

Quantitative Physiological Characterization of a Quinoxalinedione non-NMDA Receptor Antagonist

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The effects of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, or FG 9065) on excitatory amino acid responses in cultured neurons from rat hippocampus were studied using tight-seal whole-cell recording techniques. CNQX reduced the magnitude of peak inward currents produced by exogenously applied kainate, quisqualate, and *N*-methyl-D-aspartate (NMDA) with K_i 's of 2.5, 3.5, and 96 μ M, respectively. The antagonism was competitive against kainate and quisqualate, but noncompetitive against NMDA. Glycine markedly reduced CNQX antagonism of NMDA responses. The same recording technique using pairs of monosynaptically connected neurons demonstrated reversible diminution of excitatory postsynaptic potentials in 7 of 7 pairs, using CNQX at concentrations as low as 10 μ M. CNQX applied alone did not evoke inward or outward currents at membrane potentials near the resting membrane potential and did not affect the current-voltage relationship at membrane potentials between -90 and -30 mV. These observations represent the first quantitative characterization of glutamate receptor antagonism by CNQX with respect to physiological rather than biochemical parameters and demonstrate that CNQX is far more potent and more selective than currently available non-NMDA antagonists. The results suggest that CNQX will be a useful pharmacologic tool for the study of synaptic transmission in a variety of systems in which glutamate or related excitatory amino acids are involved.

Abundant evidence suggests that L-glutamate is an endogenous excitatory neurotransmitter acting at a variety of receptors in the vertebrate CNS (Mayer and Westbrook, 1987). Four receptor subclasses have been identified based upon the selectivity of the agonists *N*-methyl-D-aspartic acid (NMDA), kainic acid (KA), quisqualic acid (QA), and 2-amino-4-phosphonobutyric acid (AP-4) (Cotman and Iverson, 1987).

In many preparations, KA and/or QA receptors appear to be responsible for fast excitatory synaptic transmission (Collingridge et al., 1983; Rothman and Samaie, 1985; Nelson et al., 1986). However, examination of the role of KA and QA recep-

tors in physiological events has been hampered by the lack of potent, selective antagonists for these receptors. The blockers now used to antagonize KA and QA, usually γ -D-glutamylglycine (Francis et al., 1980) or kynurenic acid (Robinson et al., 1984) are relatively weak and are actually more potent against NMDA responses. It is likely that many events triggered by activation of KA or QA receptors in some preparations are not significantly reduced by these antagonists because of their low potency.

Honoré and colleagues (Drejer and Honoré, 1988; Honoré et al., 1988) have recently described 2 quinoxalinediones, which potently block many KA and QA responses. These compounds, 6,7-dinitroquinoxaline-2,3-dione (DNQX, or FG 9041) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, or FG 9065) have largely been tested in binding or other neurochemical paradigms. These types of experiments do not necessarily give direct information about mechanism(s) of action at physiologically relevant sites. The experiments described below provide quantitative, physiological information about KA, QA, and NMDA blockade by CNQX. They also demonstrate blockade of synaptic potentials by CNQX at concentrations far less than those of other available antagonists.

Materials and Methods

Cell culture. Neonatal rat hippocampal cell cultures were prepared using methods similar to those previously established for cortical neurons (Huettner and Baughman, 1986). Briefly, hippocampi were removed from postnatal day 1 Sprague-Dawley rats, minced, and incubated for 20 min in 3 ml Leibovitz's L-15 equilibrated with 95% O₂/5% CO₂ containing 1 mg/ml papain and 0.2 mg/ml BSA. The cells were triturated with fire-polished Pasteur pipettes in growth medium and centrifuged through 2 ml growth medium containing 10 mg/ml albumin and 10 mg/ml trypsin inhibitor. The cells were resuspended in growth medium and plated onto established cortical glial cultures, 2.5×10^5 cells per 35 mm petri dish. Approximate yield was $2-3 \times 10^6$ cells per rat pup.

Glial cells were harvested from the cortices of 2- to 6-d-old rat pups (McCarthy and de Vellis, 1980) and placed in T-25 flasks. Passaged glial cells were plated onto poly-L-lysine-coated petri dishes 4 d prior to neuronal plating. One day after plating neurons, cultures were treated with 5-fluoro-2-deoxyuridine (15 μ g/ml) and uridine (35 μ g/ml). Growth medium for all cultures was Eagle's MEM lacking glutamine and supplemented with 10% Nuserum (Collaborative Research). Penicillin (20 units/ml) and streptomycin (20 μ g/ml) were added to inhibit bacterial growth.

Electrophysiology. Experiments were performed on the stage of an inverted microscope at room temperature. Cell cultures were used 1–4 weeks after plating. Growth medium was replaced by an extracellular recording solution containing (in mM) 140 NaCl; 3 KCl; 4 CaCl₂; 4 MgCl₂; 10 HEPES; and 5.5 glucose, pH 7.3. All NMDA responses were obtained using a recording solution containing 5 μ M or 1 mM glycine, 5 mM CaCl₂, and no added magnesium. Tetrodotoxin (1 μ g/ml) was added to all recording solutions in voltage-clamp experiments to block regenerative sodium conductances. All drugs and reagents were obtained

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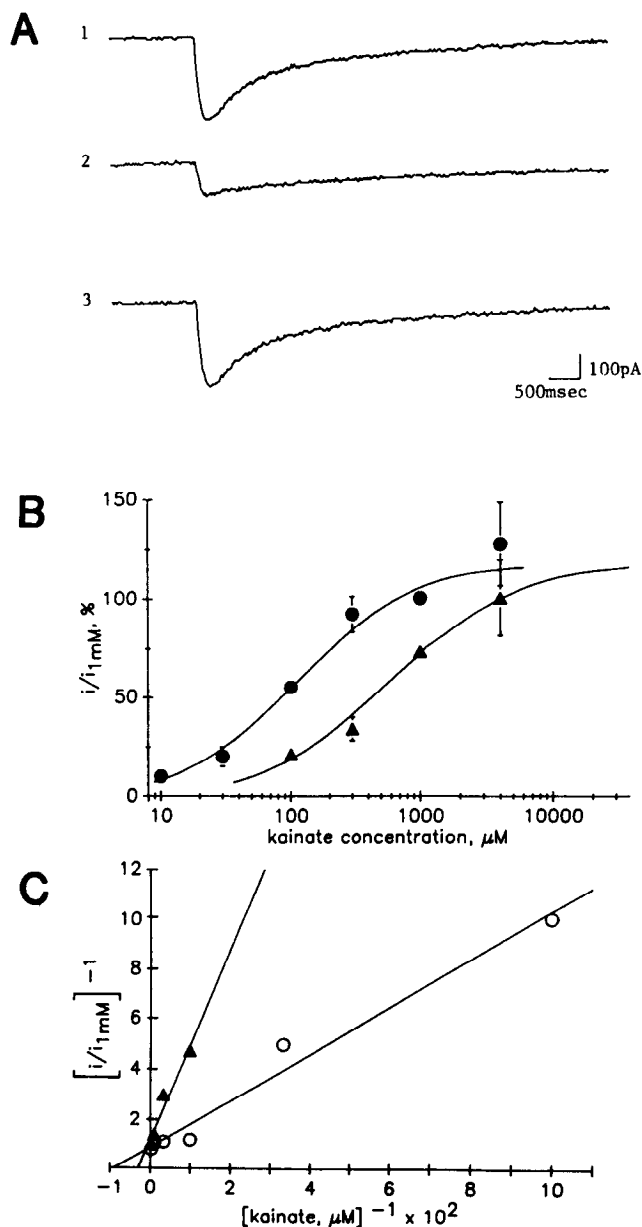


Figure 1. Effect of CNQX against KA responses. *A*, Current traces from a neuron in response to 100 μM KA alone (1) and with 5 μM CNQX (2). Recovery of response to 100 μM KA (3). *B*, Semilog dose-response curves for KA (circles) and KA after 5 μM CNQX (triangles). Error bars are SEM. Each point represents 4–7 cells. *C*, Double-reciprocal plots of data from *B*, excluding the point at 10 μM . For KA alone (circles): $(i/i_{1\text{mM}})^{-1} = 94.1 \times [\text{KA}, \mu\text{M}]^{-1} + 0.862$; $r = 0.99$. $K_d = \text{slope}/y\text{-intercept} = 110 \mu\text{M}$. For KA with 5 μM CNQX (triangles): $(i/i_{1\text{mM}})^{-1} = 378 \times [\text{KA}, \mu\text{M}]^{-1} + 1.15$; $r = 0.99$. Apparent $K_d = (1 + I/K_i) \times K_d$; therefore, $K_i = 2.5 \mu\text{M}$. See Materials and Methods, equations 4–7. In Figures 1–3 lines are derived from a least-squares fit of the data.

from Sigma (St. Louis, MO), except CNQX, which was a gift of Tage Honoré, and γ -amino-methyl sulfonic acid (GAMS), which was purchased from Tocris Neuramin (London, England).

Intracellular recording utilized the whole-cell patch-clamp configuration (Hamill et al., 1981). Electrodes (4–7 M Ω DC resistance) were filled with an intracellular recording solution containing (in mM) 130 potassium acetate, 10 KCl, 10 HEPES, 1.1 EGTA, and 2 Mg-ATP. They were connected to an Axoclamp 2A amplifier (Axon Instruments) used in either the continuous single-electrode voltage-clamp mode with series resistance compensation or bridge mode (for the synaptic pair experiments). Data were collected on a chart recorder or digitized at 6 or 30

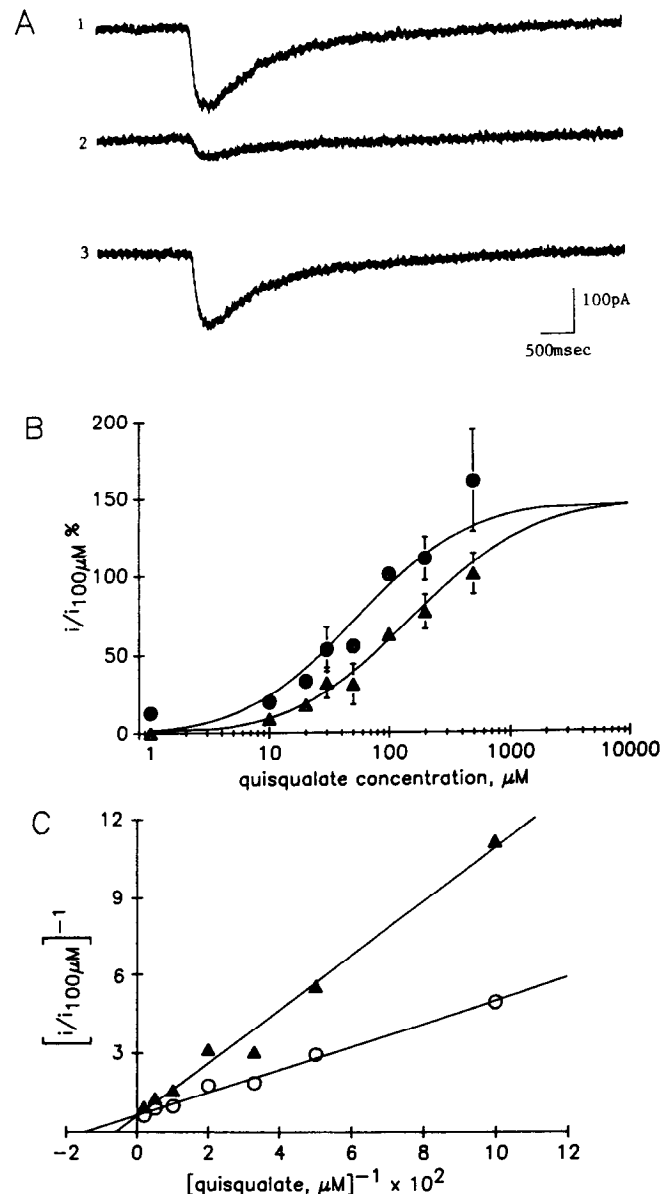


Figure 2. Effect of CNQX against QA responses. *A*, Current trace from a neuron in response to 50 μM QA alone (1) and with 5 μM CNQX (2). Recovery of response to 50 μM QA (3). *B*, Semilog dose-response curves for QA (circles) and QA after 5 μM CNQX (triangles). Each point represents 3–16 cells. *C*, Double-reciprocal plots of data from *B*, excluding the point at 1 μM . For QA alone (circles): $(i/i_{100\mu\text{M}})^{-1} = 44 \times [\text{QA}, \mu\text{M}]^{-1} + 0.654$; $r = 0.99$. $K_d = 67 \mu\text{M}$. For QA with 5 μM CNQX (triangles): $(i/i_{100\mu\text{M}})^{-1} = 102 \times [\text{QA}, \mu\text{M}]^{-1} + 0.624$; $r = 0.99$. $K_i = 3.5 \mu\text{M}$. See Materials and Methods, equations 4–7.

kHz (Modular Instruments M-100 interface) and stored on disk for later analysis.

For drug application, pipettes identical to those used for patch electrodes were filled with agonists or antagonists dissolved in extracellular recording solution. Brief pulses of air pressure ($2\text{--}7 \times 10^4$ Pa) ejected drugs (Choi and Fischbach, 1981). At high concentrations of agonist, leakage required use of smaller tipped pipettes (10–12 M Ω DC resistance).

For dose-response experiments, cells were voltage-clamped at resting membrane potential and drug pipettes were placed $<50 \mu\text{m}$ from the cell body. Agonist was applied for 100 msec, both before and immediately after a 2 sec application of CNQX. Averages of 2 or more peak current responses from each concentration of agonist, with and without antagonist, were normalized to responses produced by application of a

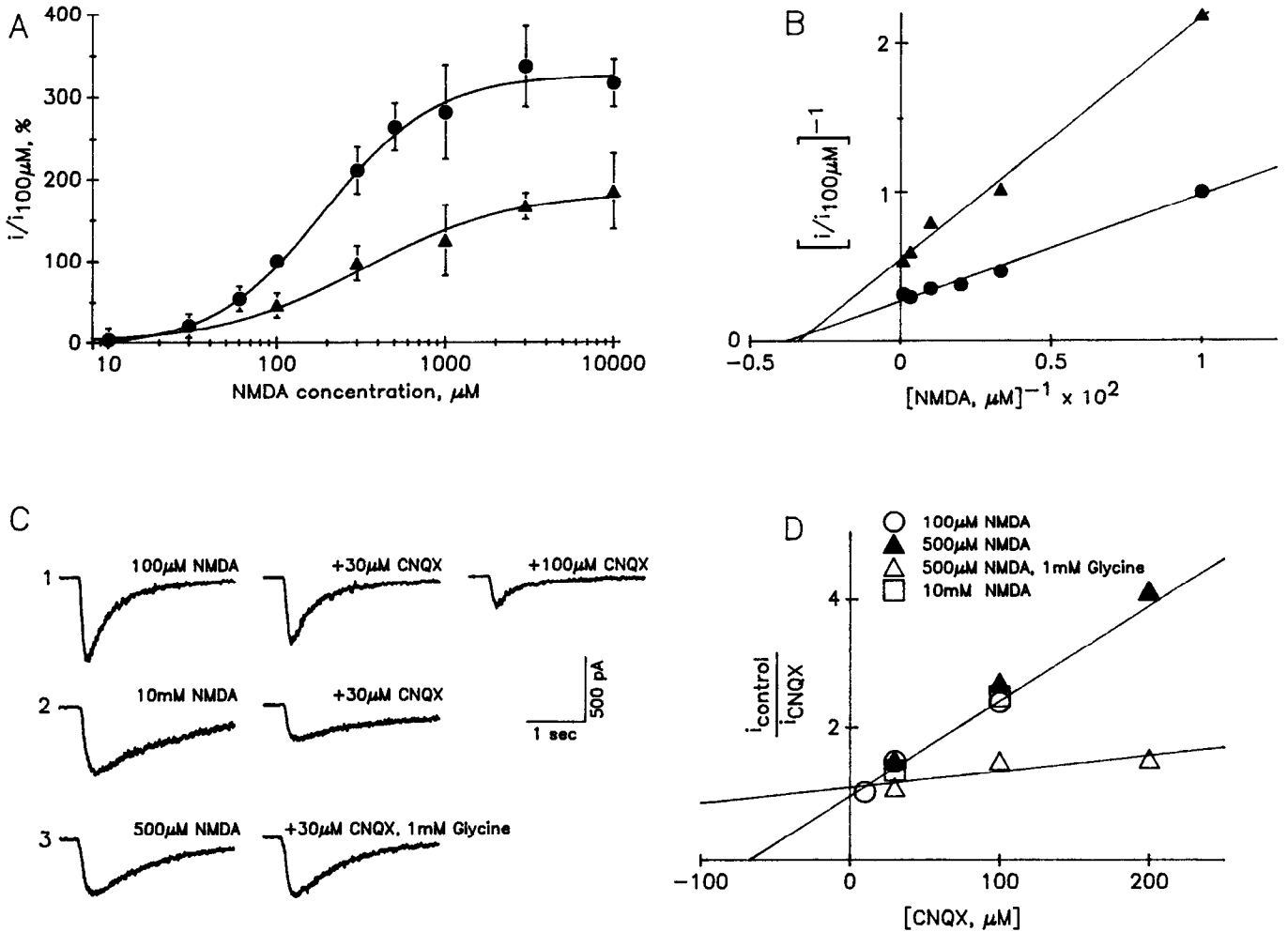


Figure 3. Effect of CNQX against NMDA responses in conditions of low magnesium and 5 μM glycine. *A*, Semilog dose–response curve for NMDA (circles) and after 100 μM CNQX (triangles). High concentrations of NMDA cannot overcome CNQX antagonism. Each point represents 3–12 cells. *B*, Double-reciprocal plot of data from *A*, excluding points at 10, 30, and 60 μM . For NMDA alone (circles): $(i/i_{100\mu\text{M}})^{-1} = 71.3 \times [\text{NMDA}, \mu\text{M}]^{-1} + 0.27$; $r = 0.99$. For NMDA with 100 μM CNQX (triangles): $(i/i_{100\mu\text{M}})^{-1} = 161.6 \times [\text{NMDA}, \mu\text{M}]^{-1} + 0.55$; $r = 0.99$. $K_d = 260 \mu\text{M}$, $K_i = 96 \mu\text{M}$. See Materials and Methods, equations 8, 9. *C*, Current traces from 3 neurons in response to NMDA. 1, Responses to 100 μM NMDA alone and with 30 and 100 μM CNQX. 2, Responses to 10 mM NMDA alone and with 30 μM CNQX. CNQX antagonism could not be overcome by high concentrations of NMDA. 3, Responses to 500 μM NMDA alone and with 30 μM CNQX in the presence of 1 mM glycine in the extracellular solution. CNQX antagonism is significantly reduced. *D*, Plot of i/i_{CNQX} vs CNQX concentration demonstrating reduction of NMDA responses independent of NMDA concentration. Slope of the top line gives a $K_i = 71 \mu\text{M}$ (see Materials and Methods, equation 9); the bottom line gives an apparent $K_i = 410 \mu\text{M}$; experimental conditions were the same except for 1 mM glycine in the extracellular solution.

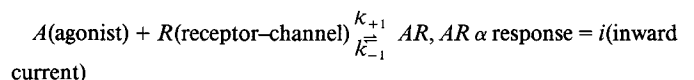
Table 1. K_d s for excitatory amino acid agonists in different preparations (in μM)

Glutamate	Kainate	Quisqualate	NMDA	Method
30	200	—	—	Cultured chick spinal neurons (Vlachova et al., 1987)
1100	500	93	—	Rat hippocampal neurons (Kiskin et al., 1986)
660	110	67	260	Cultured rat hippocampal neurons (present study)

The K_d of 660 μM glutamate is included for comparison; it was obtained using the same methods as described in Materials and Methods.

control concentration (i_{control}) of the same agonist (1 mM for kainate; 100 μM for NMDA and quisqualate). Therefore, each cell was its own control for the variability of absolute values of peak currents as a function of cell size. Average responses were collected from 3–18 cells at a given concentration to establish one data point on the dose–response curve.

Data analysis. We assume that the receptor–channel interaction can be represented in chemical terms, obeying laws of mass action. In addition, we assume that the agonist (or inhibitor)–receptor interaction equilibrates rapidly and that the observed physiological response resulting from agonist–receptor binding is proportional to receptor occupancy:



At equilibrium, the forward and reverse fluxes are equal such that

$$k_{+1}[A][R] = k_{-1}[AR]. \quad (1)$$

If p is the fraction of receptors occupied to give AR , $(1 - p) = R$ and rearrangement of equation 1 gives

$$p = \frac{K_a \times [A]}{(1 + K_a \times [A])}, \text{ where } K_a = \frac{k_{+1}}{k_{-1}} = \frac{1}{K_d} \quad (2)$$

Since p is proportional to the observed response (i.e., i/i_{control}), a proportionality constant yields

$$\frac{i}{i_{\text{control}}} = C \times \frac{K_a \times [A]}{(1 + K_a \times [A])} \quad (3)$$

Taking the reciprocal of both sides gives the following linear relationship (Segel, 1976; Barlow, 1980):

$$\frac{i_{\text{control}}}{i} = K_d \times C' \times \frac{1}{[A]} + C' \quad (4)$$

If i_{control}/i is plotted against $1/[A]$,

$$K_d = \text{slope}/y\text{-intercept} \quad (5)$$

In the presence of a competitive antagonist, $[I]$,

$$\frac{i_{\text{control}}}{i_{\text{inhibited}}} = K_d \times C' \times \left(1 + \frac{[I]}{K_i}\right) \times \frac{1}{[A]} + C' \quad (6)$$

$$\text{"apparent } K_d\text{"} = \left(1 + \frac{[I]}{K_i}\right) \times K_d, \text{ where } [I] = [\text{CNQX}] \quad (7)$$

If I is a noncompetitive antagonist,

$$\frac{i_{\text{control}}}{i} = K_d \times \left(1 + \frac{[I]}{K_i}\right) \times C' \times \frac{1}{[A]} + \left(1 + \frac{[I]}{K_i}\right) \times C' \quad (8)$$

and

$$\frac{i}{i_{\text{inhibited}}} = \frac{1}{K_i} \times [I] + 1 \quad (9)$$

These equations enable calculation of the K_i by plotting i_{control}/i versus $1/[A]$ in the presence of fixed $[I]$ or by varying $[I]$ in the presence of fixed $[A]$. The semilog dose-response curves were fit by probit analysis (Finney, 1971).

Synaptic responses. In order to test the effect of CNQX on excitatory postsynaptic potentials (EPSPs), current-clamp recordings from 2 synaptically connected neurons were obtained with 2 whole-cell electrodes. Bicuculline, 100 μM , was added to the extracellular solution to eliminate inhibitory postsynaptic potentials. Consistent EPSPs with onset ≤ 5 msec after the peak of the presynaptic action potential were considered monosynaptic. Action potentials were triggered every 10 sec by depolarizing current injection and only pairs of cells in which $>90\%$ of action potentials resulted in an EPSP were used in the analysis. CNQX, 10 and 100 μM , was applied near the postsynaptic neuron during a continuous train of evoked EPSPs.

Results

For dose-response experiments, the 137 hippocampal neurons used had resting membrane potentials of -54.8 ± 4.5 mV (± 1 SD) and input resistances of 211 ± 157 M Ω (± 1 SD). KA, QA, and NMDA evoked inward currents in every neuron at resting membrane potential (Figs. 1A, 2A, 3C). An S-shaped semilog dose-response curve was obtained for each agonist (Figs. 1B, 2B, 3A). At 5 μM , CNQX reversibly reduced inward currents produced by low concentrations of KA and QA (Figs. 1A, 2A). These reductions were a function of agonist concentration and resulted in a rightward shift of the KA and QA dose-response curves in the presence of CNQX (Figs. 1B, 2B), with the same

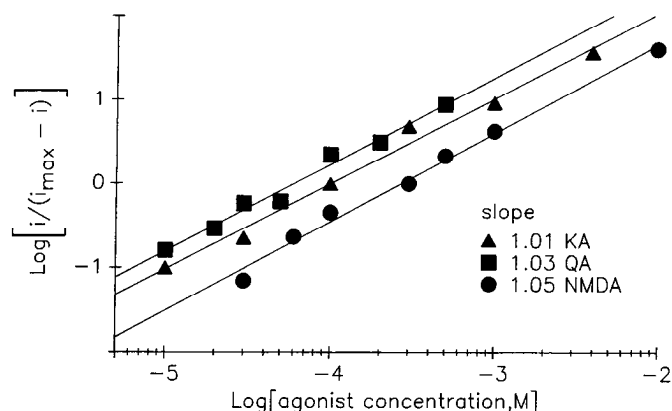


Figure 4. Hill plots for KA, QA, and NMDA. For all 3 agonists Hill coefficients were near unity, indicating that one agonist molecule reacts with one receptor-channel complex to produce a response, see Discussion.

predicted maximal response as the dose-response curves without CNQX. Double-reciprocal plots of response versus agonist concentration, with and without CNQX, were linear for KA and QA with correlation coefficients >0.99 and similar y -intercepts. This is consistent with competitive inhibition by CNQX. K_d s for KA and QA, calculated from equation 2, were 110 and 67 μM (Figs. 1C, 2C). The K_i s calculated from equation 4 for CNQX versus KA and QA, were 2.5 and 3.5 μM , respectively. The K_d s calculated from our experimental results are comparable to those from others using similar physiological measurements and *in vitro* preparations (Table 1).

Low concentrations of CNQX had little effect on NMDA currents, but higher concentrations could diminish NMDA responses (Fig. 3C1). Unlike the situation for KA and QA, extremely high concentrations of NMDA were unable to overcome CNQX antagonism (Fig. 3, A, C2). This finding suggested that blockade of NMDA by CNQX might be noncompetitive. The semilog dose-response curves for NMDA alone and with 100 μM CNQX demonstrated different maximal responses using NMDA at concentrations as high as 10 mM. Double-reciprocal plots of the dose-response data yielded lines with similar x -intercepts (and therefore K_d s) and different slopes and y -intercepts, which is consistent with noncompetitive inhibition by CNQX (Fig. 3B). The K_d for NMDA obtained from Figure 3B was 260 μM ; the K_i for CNQX against NMDA was 96 μM . A plot of i/i_{CNQX} against concentration of CNQX was linear and independent of NMDA concentration, which is also consistent with noncompetitive antagonism (Fig. 3D; equation 8). The CNQX K_i for NMDA calculated from Figure 3D was 71 μM . CNQX may interact at the glycine modulatory site of the NMDA receptor as glycine significantly reduced CNQX effects on NMDA responses (Fig. 3, C3, D). It is important to emphasize that all experiments to establish the K_i for CNQX against NMDA were obtained in no added magnesium solutions with 5 μM glycine, and consequently only apply to these conditions.

The equations used above to obtain binding and inhibitory constants are all derived assuming that only a single agonist molecule binds to the receptor and opens a channel. We tested this assumption by making Hill plots of our data. These were all linear with Hill coefficients of 1.01 for KA, 1.03 for QA, and 1.05 for NMDA (Fig. 4). Thus, the response (inward current) is first order with respect to each agonist.

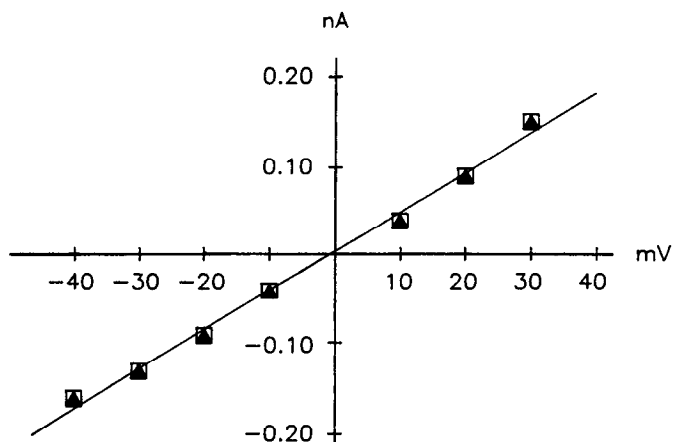


Figure 5. Current-voltage relationship with (open squares) and without (closed triangles) $100 \mu\text{M}$ CNQX. Abscissa, voltage steps from resting membrane potential of -62 mV (in mV); ordinate, current input (in nA). CNQX has no detectable effect on passive membrane characteristics.

Although unlikely, it was also possible that direct effects of CNQX on passive membrane properties might complicate our analysis. Therefore, we examined the effect of CNQX alone on current-voltage curves of our cultured neurons. The current-voltage relationship for 4 neurons was linear between -90 and -30 mV and was unchanged during application of $100 \mu\text{M}$ CNQX (Fig. 5). No currents were evoked by applications of CNQX at potentials near resting membrane potential. Therefore, the drug has no direct effects on passive neuronal properties. No morphologic changes were observed in neurons exposed to $200 \mu\text{M}$ CNQX for up to 24 hr, suggesting that the drug is not toxic to cultured neurons.

Biochemical techniques have already indicated that CNQX is far more potent than any other available non-NMDA antagonist. Therefore, we were interested in comparing it with other antagonists using our physiological assay. We selected the most potent non-NMDA antagonist already characterized, GAMS (Jones et al., 1984), and compared the ability of CNQX and GAMS to decrease KA induced currents. CNQX at a concentration of $10 \mu\text{M}$ reduced the peak inward current in response to a 100 msec application of $100 \mu\text{M}$ KA to $39 \pm 9\%$ of the control response ($n = 9$). Using GAMS on the same cells at $10 \mu\text{M}$ ($n = 3$) and $100 \mu\text{M}$ ($n = 3$) resulted in no detectable KA

antagonism (Fig. 6A). At $500 \mu\text{M}$ GAMS diminished the KA current to $82 \pm 9\%$ of control (Fig. 6B, $n = 3$). Using the KA K_d determined earlier, we calculated that the K_i for GAMS was 1.7 mM against KA. Thus, CNQX ($K_i = 2.5 \mu\text{M}$) was over 500 times more potent than GAMS in blocking KA currents.

Glutamate, acting at non-NMDA receptors probably mediates fast EPSPs in hippocampal cultures (Rothman and Sammaie, 1985; Forsythe and Westbrook, 1988). In 7 pairs of monosynaptically connected neurons, CNQX reversibly reduced the amplitude of the EPSPs. CNQX, $10 \mu\text{M}$, reversibly reduced the EPSP amplitude by $71.5 \pm 4.9\%$ ($n = 3$) (Fig. 7A) and $100 \mu\text{M}$ CNQX produced a 100% ($n = 4$) reduction in the EPSP amplitude (Fig. 7B).

Discussion

Honoré and colleagues (Drejer and Honoré, 1988; Honoré et al., 1988) and Collingridge and coworkers (Blake et al., 1988) have reported that CNQX is a potent and selective antagonist of non-NMDA excitatory amino acid receptors. They have shown that CNQX blocks excitatory amino acid binding, KA- and QA-activated GABA release from cultured neurons (Drejer and Honoré, 1988), depolarization of neurons by iontophoretic application of KA and QA, and extracellular EPSPs in hippocampal slices (Blake et al., 1988). Although these studies are in agreement, none directly demonstrates a reduction in agonist-gated inward currents, which presumably underlies the action of CNQX.

This report is largely consistent with those mentioned above in demonstrating potent, competitive antagonism of KA and QA by CNQX. However, our results significantly extend the previous observations on the compound by using physiological measurements and conditions to characterize the effects of CNQX on membrane receptors that are definitely coupled to ion channels. We have shown that CNQX potently diminishes inward currents produced by KA and QA and shifts the KA and QA dose-response curves to the right, with no change in the maximal response, compatible with competitive inhibition. The intersecting linear double-reciprocal plots of KA and QA responses in the presence of CNQX are also consistent with competitive inhibition because only the apparent K_d is shifted by CNQX (Figs. 1C, 2C).

We were initially surprised that the CNQX inhibition of NMDA appeared to be noncompetitive. We found that high concentrations of NMDA (10 mM) could not overcome CNQX

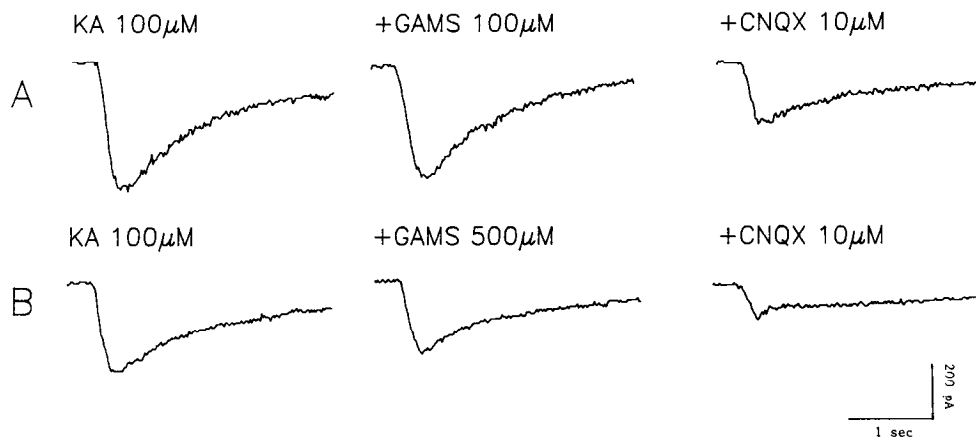


Figure 6. Comparison of the effects of GAMS and CNQX against KA on 2 neurons. Current traces in response to $100 \mu\text{M}$ KA for 100 msec. A (left to right), Control and with $100 \mu\text{M}$ GAMS and $10 \mu\text{M}$ CNQX; B (left to right), Control and with $500 \mu\text{M}$ GAMS and $10 \mu\text{M}$ CNQX.

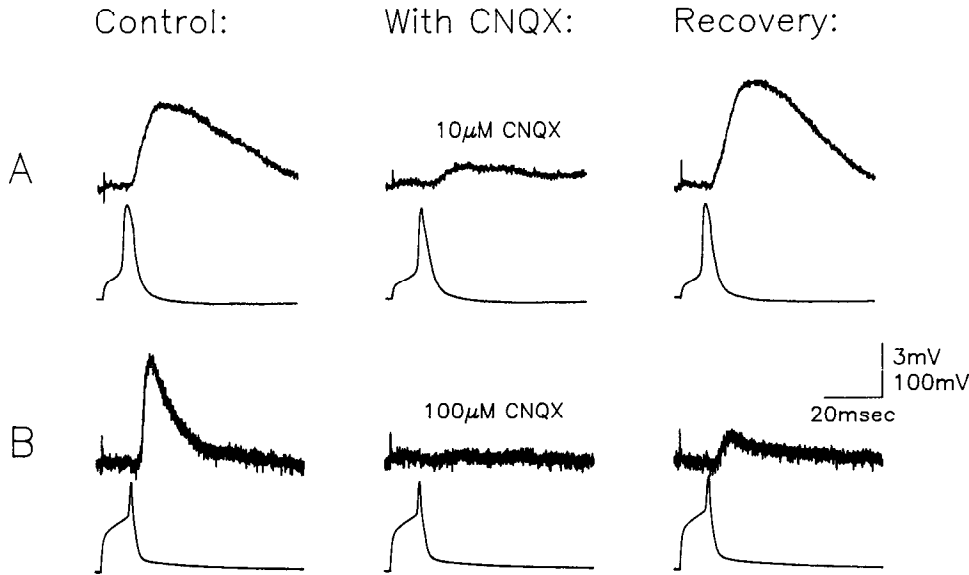


Figure 7. Effect of CNQX on synaptic responses. *Top trace* in each part of the figure is an EPSP which was produced by directly stimulating a nearby neuron through a patch electrode. *Bottom trace* shows the presynaptic action potential. *A (left to right)*, an EPSP before, during, and after 10 μM CNQX; *B (left to right)*, an EPSP before, during, and after 100 μM CNQX. There is incomplete recovery following 100 μM CNQX.

blockade and that the percent reduction of NMDA responses was independent of NMDA concentration. Drejer and Honoré (1988) have previously suggested that NMDA blockade by CNQX, and the related compound, FG 9041, is competitive. The most likely explanation for this discrepancy is that Drejer and Honoré's experiments were done with low antagonist concentrations. Under those conditions, noncompetitive antagonism can appear to be competitive (see Barlow, 1980, pp. 160–162). Recently, other investigators have described noncompetitive blockade of NMDA by DNQX (FG 9041) (Birch et al., 1988a).

Our observation that glycine can reduce the block of NMDA by CNQX suggests that both glycine and CNQX interact at the glycine modulatory site of the NMDA receptor (Johnson and Ascher, 1987). Similar findings have recently been reported for the excitatory amino acid antagonists HA-966 and kynurenic acid (Birch et al., 1988b; Fletcher and Lodge, 1988; Pullan and Cler, 1988; Ransom, 1988; Watson et al., 1988). The glycine site may be a common site of action for a variety of agonists and antagonists.

Using our physiological assay, we compared CNQX with the most potent non-NMDA antagonist previously available, GAMS. A complete dose-response curve was not done, but using the results obtained against 100 μM KA, GAMS had an estimated K_i of 1.7 mM. Thus, CNQX ($K_i = 2.5 \mu\text{M}$) is over 500 times more potent when examined directly at the KA receptor-channel complex. This finding is in general agreement with the outcome of binding and release experiments (Honoré et al., 1988).

Our methods of analysis, using the standard equations of biochemical pharmacology, make a number of assumptions that must be satisfied if our calculations are to be meaningful. These include the following:

1. The inward currents we measure must be proportional to the number of occupied receptors.
2. Antagonist and receptor reach binding equilibrium at the time we measure inward current.
3. The agonist-receptor interaction is first order with respect to agonist.
4. Receptors are equally accessible to agonist and antagonist.

5. Concentrations of agonist and antagonist that reach the cell surface are similar to concentrations within the pipette.

It is likely that assumption 1 is largely fulfilled. Drugs were applied at the neuron soma, which was voltage-clamped. The currents generated should be a direct measure of agonist-gated channels and not voltage-gated channels. In order to apply the Henri-Michaelis-Menten equation, a system must be in equilibrium or at steady state (Segel, 1976). At present, there is no data available that would allow us to determine that equilibrium or steady state is actually attained with our brief agonist applications. The fact that our data are well fit by double-reciprocal plots supports either assumption. The Hill coefficients near unity for all agonists suggests that a first-order agonist-receptor interaction is likely.

Chemical differences between compounds may affect their ability to reach sequestered sites. However, in the aqueous extracellular culture medium, it is likely that soluble compounds of similar size (i.e., the excitatory amino acid agonists and antagonists) have equal access to membrane-bound receptors.

Ideally, bath perfusion of the entire culture would insure that all receptors were exposed to a known concentration of CNQX prior to agonist application, but a limited supply of CNQX made this impossible. For our calculations to be valid, the drug concentration in the pipette should approximate the peak local concentration at the neuronal surface. Other investigators (Choi and Fischbach, 1981; Kidokoro et al., 1982) have shown that this is true using similar methods. Two different laboratories using rapid perfusion techniques *in vitro* derived K_i s for KA and QA similar to ours (Table 1). This is further evidence that pressure application exposes cells to a drug concentration close to that in the pipette.

Evidence has steadily accumulated over the past 5 years that KA and/or QA receptors are important for fast excitatory transmission in the mammalian CNS (Collingridge et al., 1983). Consistent with this hypothesis, direct intracellular recording in our cultures has shown that CNQX very potently diminishes EPSPs. CNQX, 100 μM , effectively eliminated excitatory transmission while 10 μM decreased EPSPs by over 70%. Similar low concentrations dramatically reduced intracellularly recorded EPSPs (Neuman et al., 1988) and extracellularly recorded EPSPs (Blake

et al., 1988; Neuman et al., 1988) in hippocampal slices. In comparison, millimolar concentrations of other antagonists have been required to decrease synaptic potentials thought to be mediated by non-NMDA receptors (Rothman and Samaie, 1985; Nelson et al., 1986; Huettner and Baughman, 1988). In addition to being a potent antagonist, CNQX is likely quite specific for KA and QA receptors. In the presence of 100 μ M CNQX in hippocampal cultures, it was not difficult to identify large inhibitory synaptic currents mediated by GABA (K. Holland, personal communication). Interestingly, excitatory synaptic potentials in the isolated spinal cord seem resistant to iontophoretically applied CNQX (Honoré et al., 1988). While this could reflect participation of other types of receptors, it may also be an artifact of iontophoretic administration. Insufficient CNQX released from the iontophoretic pipette or inadequate tissue penetration may explain the results.

The development of CNQX and related non-NMDA antagonists (Honoré et al., 1988) is a major advance. For the first time it should be possible to specifically reduce fast synaptic currents and potentials mediated by excitatory amino acids without nonselectively blocking all synaptic transmission. In addition, the 5- to 6-fold reduction of CNQX antagonism of NMDA responses in the presence of glycine suggests that glycine may augment the selectivity of CNQX against non-NMDA-mediated responses. At certain synapses it will also be possible to dissect out components mediated by either NMDA or non-NMDA receptors (Blake et al., 1988). Equally appealing is the therapeutic potential of these drugs. Thus far, NMDA antagonists have made it possible to identify a role for NMDA receptors in a variety of pathologic processes in the CNS (Rothman and Olney, 1987). We should be able to learn soon whether non-NMDA receptors participate in these processes. If non-NMDA receptors are also important, use of these or similar agents may allow specific intervention for the benefit of patients with these conditions.

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