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Quinoxalinediones selectively block quisqualate and kainate receptors and synaptic events in rat neocortex and hippocampus and frog spinal cord *in vitro*

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1 Two quinoxalinediones, FG9041 and FG9065, which had previously been shown to displace binding to the quisqualate receptor, were tested on rat neocortex and frog spinal cord *in vitro* against depolarizations induced by quisqualate, kainate and N-methyl-D-aspartate (NMDA). In both preparations effects of quisqualate were reduced the most and those of NMDA the least.

2 The near unitary slopes of the Schild plots were consistent with a competitive type of interaction. pA_2 values for FG9041 were estimated to be 6.6, 6.1 and 5.1 in frog cord and 5.9, 5.3 and about 4 in rat neocortex for quisqualate, kainate and NMDA antagonism, respectively. FG9065 gave equivalent pA_2 values of 6.2, 5.6 and 4.5.

3 At concentrations, which were without effect on depolarizations induced by NMDA, FG9041 and FG9065 reduced or blocked synaptically-evoked field potentials in hippocampal and neocortical slices superfused with normal magnesium-containing medium. Since these synaptic components are also insensitive to NMDA antagonists, these results are consistent with their mediation by postsynaptic receptors of the quisqualate (or kainate) type.

4 By contrast, quinoxalinediones had only limited effects on spontaneous epileptiform activity seen in both neocortical and hippocampal preparations when superfused with magnesium-free medium. These burst discharges were, however, abolished by NMDA antagonists.

5 In the frog spinal cord the early component of the dorsal root to ventral root reflexes was selectively reduced by FG9041 whereas NMDA antagonists reduced the longer latency components.

6 Our results suggest that the quinoxalinediones are likely to be useful pharmacological probes for elucidating the role of non-NMDA receptors in the vertebrate central nervous system.

Introduction

It is considered likely that amino acids, such as glutamate and aspartate, are the major neurotransmitters at excitatory synapses in the vertebrate CNS (Curtis & Johnston, 1974; Watkins & Evans, 1981; Fonnum, 1984). However, pharmacological investigation of this has been hampered by the lack of potent antagonists, selective for the various glutamate receptors. Electrophysiological (McLennan, 1983; Mayer & Westbrook, 1987) and radioligand binding (Foster & Fagg, 1984; Cotman *et al.*, 1987; Watkins & Olverman, 1988) studies have defined at least three glutamate receptor subtypes, named after agonists which are believed to activate them selectively, namely N-methyl-D-aspartate (NMDA), quisqualate and kainate. Potent and selective NMDA antagonists of both the competitive and non-competitive types are well documented (for reviews see Lodge *et al.*, 1988; Watkins & Olverman, 1988). So far only weak non-NMDA receptor antagonists have been available (Watkins & Olverman, 1988). These include glutamic acid diethylester (GDEE; McLennan & Lodge, 1979), kynurenate (Perkins & Stone, 1982), piperidine dicarboxylates (Davies & Watkins, 1983), glutamyl dipeptides (Davies *et al.*, 1984) and benzoylpiperazine dicarboxylates (Davies *et al.*, 1984), which all exhibit broad spectrum for the three receptor subtypes. Hence electrophysiological investigations have been concentrated around NMDA-receptor mediated synaptic events. Although synaptic excitations involving NMDA

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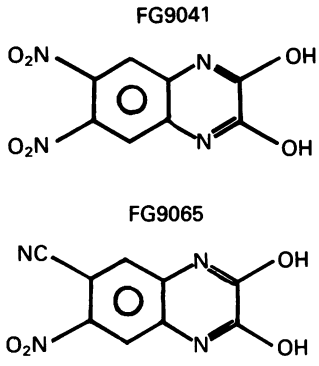

Figure 1 Structures of the two quinoxalinediones FG9041 and FG9065.

receptors are widely documented from *in vivo* studies (Davies & Watkins, 1983; Headley *et al.*, 1987; Salt, 1986), much of the evoked activity recorded from brain slices in normal medium is insensitive to NMDA antagonists (Collingridge & Bliss, 1987; but see Thomson *et al.*, 1985). However, removal of magnesium from bathing solutions of cortical (Thomson *et al.*, 1985) and hippocampal (Coan & Collingridge, 1985) slices leads to enhanced synaptic activity and induces spontaneous epileptiform potentials (Aram & Lodge, 1985), both of which are sensitive to NMDA antagonists. Magnesium, a non-competitive NMDA antagonist (Ault *et al.*, 1980), normally limits synaptic activity mediated by NMDA receptors. Broad spectrum glutamate antagonists reduce synaptic potentials that are resistant to NMDA antagonists, but elucidation of the role of non-NMDA receptors in synaptic activity requires the development of more potent and selective quisqualate and/or kainate antagonists.

Recently two quinoxalinediones, 6,7,dinitroquinoxaline-2,3-dione (FG9041) and 6-cyano-7-nitroquinoxaline-2,3-dione (FG9065), the structures of which are shown in Figure 1, have emerged as potentially selective antagonists of quisqualate (Honoré *et al.*, 1987). Both compounds inhibited binding of the quisqualate receptor agonist, [^{3}H]-AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) to rat cortical membranes with IC_{50}s of (FG9041) 500 nM and (FG9065) 300 nM (Honoré, *et al.*, 1987). Equivalent values for high affinity [^{3}H]-kainate and [^{3}H]-CPP (3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid) binding were approximately 10 and 100 times greater, respectively. In the same [^{3}H]-AMPA binding assays, IC_{50}s for GDEE and γ-D-glutamylaminomethylsulphonic acid (GAMS) were greater than 100 μM. Furthermore these two quinoxalinediones showed no affinity for other neutotransmitter receptors. Similarly in more functional biochemical assays, e.g. amino acid stimulated γ-aminobutyric acid (GABA) release and ^{22}Na-flux, micromolar concentrations of FG9041 and FG9065 selectively blocked the effects of quisqualate and kainate (Drejer & Honoré, 1988). Preliminary data from electrophoretic studies on rat spinal cord neurones *in vivo* suggest that FG9041 and FG9065 *in vivo* reduced responses to quisqualate and kainate approximately equally with little effect on the response to NMDA (Honoré *et al.*, 1987).

The electrophysiological experiments presented here had two aims. Firstly to quantify the selectivity and potency of FG9041 and FG9065 as antagonists of excitatory amino acid-induced depolarization of rat neocortical and frog spinal neurones *in vitro*. Secondly, to investigate and compare the effects of these drugs on normal synaptic activity and enhanced activity in magnesium-free media.

Methods

Preparation of rat neocortical and hippocampal slices

Methods for preparation of and recording from neocortical slices have been described in detail previously (Aram & Lodge, 1988). Briefly, brains of killed Wistar rats (150–250 g) were rapidly removed to ice-cold artificial cerebrospinal fluid (aCSF). Serial 500 μm slices of neocortex were cut using a Vibroslice (Camden Instruments) so that each slice comprised cerebral cortex, corpus callosum and underlying striatal tissue. To prepare hippocampal slices, hippocampi were dissected out and cut transversely at 500 μm thickness with a McIlwain chopper. Two or three slices of either neocortex or hippocampus were transferred to the recording chamber maintained at 29 ± 1°C. They were superfused with aCSF at 2 ml min^{-1}. Composition of aCSF in mM was: NaCl 124, $NaHCO_3$ 25.5, KCl 3.3, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1 or 2, D-glucose 10, equilibrated with 95% O_2 plus 5% CO_2 to maintain a pH of 7.3–7.4. In some experiments the slices were superfused with medium with no added magnesium.

Tungsten bipolar stimulating electrodes were positioned in the white matter usually near the cingulum for recording in cell layers of the cingulate and somatosensory cortex. Square wave pulses (0.1 ms) were delivered every 10 s. The position of the recording electrode was estimated by reference to the atlas of Paxinos & Watson (1982). For recording evoked potentials in the hippocampus the stimulating electrode was placed on the Schaffer collateral-commisural pathway and the recording electrode in CA1 pyramidal layer. Extracellular recordings of spontaneous and evoked field potentials were made

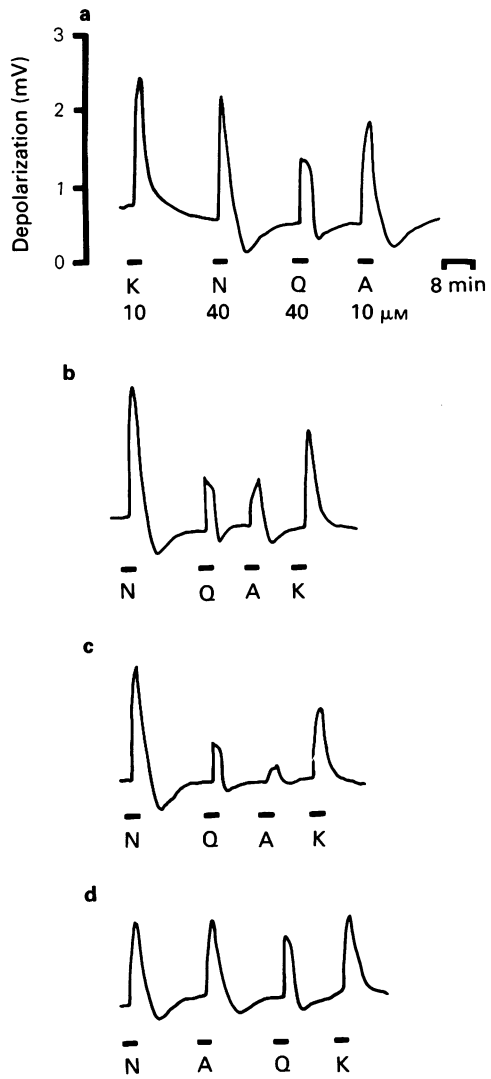

Figure 2 Neuronal depolarizations induced by 5 ml aliquots of 10 μM kainate (K) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (A; AMPA), and 40 μM N-methyl-D-aspartate (N; NMDA) and quisqualate (Q) in a cortical wedge preparation in the absence (a) and presence of (b) 1 and (c) 3.16 μM FG9065. Depolarizations to AMPA were most sensitive to the antagonist. (d) Partial recovery of responses to AMPA, quisqualate and kainate were seen on washing the preparation in control medium although the response to NMDA continued to decline.

using 0.5 M sodium acetate-filled glass microelectrodes (R = 4–12 MΩ). Conventional amplification and filtering (d.c.-500 Hz) techniques were employed to record extracellular field potentials. To aid quantification, evoked potentials were summed (8 sweeps; duration 50–200 ms; 256 bins; 0.1–0.5 Hz) and plotted onto a Gould chart recorder. Maximum amplitudes and half decay times were measured from these records.

Preparation of rat cortical wedges

Cortical wedges were prepared as described (Harrison & Simmonds, 1985). Freshly cut 500 μm neocortical slices, prepared as above, were further cut by hand at the midline and just lateral to each cingulum to produce wedge-shaped slices of tissue comprising both grey and white matter. These were positioned in a divided bath so that they passed through a silicone-greased slot between the two compartments, in such a way that one compartment contained entirely grey matter and the other mostly white matter.

Each compartment was superfused independently with aCSF (as above) at a rate of 2 ml min^{-1} at room temperature (19–22°C). Initially the grey matter side was superfused with normal medium for 30 min before replacing with one containing no added magnesium (to maximize NMDA-evoked depolarizations or to record spontaneous synaptic activity). In order to study the action of quinoxalinediones as amino acid antagonists, tetrodotoxin (TTX; initially 2.5 μM and maintained at 0.1 μM) was added to the medium to block synaptic influences. To study the pharmacology of spontaneous epileptiform activity in these cortical wedges, TTX was not added. The d.c. potential between the two compartments was monitored via paper wick Ag/AgCl electrodes embedded in 3% agar containing 0.9% saline. Potentials were amplified, filtered (d.c.-5 Hz for effects of agonists; d.c.-500 Hz for epileptiform activity) and continuously displayed on a chart recorder. After a 1–2 h stabilization period, 5 ml aliquots of agonist were added to the medium perfusing the grey matter (5–40 μM quisqualate or kainate, 10–80 μM NMDA) to obtain control responses before addition of the quinoxalinediones to the superfusing medium. Dose-response curves and Schild plots were constructed and pA_2 and IC_{50} values estimated with the aid of computer programmes.

Frog hemisected spinal cord

Preparation and recording from frog spinal cords were as described previously (Martin & Lodge, 1985). Dissection and sagittal hemisection were performed under chilled magnesium-free frog Ringer. The cords were kept overnight at 4°C, then placed medial surface down in Perspex channels and superfused at 1 ml min^{-1} with Ringer solution, at 10–12°C, of the following composition in mM: NaCl 110,

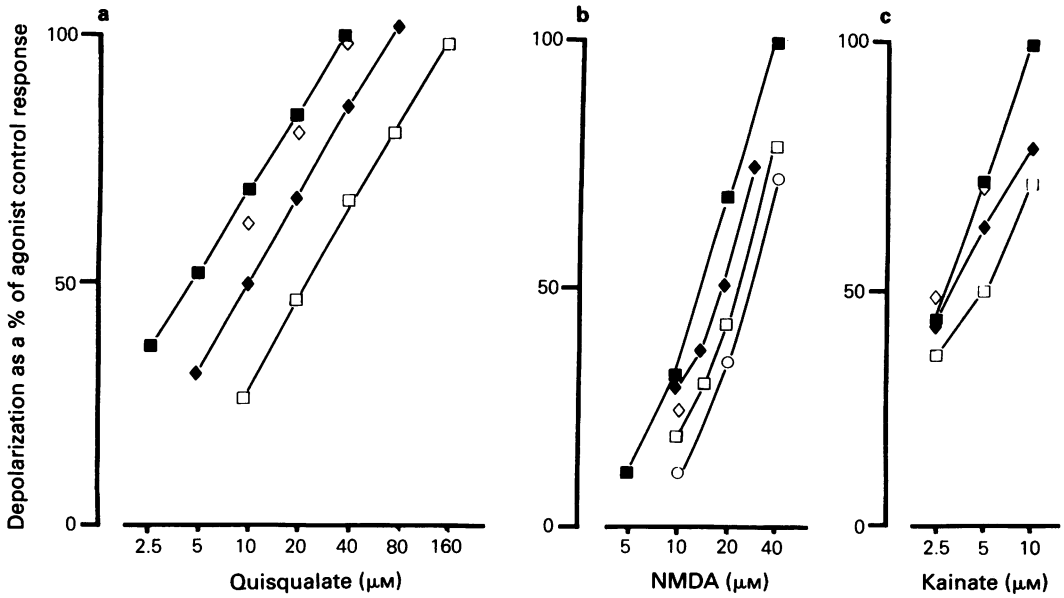

Figure 3 Antagonism of (a) quisqualate, (b) N-methyl-D-aspartate (NMDA) and (c) kainate by FG9065 in three separate experiments on cortical slices. Depolarization of the cortical wedge preparations was expressed as a % of the initial control response to either 40 μM quisqualate, 40 μM NMDA or 10 μM kainate. Dose-response curves were shifted to the right from the controls (■), by 1 μM (◆), 3.16 μM (□) and 10 μM (○) FG9065. Partial recovery (◇) was obtained at the end of the experiment after 60–90 min wash in control medium.

KCl 2, $CaCl_2$ 2, $NaHCO_3$ 10, glucose 11, Tris buffer 10 and adjusted to pH 7.5 with HCl. To examine amino acid depolarizations in the absence of synaptic activity, TTX was added as above.

A lumbar ventral root, insulated from the cord with petroleum jelly/liquid paraffin, was placed in contact with a paper wick Ag/AgCl electrode embedded in 3% agar in saline; d.c. potentials were recorded between this electrode and an indifferent electrode positioned close to spinal cord. Amplification, filtering, recording and analysis were as described above.

Potentials were also recorded in ventral roots (VR) following electrical stimulation (1 ms pulses; 20× threshold; 0.5 Hz) of the segmental dorsal root (DR). These DR-VR reflexes were recorded, amplified, filtered (d.c.-1 kHz), stored on a digital oscilloscope and plotted on a chart recorder. To differentiate between the pharmacological effects on early and late components of the DR-VR potential, peak amplitudes were measured at 5–10 ms and 500–550 ms after the stimulus artefact.

Sources of compounds used

FG9041 and FG9065 are newly synthesised compounds from Ferrosan Research Division. NMDA and D-2-amino-5-phosphonovaleric acid (D-AP5) were purchased from Tocris Chemicals, and kainic and kynurenic acids from Sigma Ltd. AMPA and quisqualate were gifts from Dr Robin Allen and from Dr H. Shinozaki, respectively.

Results

Pharmacology of FG9065 and FG9041 in rat cortical wedges

Quisqualate, kainate and NMDA produced dose-dependent depolarizations of cortical neurones. Responses were reproducible, provided that concentrations of agonists were kept low. Doses of NMDA greater than 80 μM and kainate greater than 20 μM were followed by long lasting reductions in response to all three agonists. This made it difficult to obtain full dose-response curves to all three agonists (see Martin & Lodge, 1985) and hence as controls, the maximum concentrations normally used were NMDA 40 μM, quisqualate 40 μM and kainate 20 μM. FG9041 and FG9065 reduced responses to all three agonists, those to quisqualate being the most sensitive and those to NMDA the least (Figure 2). As can be seen in Figure 3, dose-response curves were shifted to the right in a near parallel manner for all three agonists. In some preparations those for

Table 1 Potency of quinoxalinediones as amino acid antagonists

	Rat cortical wedges				*Frog cord*	
	FG9041		*FG9065*		*FG9041*	
Agonist	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*
Quisqualate	5.9	0.9 ± 0.1	6.2	0.8 ± 0.1	6.6	1.0 ± 0.1
Kainate	5.3	1.0 ± 0.1	5.5	0.8 ± 0.1	6.1	1.1 ± 0.1
NMDA	4.5		4.5		5.1	1.2 ± 0.2

Dose-response curves ($n = >4$) were constructed for each agonist in the absence and presence of various concentrations of the quinoxalinediones. At least 30 min equilibration was allowed before testing the agonists. Dose-ratios were measured and used to compute the pA_2 values. The values for N-methyl-D-aspartate (NMDA) antagonism on cortical wedges are estimates based on the findings that 31.6 μM FG9041 and FG9065 (the highest doses tested) produced dose-ratios close to 2 for NMDA.

kainate appeared to become flatter in the presence of FG9065 suggesting that higher concentrations of kainate were more sensitive to the antagonist. Schild plots were used to calculate the pA_2 values and these are presented in Table 1.

Depolarization by the quisqualate receptor selective ligand, AMPA (Krogsgaard-Larsen *et al.*, 1980), was reduced by FG9065 to a somewhat greater extent than quisqualate itself. Concentrations of 1 and 3.16 μM FG9065 reduced responses to AMPA by 50 and 80%, to quisqualate by 38 and 60%, to kainate by 26 and 36% and to NMDA by <5 and 10%, respectively. An example of the selectivity of FG9065 on such responses is given in Figure 2.

Extracellular field potentials in neocortical slices

Evoked potentials in cingulate and sensorimotor cortex were observed throughout layers I–VI following stimulation of the white matter; the optimal positioning of the stimulating electrode was in the white matter immediately ventral to the recording site. Near threshold the latency of evoked potentials was variable and therefore in order to make satisfactory averages suprathreshold stimuli were used.

The nature of the evoked potential was variable. Sometimes the potentials occurred at short latency (8.4 ± 0.4 ms) even near threshold and were gradually increased in size and duration with stimulus strength, a late (24 ± 2 ms) component often being recruited. The second part was also graded in amplitude. Such potentials were normally less than 1 mV in amplitude and up to 100 ms in duration. In other slices, larger potentials (1–3 mV and >100 ms duration) appeared abruptly with increasing stimulus strength. These were of longer latency which near threshold fluctuated between 50 and 200 ms.

Effect of D-AP5 In all 14 experiments, addition of 10–20 μM D-AP5 (or 20–40 μM DL-AP5) reduced part of the evoked potentials recorded in 1 mM magnesium-containing medium throughout all layers of the neocortex. In the case of the smaller graded type of potential, the later components particularly any secondary events, were AP5-sensitive and the principal effect of this competitive NMDA antagonist was to shorten the duration of the potential. In the case of the larger types of potential, AP5 had a more marked effect, reducing the amplitude and duration so that only an early component remained. Measurements of the effect of AP5 on the maximum amplitude and half decay times showed that at higher stimulus strengths a greater proportion of the potential appears to be mediated by NMDA receptors.

Effects of FG9065 and FG9041 In 9 experiments FG9065 was tested on potentials evoked in all neocortical layers (but primarily in layers II/III and V) in normal medium ($n = 4$) or after addition of DL- or D-AP5 ($N = 5$). Throughout the neocortex, FG9065, 0.2–1 μM, reduced or abolished all types of potentials in a dose-dependent manner. In one experiment a dose of 0.2 μM FG9065 abolished evoked potentials in all six cortical layers. Small increases in stimulus strength reversed the effect of such low doses of FG9065, but after 30–60 min of superfusion with 1 μM FG9065 stimulus amplitudes up to ten times threshold often failed to evoke any potential. Effects of FG9065 were usually reversed within one hour of introducing drug-free solution.

The effects of FG9065 (0.5 μM) and D-AP5 (20 μM) on evoked potentials in 1 mM magnesium were compared on a further 3 slices. A typical example is shown in Figure 4. It can be seen that AP5 abolished a long lasting potential recorded from layer V, although a small early potential remained both at 2× and 7× threshold (T). FG9065, by contrast, abolished all components at $2 \times T$ but at $7 \times T$ a small potential remained which was of variable latency (hence the ripple on the averaged record illustrated in Figure 4).

FG9041 had similar effects to those of FG9065, field potentials evoked in layer II/III being reduced

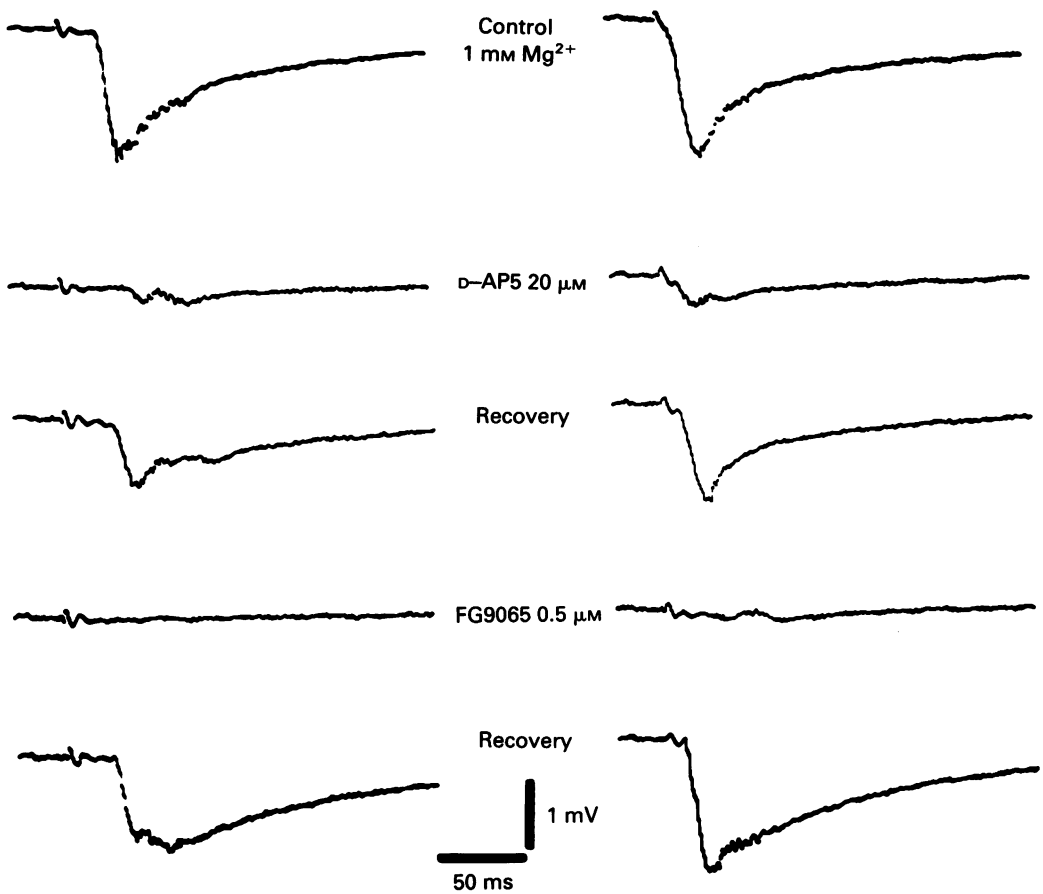

Figure 4 Effect of D-2-amino-5-phosphonovaleric acid (D-AP5) and FG9065 on potentials evoked in layer V of the cerebral cortex following stimulation at 2× (left hand column) and 7× (right hand column) threshold (T) in the underlying white matter. This slice was maintained in 1 mM magnesium-containing medium. Each trace is the average of 8 consecutive potentials recorded extracellularly and illustrates that at 7 × T there is a small early component which is insensitive to 20 μM D-AP5 but this early part is abolished by 0.5 μM FG9065 leaving only a small potential of variable latency in each of the 8 original traces.

or abolished. In all 4 experiments, 10–15 min of superfusion with 1–5 μM FG9041 reduced all components of evoked field potentials. At this time increasing the stimulus amplitude could evoke potentials of similar shape and size. After 30–60 min had elapsed for full equilibration, stimulus amplitudes 10× threshold failed to evoke any potential. One hour washout of FG9041 produced reasonable recovery.

The non-selective excitatory amino acid antagonist, kynurenic acid (0.5–2 mM), was tested on 8 slices after the late parts of the evoked potentials had been reduced by D-AP5. It had similar actions to those described above for the quinoxalinediones, in that the remaining early components were gradually reduced as the kynurenate concentration was increased.

Effects of FG9065 against epileptiform activity in neocortex

When neocortical slices are superfused with magnesium-free medium, large long-lasting epileptiform potentials with many afterpotentials superimposed onto the decay phase appear spontaneously. Similar potentials can be evoked following stimulation of the underlying white matter. This seizure-

Table 2 Effect of quinoxalinediones on epileptiform activity

	Reduction by 1 μM		*Estimated ED_{50}*	
	FG9065	*FG9041*	*FG9065*	*FG9041*
Frequency of bursts (min^{-1})	2 ± 4%	9 ± 4%	>10	7.4 ± 0.7
Afterpotentials per burst	5 ± 3%	48 ± 8%	4 ± 0.3	1.3 ± 0.3

One hour equilibration was allowed before measuring the effect of each dose of drug. The number of bursts and afterpotentials were counted in a 5 min period and compared with similar values of the stable control epileptiform activity. Each drug concentration was tested on 3–6 preparations. The estimated ED_{50} values are only a guide to show that the action of quinoxalinediones on burst frequency is likely to include effects on N-methyl-D-aspartate and/or kainate receptors.

like activity in the neocortex has been shown previously to be sensitive to both competitive and non-competitive NMDA antagonists and to clinically relevant concentrations of anticonvulsant drugs (Aram & Lodge, 1985; 1988).

FG9065, 0.316–10 μM, was tested in 10 experiments. There was no effect on epileptiform activity with doses less than 1 μM, which are selective for non-NMDA receptors in this tissue (see above). At 1 μM and 3.16 μM FG9065, there were respectively

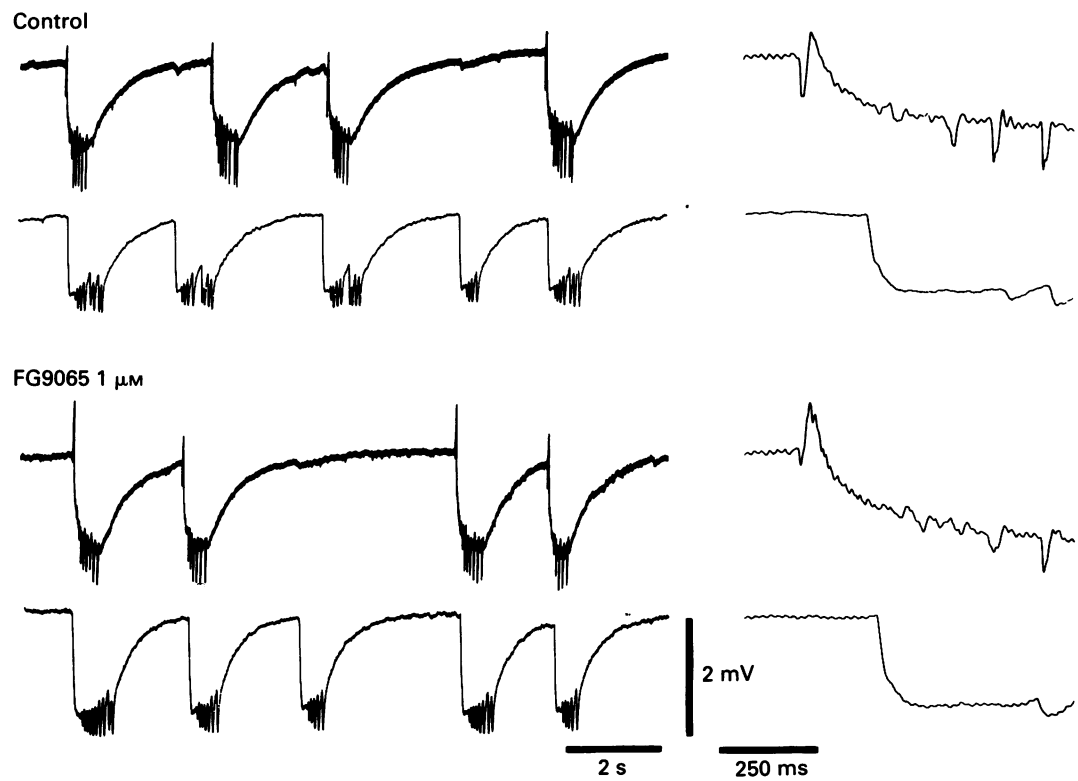

Figure 5 Lack of effect of 1 μM FG9065 on epileptiform activity recorded from a slice superfused with magnesium-free medium. Records were obtained simultaneously from two electrodes placed in opposite hemispheres. It can be seen in the control traces that on three occasions the bursts are almost synchronous in the two records but three other bursts occur independently. On the right is an expanded time-base to show the typical latency (150–220 ms) between bursts in the upper and lower traces. After administration of FG9065 1 μM for one hour, there was no reduction in the frequency or duration of the epileptiform bursts nor in the frequency of coincidence (coupling) between the two seizure sites. The latency between the two bursts was little changed.

15% and 55% reductions in the number of after-potentials per epileptiform burst but only a small reduction in the frequency of the bursts (see Table 2 and Figure 5). FG9065 10 μM produced a 27% reduction in burst frequency. FG9041 proved to be more potent than FG9065 in reducing epileptiform activity but even at 3.16 μM burst frequency was only reduced by 15%.

Because FG9065 blocked potentials in normal medium evoked from the underlying white matter (see above), it was of interest to observe its effects on epileptiform activity evoked between different areas of the neocortex. We have previously shown that in these slices epileptiform activity in one area of the cortex can trigger similar activity in another distant part of the slice (Aram & Lodge, 1988). When tested on such coupled potentials, FG9065 (1 μM) had no effect on the latency or the coupling between the two sites (Figure 5). Thus, in this example 60% of the control bursts at site 1 were coupled to epileptiform events at site 2, there being 38 bursts and 8.4 after-potentials per burst in a 5 min control sample. After one hour superfusion with 1 mM FG9065, 70% of the bursts at site 1 were coupled to events at site 2 and there were now 33 bursts per 5 min and 10 after-potentials per burst. These results suggest that quisqualate receptors do not make a significant contribution to these intracortical synaptic events in the absence of added magnesium. When magnesium was subsequently replaced evoked potentials, recorded at the same sites following corpus callosum stimulation, were abolished by one hour superfusion with 1 μM FG9065.

Effect of FG9065 on synaptic activity in hippocampal slices

Orthodromic field potentials were recorded in the CA1 pyramidal cell layer following stimulation of the Schaffer collateral-commissural pathway. These were characterized by a positive-going field potential superimposed on which, at adequate stimulus intensities, was a negative-going population spike. Actions of drugs were assessed on the amplitude of the population spike.

In all experiments ($n = 10$) the amplitude of the field potentials was markedly reduced by the presence of FG9065 (>250 nM) in a dose-dependent fashion. At 500 mM there was a 41% ($n = 8$) and at 1 μM a 78% ($n = 6$) reduction of the population spike amplitude. An increase in strength of stimulus intensity could restore the size of the response to pre-drug values. As found previously (Collingridge *et al.*, 1983) 20–40 μM D-AP5 caused no appreciable reduction in the size of the responses. All drug effects on field potentials recovered to near control values on returning the slices to control medium.

An example of these effects is presented in Figure 6 where it can be seen that 0.5 μM and 1.0 μM FG9065 and 1.0 μM FG9041 reversibly attenuated the evoked potentials whereas 20 μM AP5 was almost without effect. These results suggest that FG9065 is somewhat more potent as a synaptic blocker than FG9041, which agrees with the order of potency as quisqualate antagonists.

Removal of magnesium from the superfusing medium was followed initially by an enhancement of the size of the evoked population spike and subsequently by the appearance of several later population spikes. This type of epileptiform activity was abolished by 20–40 μM AP5 leaving only the first population spike. In contrast FG9065 500 nM–1 μM produced little change on control epileptiform activity. In some preparations there was a slight reduction in the size of the first population spike, although in the presence of AP5 the remaining early population spike was blocked by FG9065.

Antagonist properties of FG9041 on frog spinal cord neurones

FG9041 reduced depolarizing responses to quisqualate, kainate and NMDA but was most effective against quisqualate. The dose-response curves were shifted to the right in a dose-dependent and near parallel manner. Between 1 and 3.16 μM FG9041, responses to 40 μM NMDA were unaffected whereas those to 40 μM quisqualate were considerably attenuated. Schild plots for FG9041 gave slopes close to unity consistent with competitive blocking action. pA_2 values are shown in Table 1. From these it can be estimated that FG9041 was approximately 3 and 30 fold more potent as a quisqualate antagonist than as an antagonist of kainate and NMDA, respectively.

Effects of FG9041 on frog DR-VR reflexes

Dorsal root stimulation in frog hemisected spinal cord preparations generated a depolarization of several millivolts amplitude and several seconds duration in the corresponding ventral root as described previously (Evans *et al.*, 1979). FG9041 3.16 μM reduced the rate of rise and the peak amplitude of the DR-VR reflex with relatively little effect on the prolonged decay phase. The mean reduction ($n = 4$) of the amplitude in the short latency (5–10 ms) was 76.1 $\pm$ 2.5% (mean $\pm$ s.e.mean), whereas the long latency component was reduced by 28.3 $\pm$ 11%. This differential effect on early and late components was reversed when AP5 and ketamine

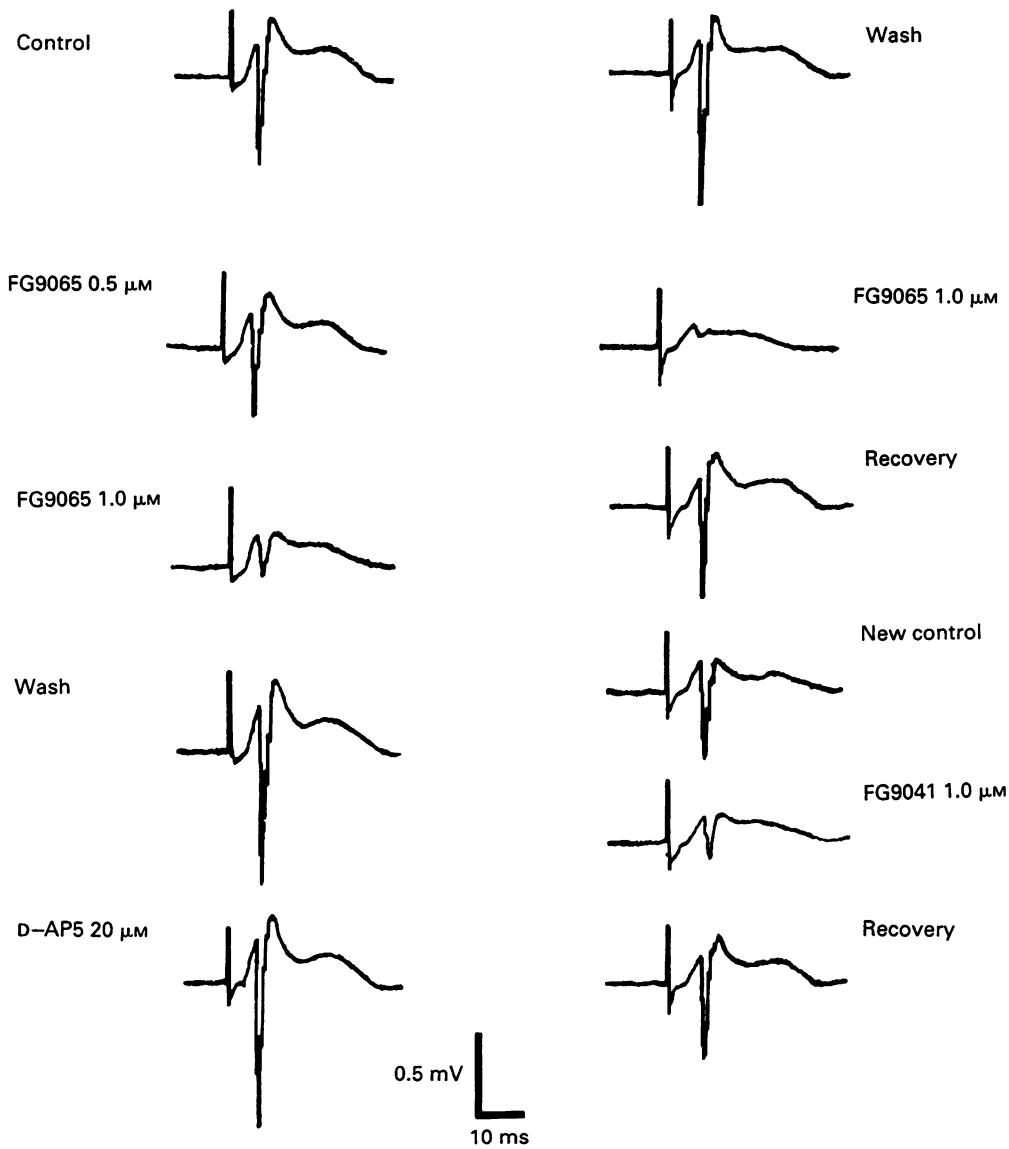

Figure 6 Effect of FG9065 and FG9041 on evoked potentials in the CA1 pyramidal layer of a hippocampal slice maintained in 2 mM magnesium-containing medium following stimulation of the Schaffer collateral-commisural pathway. Each trace is the average of 8 extracellularly recorded potentials. Increasing concentrations of FG9065 from 0.5 to 1 μM reversibly reduced the amplitude of the population spike. D-2-amino-5-phosphonovaleric acid (D-AP5) 20 μM had no effect although subsequent superfusion with 1 μM FG9065 almost abolished the response. After partial recovery, the recording and stimulation conditions were adjusted and the new controls are shown. FG9041 1 μM also reduced the population spike but to a somewhat smaller extent than the same concentration of FG9065.

were tested at doses which were selective for NMDA antagonism. The early component was reduced by 17.9 ± 3% and 22.2 ± 9% and the late component by 87.1 ± 1% and 70.7 ± 6% by AP5 (10 μM) and the non-competitive NMDA antagonist, ketamine (31.6 μM), respectively. An example of this difference between the effects of FG9041 and AP5 is illustrated in Figure 7.

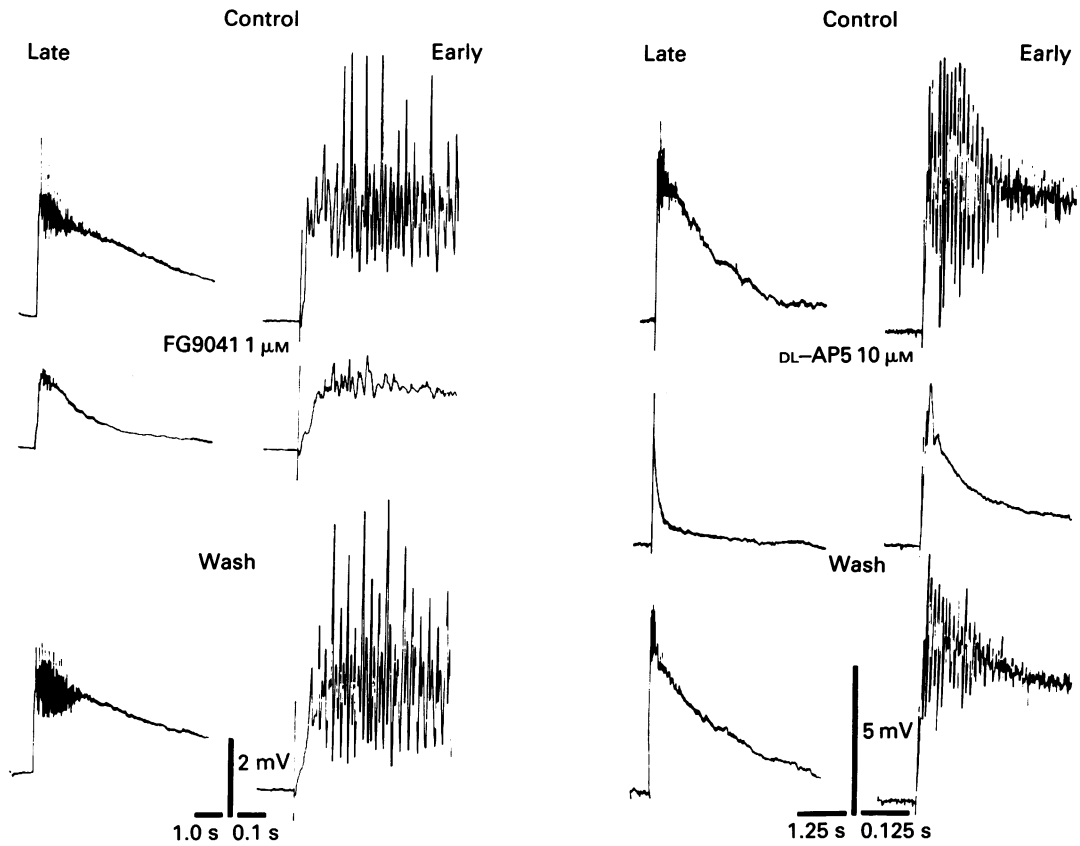

Figure 7 Effect of FG9041 (left hand traces) and DL-2-amino-5-phosphonovaleric acid (DL-AP5; right hand traces) on ventral root reflexes in two hemisected spinal cords of the frog following 0.03 Hz stimulation of the segmental dorsal root. The two preparations were bathed in frog Ringer which contains no added magnesium. Each record has been re-plotted at a faster time base to show the early components of the reflexes. This early component (< 50 ms) was sensitive to 1 μM FG9041 and apparently unaffected by 10 μM DL-AP5 which preferentially reduced the late component.

Discussion

Quinoxalinediones as quisqualate antagonists

Submicromolar concentrations of the quinoxalinediones, FG9065 and FG9041, resulted in a selective reduction of quisqualate-induced depolarizations in both frog cord and rat cortex. The parallel shifts of the dose-response curves and the near unitary values of the Schild plots are consistent with the proposal that the quinoxalinediones act as competitive antagonists at the quisqualate recognition site (Honoré *et al.*, 1987). AMPA appears to be a more potent and selective ligand for the FG9065-sensitive or quisqualate receptor than quisqualate itself. The 30 to 100 fold difference in potency between effects on quisqualate- and NMDA-induced depolarizations is similar to that found in binding studies (Honoré *et al.*, 1987). The potency and selectivity of these two quinoxalinediones is greater than that for existing broad spectrum antagonists (Davies *et al.*, 1984; Ganong & Cotman, 1985; Evans *et al.*, 1987; Kemp *et al.*, 1987; Stone & Burton, 1987) and should be sufficient to allow the drugs to be useful in distinguishing between synaptic events mediated by NMDA and quisqualate receptor types.

Effects of quinoxalinediones on kainate depolarizations are more equivocal. Firstly kainate responses are less reproducible than those of quis-

qualate and NMDA and often produce long lasting reductions in sensitivity to any agonist. Secondly the dose-response curves were often shifted in a non-parallel manner. This decrease in steepness of the dose-respone curves for kainate in the presence of FG9065 may suggest that, at high concentrations, this amino acid is also acting at the quisqualate receptor. The difference in pA_2 values between kainate antagonism and quisqualate antagonism appears therefore to be too small for either quinoxalinedione to be useful for distinguishing between these receptors as mediators of synaptic excitations. Submicromolar concentrations of FG9065, however, would be expected to have their major effect on quisqualate receptors. Interestingly the selectivity on both these *in vitro* preparations was greater than that seen on rat spinal neurones *in vivo*, where kainate- and quisqualate-induced increases in firing rate were equally sensitive to the quinoxalinediones (Honoré *et al.*, 1987). The electrophysiological effects of kainate and quisqualate are in contrast to the selectivity found in binding studies, there being a 5–6 fold difference for displacing [^{3}H]-AMPA and [^{3}H]-kainate from cortical membranes. Such discrepancies call into question the relevance of the high affinity kainate binding site to the electrophysiological effects of this amino acid and suggest that the [^{3}H]-AMPA binding site may, at least in part, mediate the depolarizing effects of both quisqualate and kainate.

Role of quisqualate receptors in synaptic activity in brain and cord

Evoked field potentials in cortical slices could be abolished by the combined use of D-AP5 and kynurenic acid or FG9065, suggesting that excitatory amino acid receptors mediate the majority of afferent inputs into the cerebral grey matter. The partial reversal of this block by increasing stimulus amplitudes is consistent with the competitive nature of these antagonists on cortical neurones (Harrison & Simmonds, 1985; Honoré *et al.*, 1987; Kemp *et al.*, 1987). Since low concentrations of D-AP5 alone reduce parts of the cortical potentials, activation of NMDA receptors seems to contribute at low frequency stimulation despite the presence of 1 mM magnesium and the absence of depolarizing agents or GABA antagonists (see also Thomson *et al.*, 1985; Aram *et al.*, 1987; Jones, 1987). Although D-AP5 always shortened the duration of the evoked potential, this was often accompanied by a reduction in the amplitude of the early component. Addition of the quisqualate antagonist FG9065 alone or in combination with D-AP5 led to abolition of evoked synaptic activity in all layers, implying the importance of non-NMDA receptors in mediating synaptic transmission in the neocortex. In view of the low concentrations of FG9065, which sometimes abolished these events, the subsynaptic receptors are likely to be of the quisqualate type. The complete block of the evoked responses by quisqualate-selective doses of FG9065 suggest that in our slices, prior activation of such receptors is necessary to recruit NMDA receptors. Together with the observation that pure NMDA receptor-mediated synaptic potentials can be demonstrated in cortical neurones (Thomson *et al.*, 1985; Jones, 1987), the present results may suggest that quisqualate receptors mediate an excitatory event at a separate, perhaps earlier, synapse in the pathway from white matter to layer III cortical neurones. Although the failure of FG9065 to prevent coupling between epileptic foci in different parts of the cerebral cortex suggest that some afferent inputs from the white matter do not utilize quisqualate receptors.

The established contribution of NMDA and non-NMDA receptors to synaptic events in the cortex is in contrast to the situation in hippocampal slices where NMDA receptors are thought not to participate in normal low frequency synaptic transmission (Collingridge *et al.*, 1983; 1987; Herron *et al.*, 1986; Kelly & Crunelli, 1988), but to be recruited by high frequency inputs (Collingridge *et al.*, 1983; Harris *et al.*, 1984; Mody & Heinemann, 1987; Stelzer *et al.*, 1987) or when the neurones are depolarized (Herron *et al.*, 1985; Dingledine *et al.*, 1986; Collingridge *et al.*, 1987). The block of potentials evoked in the CA1 region after Schaffer collateral-commisural stimulation by submicromolar concentrations of FG9065 is consistent with this and further suggests that it is the quisqualate receptor subtype that mediates excitation in these pathways. Quisqualate receptor density is particularly high over pyramidal cells in the CA1 region (Monaghan *et al.*, 1985).

The failure of 1 μM FG9065 to affect appreciably epileptiform activity in magnesium-free medium especially in the neocortex is in contrast to its effects on evoked potentials in normal magnesium. This suggests that quisqualate receptors are not important in the generation of such activity. But attenuation of epileptiform activity was seen at 3.16 μM FG9065, a dose which is likely to affect all three receptor subtypes. On spontaneous epileptiform bursts in the neocortex, the preferential reduction by FG9065 and FG9041 of the number of after-potentials per burst rather than burst frequency is more reminiscent of the actions of phenobarbitone and carbamazepine on this type of epileptiform activity (Aram & Lodge, 1988) than of NMDA antagonists which reduce the two parameters in parallel (Aram & Lodge, 1985). In this tissue, phenobarbitone (Simmonds & Horne, 1987) but not carbamazepine (Aram & Lodge, 1987) is a quisqualate antagonist.

The differential effects of FG9041 and AP5 or ketamine on early and late parts of the spinal reflexes in the frog cord is in agreement with previous observations (Evans *et al.*, 1979), suggesting that the monosynaptic reflex is mediated by quisqualate (or kainate) receptors, whereas the prolonged and presumably polysynaptic components utilize NMDA receptors. In this preparation the NMDA-mediated part of the synaptic input is still present after block of non-NMDA receptors by FG9041. It should be noted that magnesium is not a normal constituent of frog Ringer, so there is no voltage-dependent block of the NMDA coupled channels (Nowak *et al.*, 1984; Mayer & Westbrook, 1987) to be overcome by prior depolarization via another receptor subtype.

It would appear that the two quinoxalinediones investigated here have selective non-NMDA actions which have helped to elucidate the role of quisqualate and kainate types of receptor in normal and epileptiform activity in forebrain and spinal cord. Antagonists with greater resolution betwen quisqualate and kainate receptors and particularly with selectivity for kainate receptors would help to separate the putative role of these receptor subtypes.

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