This value was then multiplied by the corresponding point on the control dose–response curve (*open circles*). These points were then fitted visually (*broken curve*) and yielded an $ED_{50(kainate,pre)}$ of 300 μM. *D*, Coapplication of quisqualate shifts the kainate dose–response curve to the right without altering the maximum response. The amplitude of the peak current produced by a test kainate concentration plus 10 μM quisqualate was expressed as a percentage of the peak current produced by 1 mM kainate in the same neuron (*open circles*). These data were visually fit to the curve shown (*broken curve*) with an $ED_{50(kainate,co)}$ of 1 mM. Control kainate dose–response curve (*solid circles* and *solid curve*) are as in *C*. *Error bars* in *C* and *D* represent ±SEM, and those not shown are smaller than the symbol (n = 4–15 for each point). *E* and *F*, Interaction between kainate and quisqualate when coapplied: response of a neuron to 1 mM kainate (*left*), 100 μM quisqualate (*middle*), and 1 mM kainate plus 100 μM quisqualate (*right*) in the absence (*E*) and presence (*F*) of 580 nM WGA in the extracellular solution. All data in this figure were obtained at −50 mV.

receptors do not appear to evoke a current, whereas a steady-state current was activated by such doses in our cultured postnatal rat hippocampal neurons. The difference in these results may reflect the relatively small size of the steady-state current in these other studies.

The difference in potency between desensitization and activation has led to the suggestion that quisqualate channels can desensitize without opening (Trussell and Fischbach, 1989) and that separate activation and desensitization sites exist (Kiskin et al., 1990). In our study, conditioning doses of quisqualate that failed to activate a steady-state current drove ~10% of the receptors into the desensitized state (Fig. 6*C*). The majority of receptors, however, were not desensitized until concentrations large enough to evoke a clear steady-state current were administered. The overlap between the dose–response curves for desensitizing the peak current and activating the steady-state current suggests that the two processes are related. This overlap also suggests that the peak and steady-state currents are mediated by the same receptors. If they reflect the activation of the same receptors, then the two currents are likely to be different states of the receptor since the $ED_{50(peak)}$ is 10-fold greater than the $ED_{50(ss)}$. This difference in ED_{50} values is unlikely to be accounted for by an underestimate in the peak current since the $ED_{50(peak)}$ is not reduced by 10-fold when desensitization is blocked by WGA (40 μM in control neurons vs 30 μM in WGA-treated neurons). However, our data do not exclude the existence of a separate desensitizing site having a dose–response curve comparable to that for the steady-state current. In this model, the steady-state current could be mediated by a separate receptor–channel complex from the peak current.

Relationship between quisqualate and kainate receptors

Previous studies have established that overlap exists between quisqualate and kainate receptor–channel complexes, though the extent of the overlap is unknown. A competitive interaction between quisqualate and kainate is observed when the two agonists are coapplied (O'Brien and Fischbach, 1986; Zorumski and Yang, 1988; O'Dell and Christensen, 1989a,b; Perouansky and Grantyn, 1989; Pin et al., 1989; Rassendren et al., 1989). In contrast, uncompetitive interaction is found when quisqualate is applied after kainate (Ishida and Neyton, 1985), and quisqualate desensitizes kainate responses when the order of application is reversed (Kiskin et al., 1986, 1990). These results were confirmed in our study. Quisqualate and kainate appeared to interact competitively when coapplied to postnatal rat hippocampal neurons and noncompetitively when quisqualate is applied first. In addition, saturating concentrations of kainate and quisqualate produced responses of equal amplitude in neurons bathed in WGA. The response to either agonist alone equaled the response to a coapplication of saturating concentrations of kainate and quisqualate in WGA-treated neurons. Finally, as might be expected, saturating concentrations of kainate induce no additional current when a saturating concentration of quisqualate is applied first in a WGA-treated neuron. The results from WGA-treated neurons suggest that the apparently noncompetitive interaction in control neurons between kainate and quisqualate when the latter is preapplied results from the desensitization of some kainate receptors by quisqualate. All the observations taken together are consistent with the hypothesis proposed earlier (Kiskin et al., 1986, 1990) that kainate and quisqualate activate the same receptor–channel complexes but kainate is incapable of desensitizing them. Thus, desensitization of the complex may be agonist specific. In agreement with these results, the quisqualate receptor clones respond to both quisqualate and kainate (Boulter et al., 1990; Keinänen et al., 1990). The relationship between the quisqualate and kainate receptor–channel complexes should be better defined as work with the quisqualate receptor clones progresses.

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