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Antagonism of synaptic potentials in ventral horn neurones by 6-cyano-7-nitroquinoxaline-2,3-dione: a study in the rat spinal cord *in vitro*

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1 The rat spinal cord *in vitro* has been used to assess the effect of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) on the dorsal root evoked extracellular ventral root reflex (DR-VRR) and the intracellular excitatory postsynaptic potential (e.p.s.p.) in ventral horn neurones and motoneurones.

2 CNQX (1–5 μM) produces a selective and dose-dependent reduction in the amplitude of the monosynaptic component of the DR-VRR recorded from lumbar spinal segments.

3 With low intensity dorsal root stimulation CNQX selectively attenuates the amplitude of the short latency intracellular e.p.s.p. (70% reduction, $P<0.005$) and its rise-time (75%, $P<0.01$) without affecting the half-time to decay.

4 When high intensity stimulation is used CNQX significantly attenuates the amplitude of the e.p.s.p. (56%, $P<0.005$), rise-time (76%, $P<0.01$) and abolishes the short latency spike. In addition longer latency synaptic components are attenuated and the half-time to decay significantly reduced (47%, $P<0.005$).

5 The results with CNQX are compared to D-aminophosphonovalerate and discussed in relation to the recruitment of low versus high threshold afferents. The data supports an involvement of non-NMDA receptors in transmission through both mono- and polysynaptic pathways in the ventral horn.

Keywords: Spinal cord; quinoxalinediones; motoneurones; excitatory amino acids; excitatory synaptic potentials

Introduction

The quinoxalinedione derivatives 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) act as amino acid antagonists with a selectivity targeted towards receptors of the non-N-methyl-D-aspartate (NMDA) variety (Honoré *et al.*, 1988). Affinity ratios in binding studies for the quinoxalinediones indicate a strong preference for α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate receptors over NMDA receptors (Honoré *et al.*, 1988). Iontophoretic experiments in hippocampus (Andreasen *et al.*, 1989), spinal cord (Honoré *et al.*, 1988) and other areas of the central nervous system (Davies & Collingridge, 1990) confirm that these antagonists more effectively attenuate excitations produced by exogenous AMPA, quisqualate (Quis) or kainate over NMDA. The quinoxalinediones are therefore useful pharmacological tools for the elucidation of the role of non-NMDA receptors in the vertebrate central nervous system

In the amphibian spinal cord the quinoxalinediones selectively reduce the early monosynaptic component in the dorsal root evoked extracellular ventral root reflex (DR-VRR) whilst the longer latency components are relatively unaffected (Fletcher *et al.*, 1988). In rat Long *et al.* (1990) reported that CNQX was a potent antagonist of the short latency monosynaptic component of the ventral root reflex recorded from sacro-coccygeal segments of the spinal cord. These authors did not describe the effects of CNQX on longer latency components of the reflex. Taken overall the available extracellular data support a role for CNQX-sensitive receptors such as kainate and AMPA receptors in transmission from low threshold afferents onto motoneurones. The resistance of the early monosynaptic component of the ventral root reflex recorded in rat to antagonism by selective NMDA antagonists such as D-aminophosphonovalerate (D-AP5) strengthens this argument (Long *et al.*, 1988). In contrast longer latency components within the ventral root reflex generated by high threshold afferents are strongly attenuated by selective NMDA antagonists (Evans *et al.*, 1982; Evans, 1989) arguing for a role for NMDA receptors in transmission through polysynaptic pathways activated by high threshold afferents. However, the effect of the quinoxalinediones on these longer latency components of the mammalian ventral root reflex is unknown.

Intracellular data on the effects of the quinoxalinedione derivatives on synaptic potentials is accumulating. In the hippocampus, for example, CNQX has revealed an NMDA-mediated component of transmission through the Schaffer collateral-commissural pathway (Davies & Collingridge, 1989). In the spinal cord, intracellular data are available only for the dorsal horn; Yoshimura & Jessel (1990) reported that synaptic responses generated by A- delta and C fibres in substantia gelatinosa neurones are antagonized by CNQX. CNQX also antagonizes a fast excitatory postsynaptic potential produced in deep dorsal horn neurones by low frequency dorsal root stimulation (Gerber & Randic, 1989). To date there are no intracellular data available on the effects of quinoxalinedione antagonists on neurones of the mammalian ventral horn including motoneurones.

In the present study the main aim has been to assess the effects of CNQX on intracellularly recorded dorsal root-evoked synaptic potentials elicited in rat lumbar ventral horn neurones *in vitro*. The selective NMDA antagonist D-AP5 which is known to antagonize preferentially polysynaptic potentials in the mammalian ventral horn (Evans, 1989) has been used for comparison purposes. The two antagonists, CNQX and D-AP5, have also been used in combination with each other. In order to confirm earlier data on the effect of CNQX on the synchronized monosynaptic component of the ventral horn reflex and to determine an effect, if any,

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on longer latency synaptic components, the DR-VRR was recorded. Some of the preliminary results have appeared in abstract form (King & Lopez-Garcia, 1991).

Methods

The spinal cords of young male or female rats 10–12 days after birth and weighing less than 30 g were used for all experiments. For a complete description of the dissection see King *et al.* (1990). Briefly, under urethane anaesthesia (dose: $2\,g\,kg^{-1}$, i.p.) a dorsal laminectomy was performed to reveal the lumbar spinal cord with attached dorsal and ventral roots (L3–6). The cord was rapidly excised and placed in ice cooled artificial CSF for hemisection. The hemisected cord was submerged in a bath, cut surface uppermost, and superfused continuously with oxygenated (95% O_2-5% CO_2) Krebs solution (mM: NaCl 128, KCl 1.9, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, glucose 10, pH 7.4, 28–30°C) at a rate of $5\,ml\,min^{-1}$.

Drugs (NMDA and Quis, D-AP5 and CNQX from Cambridge Research Biochemicals; D-serine from Sigma) were dissolved in this solution and perfused through separate gravity feed inlets in a fixed volume (10–25 ml). Concentrations of 1–5 μM CNQX were chosen for the experiments on the effects of CNQX on synaptic responses for several reasons. Firstly with these concentrations the antagonism could be reversed over a time scale (45–60 min) realistic for maintaining a stable intracellular recording. A feature of the antagonism produced by CNQX is its slow and sometimes incomplete reversibility especially at higher concentrations (Andreasen *et al.*, 1989). Secondly CNQX at a concentration as low as 10 μM is known to have an effect on the strychnine-insensitive glycine receptor which modulates responses to NMDA (Birch *et al.*, 1988).

For intracellular recordings, 3 M potassium acetate-filled microelectrodes with a 60–100 MΩ d.c. resistance were routinely used and signals were amplified through an Axoclamp 2A system. Records were stored on Racal FM tape for subsequent off-line computer analysis. For the averaged samples illustrated, the data were digitized at a minimum rate of 3kHz. All data are expressed as mean ± s.e.mean and for statistical comparisons, Student's *t* test was used. Motoneurones were identified on the basis of the appearance of a short latency spike following low intensity (< 50 μA, 50 μs) antidromic stimulation of the segmental ventral root. Cells which did not respond to antidromic stimulation were classed as ventral horn neurones. The pattern of antagonism produced by CNQX and D-AP5 was equivalent so the data from both groups have been combined. For synaptic activation of neurones, two stimulation conditions, 'low' and 'high', were employed; low intensity was considered as less than 80 μA,60 μs while high intensity was from 100 μA,100 μs up to 500 μA,500 μs. These values are based on those of Thompson *et al.* (1990) who showed, in the same preparation, that the low intensity would activate exclusively A beta, Group I and Group II fibres whilst the high intensity would activate in addition A delta, C and Group III/IV afferent fibres.

For extracellular recording of the segmental DR-VRR the lumbar dorsal and ventral roots were pulled into tight fitting glass suction electrodes constructed from capillary glass and filled with Krebs solution. Constant current stimuli (up to 1.0 mA, 500 μs) were applied via suction electrodes on L3–L6 dorsal roots.

Results

The intracellular data base is comprised of recordings from 27 ventral horn neurones and 12 motoneurones. The mean resting membrane potential for the 39 neurones was − 73.6 ± 1.2 mV and the mean input resistance was 16.2 ± 8 MΩ. The extracellular data were obtained from simultaneous ventral root recordings during the intracellular experiments and from an additional 10 preparations from which only ventral root recordings were performed.

Antagonism of the extracellular DR-VRR

Supramaximal stimulation of dorsal roots produces a short latency (5.7 ± 0.2 ms, $n = 25$), highly synchronized population spike followed by longer latency (> 10 ms) asynchronous synaptic activity. This is recorded extracellularly from the ventral root as the DR-VRR. The effects of the antagonists CNQX (1–5 μM) and D-AP5 (10–50 μM) on the DR-VRR were tested. CNQX (1–5 μM) produced a potent and dose-dependent preferential antagonism of the highly synchronized monosynaptic component of the DR-VRR. This effect is illustrated in Figure 1 which shows an example of a DR-VRR recorded in control versus antagonist containing Krebs solution. Note the strong attenuation of the amplitude of the short latency component in the presence of 5 μM CNQX (Figure 1c) compared to 50 μM D-AP5 (Figure 1b). Superfusion of D-AP5 (50 μM) in contrast had very little effect on the short latency monosynaptic component although a modest decrease of 11% was produced in the example shown in Figure 1b; the mean reduction of this component in the presence of D-AP5 (10–50 μM) was 13 ± 4%.

CNQX up to 1 μM had little effect on the amplitude of the longer latency component of the DR-VRR (see graph of Figure 1). However the highest concentration of 5 μM CNQX produced variable reductions in the amplitude (mean value 32%; range 0–75%). D-AP5 (10–50 μM) more consistently antagonizes this component (Figure 1b) with a 27 ± 6% reduction in amplitude ($n = 15$).

Antagonism of exogenous agonists

The effect of CNQX on bath-applied Quis and NMDA was assessed intracellularly. Superfusion of 30 μM Quis produced a rapidly developing membrane depolarization of 10–20 mV (Figure 2a) which elicited high frequency cell firing and was followed by a return to baseline. A similar profile of excitation was produced by 50 μM NMDA although these responses typically decayed more slowly back to the resting membrane potential. CNQX over a range of 1–5 μM consistently produced a reversible attenuation of the depolarization induced by 30 μM Quis (Figure 2a). The dose-dependency of the reduction in the amplitude of the Quis induced depolarization in the presence of CNQX is illustrated in the graph of Figure 2. CNQX up to 5 μM had no significant effect on NMDA-induced depolarizations (Figure 2b and graph).

Antagonism of intracellularly recorded synaptic potentials

At low stimulus intensities (< 80 μA,60 μs) a short latency (5.6 ± 0.72 ms, $n = 16$) presumed monosynaptic e.p.s.p. was generated. This was followed by a longer latency (28.1 ± 4.1 ms, $n = 17$) subthreshold polysynaptic e.p.s.p. which took several hundred milliseconds (mean 611.9 ± 112.9 ms, $n = 17$) to decay completely. The half time to decay (defined as the time taken for the e.p.s.p. amplitude to decay to half its maximum value) was 104.3 ± 4.1 ms. An example from a single motoneurone is shown in Figure 3a where the stimulus strength was set just below monosynaptic spike threshold at 30 μA,50 μs. At high stimulus intensities (100 μA,100 μs to 500 μA,500 μs) a short latency (5.4 ± 0.45 ms, $n = 14$) monosynaptic spike was followed by longer latency (89.9 ± 29.4 ms, $n = 16$) polysynaptic potentials which took several seconds (mean 4.9 ± 1.3 s, $n = 11$) to decay. The mean half-time to decay for these polysynaptic responses was 322.6 ± 33.6 ms, $n = 16$. Examples of the synaptic excitation produced by high intensity stimulation are shown in Figure 4.

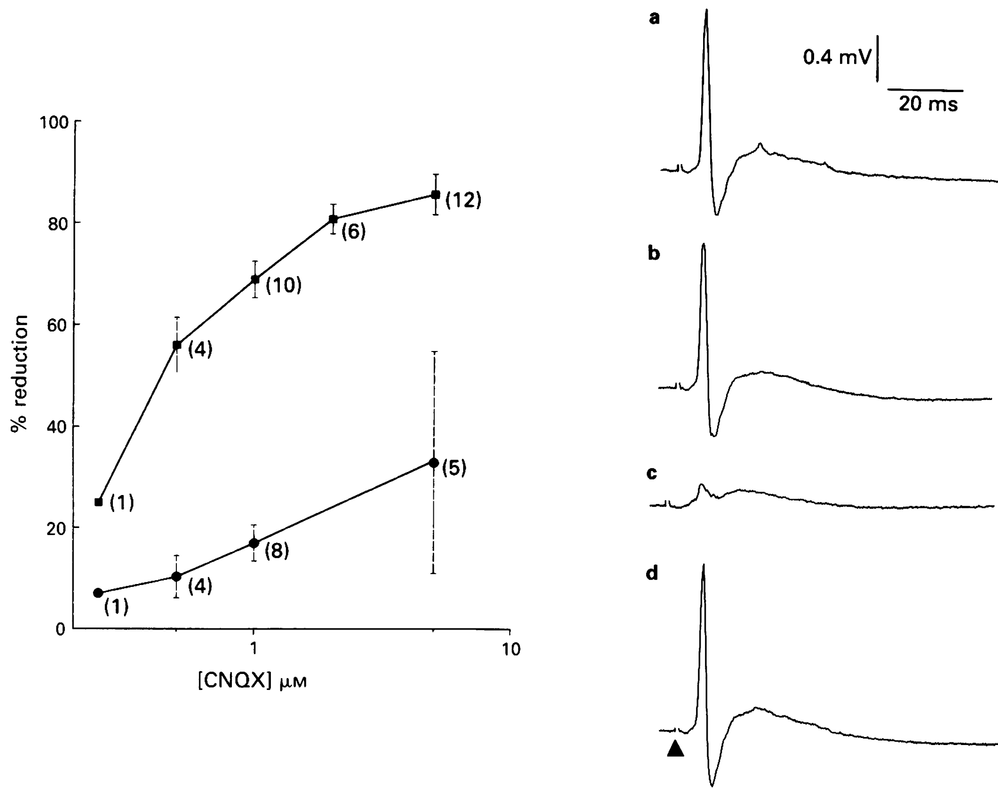

Figure 1 Antagonism of the extracellular dorsal root evoked ventral root reflex (DR-VRR) by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-aminophosphonovalerate (D-AP5). The DR-VRR (average of 5 samples) recorded in (a) control Krebs solution (b) D-AP5 (50 µM) (c) CNQX (5 µM) and (d) 30 min following return to control Krebs solution. Stimulus artifact indicated in this and other figures by (▲). Graph shows the concentration-dependent reduction in the mean amplitude of the short latency (■) and long latency (●) components of the extracellular DR-VRR by CNQX (0.25–5 µM). Values are mean with s.e.mean (vertical bars), number of trials per data point indicated in parentheses.

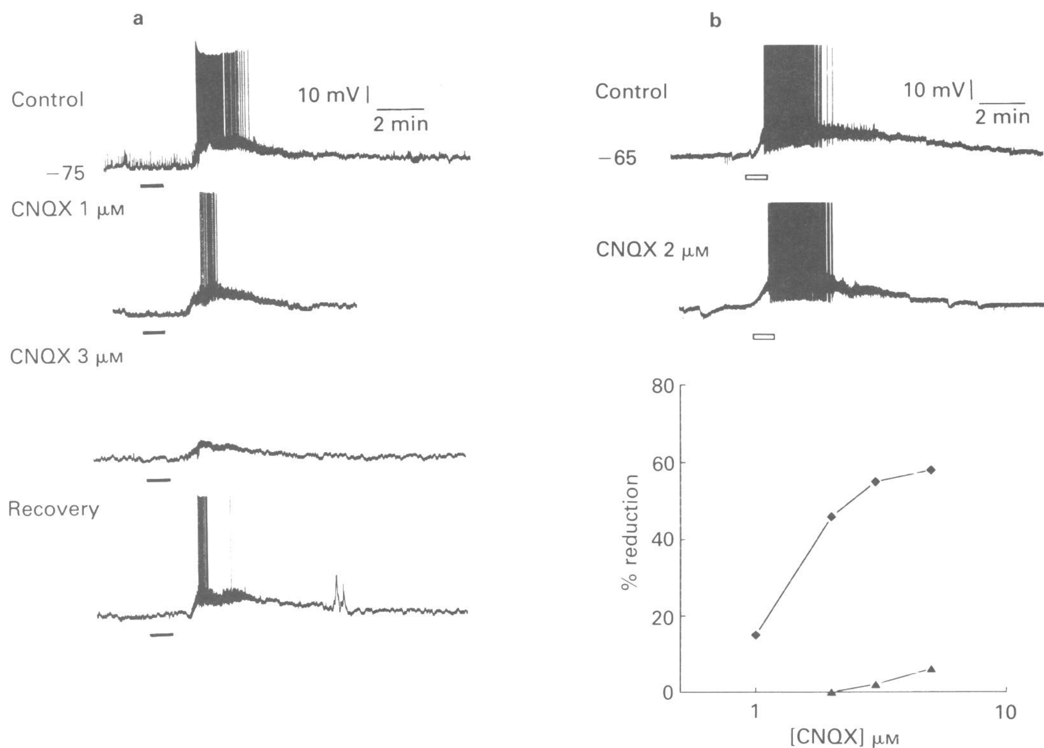

Figure 2 Antagonism of exogenous quisqualate (Quis, 30 µM) and N-methyl-D-aspartate (NMDA, 50 µM) by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). (a) Dose-dependent reduction in Quis depolarization produced by 1 and 3 µM CNQX and partial recovery following 45 min in normal Krebs solution. (b) In another neurone 2 µM CNQX fails to antagonize the NMDA depolarization. Duration of agonist superfusion is indicated by the solid (Quis) and open (NMDA) horizontal bars. Graph shows selective antagonism by 1–5 µM CNQX against Quis (◆) vs NMDA (▲) depolarizations; data points obtained from single preparations except for 3 µM ($n = 2$) and 5 µM CNQX ($n = 3$).

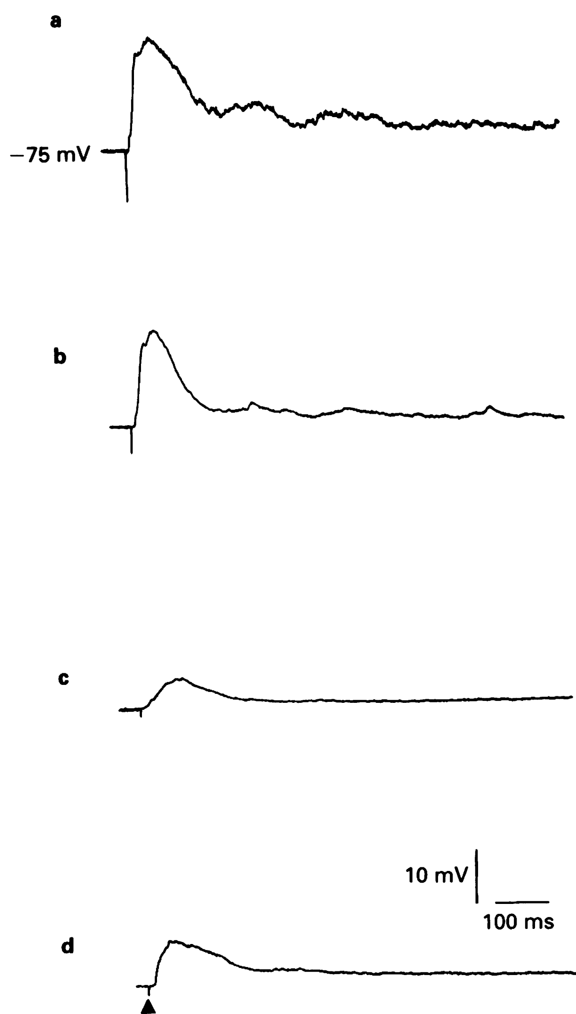

Figure 3 Effects of CNQX and D-AP5 on an e.p.s.p. produced in a motoneurone by low intensity (30 μs,50 μA) dorsal root stimulation. (a) Control Krebs solution; (b) 50 μM D-AP5; (c) 5 μM CNQX; (d) partial recovery following 45 min wash in control Krebs solution. All e.p.s.p. records in this and subsequent figures are the average of 5 sweeps. For abbreviations, see legends to Figures 1 and 2.

The effects of 1–5 μM CNQX and 50 μM D-AP5 were tested against intracellular e.p.s.ps in these two conditions; low versus high intensity stimulation of dorsal roots. When testing the antagonists, three parameters were measured; e.p.s.p. peak amplitude (mV), rise-time (mV ms^{-1}) and half-time to decay (ms). The results are summarized in Table 1.

Under conditions of low intensity dorsal root stimulation CNQX (1–5 μM) and D-AP5 (10–50 μM) had contrasting effects on the e.p.s.p. as illustrated in Figure 3. CNQX strongly attenuated the amplitude of the short latency e.p.s.p. and significantly reduced its rise-time (Figure 3 and Table 1). The mean e.p.s.p. amplitude was reduced from a control value of 12.7 ± 1.7 mV to 3.8 ± 1.0 mV ($n = 16$, $P < 0.005$ level) representing a 70% reduction. The rise-time of the e.p.s.p. reduced by 75% from 6.3 ± 1.9 to 3.9 ± 1.2 ms ($P < 0.01$). CNQX had a small and statistically insignificant effect on the half-time to decay of the e.p.s.p. (Table 1). In contrast the main action of D-AP5 was to reduce the half-time to decay from a control value of 112.5 ± 17.9 ms to 65.0 ± 7.8 ms ($n = 10$, $P < 0.01$) without substantially reducing the amplitude or rise-time of the short latency e.p.s.p. (Figure 3b, Table 1).

At high stimulus intensities CNQX (1–5 μM) reduced the amplitude and rise-time of the short latency e.p.s.p. (Figure 4a and Table 1), the effect of which is to abolish spike initiation (Figure 4a). In the presence of CNQX the short latency e.p.s.p. mean amplitude was reduced from a control value of 16.0 ± 2.2 mV to 6.9 ± 1.8 ($n = 12$, $P < 0.005$, Table 1) representing a 56% reduction while the mean rise-time was reduced by 76% ($P < 0.01$). However, in addition CNQX antagonized longer latency components of the postsynaptic potential as illustrated in the motoneurone of Figure 4a. In this example the e.p.s.p. half-time to decay decreased from a control value of 580 ms to 281 ms in CNQX. The data in Table 1 indicate a statistically significant 47% reduction ($P < 0.005$) in the mean half-time to decay for the synaptic responses in control Krebs solution (mean value of 298 ± 48.8 ms, $n = 12$) versus CNQX (mean value of 156 ± 23.4 ms). D-AP5 reduced the e.p.s.p. mean half-time to decay (Figure 4b and Table 1) indicating the expected potent effect of this antagonist against longer latency polysynaptic potentials. Table 1 shows a modest but significant 28% reduction in the mean amplitude of the short latency e.p.s.p. from a control value of 17.3 ± 1.8 mV to 12.4 ± 2.1 mV ($n = 10$, $P < 0.005$) although the mean e.p.s.p. rise-time and spike initiation were unaffected by D-AP5 (Figure 4b).

The extent of antagonism produced by 1–5 μM CNQX and 50 μM D-AP5 against the short versus long latency com-

Table 1 Effects of the antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 1–5 μM), D-aminophosphonovalerate (D-AP5, 50 μM) on dorsal root evoked e.p.s.ps produced by 'low' (<80 μA,60 μs) and 'high' (100 μA,100 μs to 500 μA,500 μs) intensity stimulation

	Control	*CNQX*	*Control*	*D-AP5*	*Control*	*CNQX + D-AP5*
Low Intensity						
Rise-time (mV ms^{-1})	6.3 ± 1.9	1.6 ± 0.5 (**, 75%)	3.9 ± 1.2	3.8 ± 1.3 (2%)	5.6 ± 1.6	1.1 ± 0.3 (**, 80%)
e.p.s.p. amplitude (mV)	12.7 ± 1.7	3.8 ± 1.0 (***, 70%)	11.9 ± 2.3	10.1 ± 2.8 (15%)	13.1 ± 2.6	2.4 ± 0.9 (***, 81%)
Half-time to decay (ms)	96.4 ± 14.1	72.1 ± 11.3 (25%)	112.5 ± 17.9	65.0 ± 7.8 (**, 42%)	82.5 ± 16.7	22.5 ± 10.3 (***, 72%)
High intensity						
Rise-time (mV ms^{-1})	7.6 ± 2.4	1.8 ± 0.6 (**, 76%)	4.4 ± 1.2	4.1 ± 1.4 (7%)	11.9 ± 3.9	2.0 ± 0.5 (**, 82%)
e.p.s.p. amplitude (mV)	16.0 ± 2.2	6.9 ± 1.8 (***, 56%)	17.3 ± 1.8	12.4 ± 2.1 (***, 28%)	23.7 ± 3.3	6.4 ± 1.6 (***, 72%)
Half-time to decay (ms)	298 ± 48.8	156 ± 23.4 (***, 47%)	272.1 ± 37	170.3 ± 27.6 (***, 55%)	320 ± 69.0	60.1 ± 9.9 (**, 81%)

Results are shown as mean ± s.e.mean values.
Statistical significance levels : $^{**}P < 0.01$; $^{***}P < 0.005$ and % reductions are shown in parentheses.

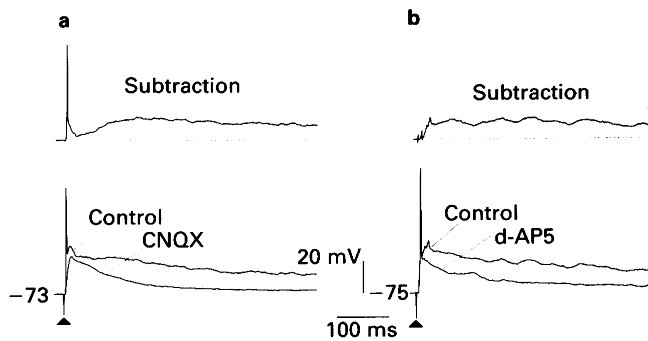

Figure 4 Antagonism by (a) CNQX (5 μM) and (b) D-AP5 (50 μM) of short latency vs long latency e.p.s.ps produced in two motoneurones by high intensity dorsal root stimulation. Note the characteristic reduction in the short latency e.p.s.p. amplitude and rise-time with CNQX. Lower panels; averaged e.p.s.p. elicited in control Krebs solution and reduction of the e.p.s.p. in presence of the antagonist. Upper panels; mathematical subtraction of the two records (control vs antagonist). Resulting profile is that part of the e.p.s.p. removed by either CNQX or D-AP5 (see text for details). Stimulus intensities used were (a) 100 μs,400 μA and (b) 100 μs,200 μA. See legends to Figures 1 and 2 for abbreviations.

ponents of the postsynaptic potential produced by high intensity dorsal root stimulation can be visualized by performing a digital subtraction protocol (Forsythe & Westbrook, 1988). An example is illustrated in Figure 4 (upper panels); the subtracted record represents the synaptic components eliminated by the antagonist. CNQX antagonized the short latency e.p.s.p. with abolition of the monosynaptic spike and additionally attenuated the longer latency subthreshold e.p.s.ps. In contrast, the predominant effect of D-AP5 was an attenuation of the longer latency subthreshold e.p.s.p. (Figure 4b) with no effect on the short latency spike.

CNQX is known to antagonize NMDA responses via an action at the strychnine-insensitive glycine receptor (Birch *et al.*, 1988). This action of CNQX is offset by D-serine and in separate experiments the antagonism produced by 5 μM CNQX against motoneurone e.p.s.ps recorded in the presence of 0.1 – 1 mM D-serine was tested. Under these conditions, CNQX still reduced both the short latency e.p.s.p. spike and the long latency subthreshold e.p.s.ps produced by high intensity dorsal root stimulation. In the example of Figure 5 the amplitude of the short latency e.p.s.p. was reduced from 25 mV to 17 mV and the initial spike abolished by CNQX. The e.p.s.p. half-time to decay decreased from 90 ms to 30 ms. The mean % reduction of the e.p.s.p. amplitude and half-time to decay produced by CNQX in the presence of D-serine was 30% and 52% ($n = 4$) respectively.

The antagonism produced by superfusion of a combination of 5 μM CNQX and 50 μM D-AP5 was assessed against e.p.s.ps produced by low and high intensity dorsal root stimulation. The degree of antagonism produced by the two antagonists applied together exceeded that produced by one antagonist alone (see Table 1). Following low intensity stimulation the postsynaptic e.p.s.p. was virtually abolished in the presence of a combination of CNQX and D-AP5 (Figure 6a), the mean amplitude was reduced from a control value of 13.1 ± 2.6 mV to 2.4 ± 0.9 mV ($n = 10$). This represents a reduction of 81% compared to an average reduction of 70% by CNQX or 15% by D-AP5 alone (Table 1). In 4 ventral horn neurones the e.p.s.p. was completely abolished by CNQX and D-AP5. With high intensity stimulation although the e.p.s.p. amplitude was strongly attenuated, a more distinct synaptic potential with an amplitude of 5 – 10 mV (mean 6.4 ± 1.6 mV) remained in the presence of the two antagonists (Figure 6b). A 72% reduction in the mean e.p.s.p. amplitude was measured (Table 1).

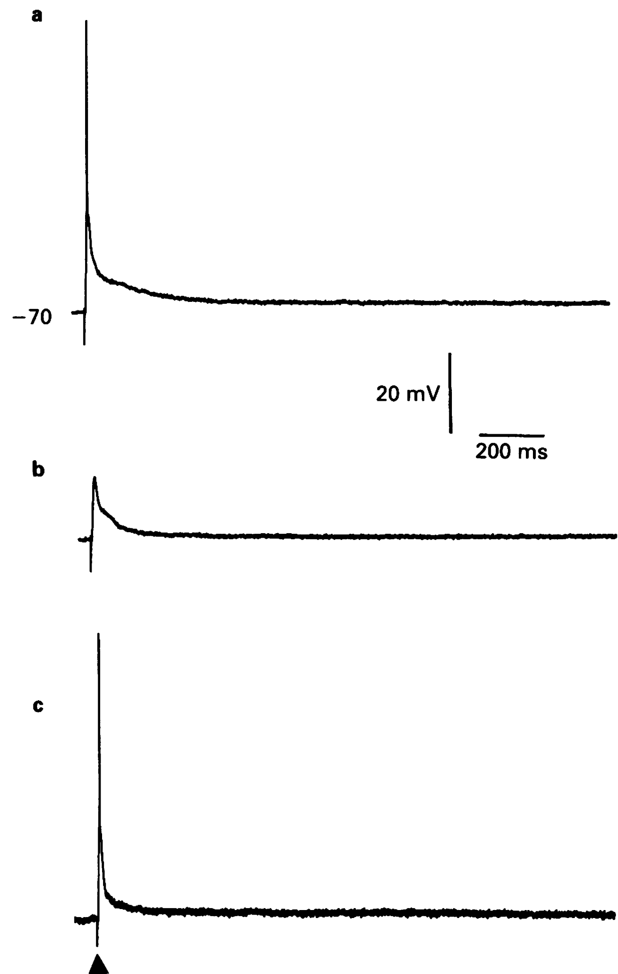

Figure 5 The effect of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the presence of D-serine. Records are of the e.p.s.p. (stimulus intensity 150 μs,150 μA) recorded from a motoneurone in normal Krebs solution containing (a) 1 mM D-serine and (b) 1 mM D-serine plus 50 μM CNQX. Partial recovery after removal of CNQX is shown in (c).

Discussion

In the lumbar segments of the rat spinal cord, as in sacrococcygeal segments (Long *et al.*, 1990), CNQX has a preferential antagonistic effect against the short latency monosynaptic component of the extracellularly recorded DR-VRR. Intracellularly in ventral horn neurones this is seen as a reduction in the amplitude and rise-time of the early e.p.s.p. below that required for spike initiation. The selectivity of CNQX against the short latency synaptic component is demonstrated clearly under conditions of low stimulus intensity where the amplitude of the subthreshold e.p.s.p. is significantly attenuated. The low stimulus intensities used in this study have been shown previously to activate predominantly low threshold, large diameter afferents, namely Group I and II afferents (Thompson *et al.*, 1990; King *et al.*, 1990) which make monosynaptic connections onto motoneurones. This intracellular data strengthens the view that the postsynaptic action of these afferents is mediated mainly by non-NMDA receptors (Evans, 1989) such as the AMPA receptor for which CNQX has a high affinity (Honoré *et al.*, 1988). Previously kynurenate, which similarly but less potently discriminates in favour of non-NMDA receptors, has been shown to be a potent inhibitor of the Ia e.p.s.p. (Jahr & Yoshioka, 1986).

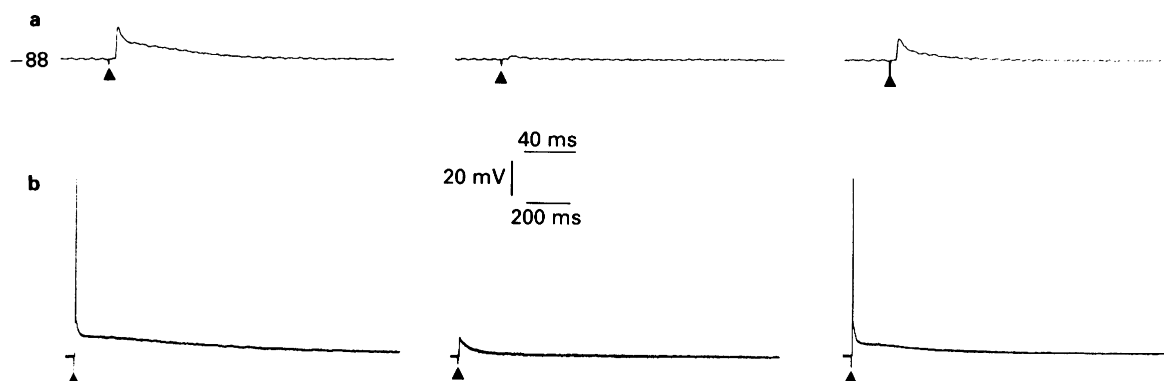

Figure 6 Antagonism produced by co-application of CNQX (5 μM) and D-AP5 (50 μM). Motoneurone e.p.s.p. elicited by (a) low intensity (50 μA,50 μs) and (b) high intensity (200 μA,200 μs) dorsal root stimulation. Left panels (upper and lower): e.p.s.ps recorded in control Krebs medium; middle panels: in presence of a combination of CNQX (5 μM) and D-AP5 (50 μM); (c) recovery. Note presence of residual e.p.s.p. especially in (b). For abbreviations see legends to Figures 1 and 2.

At high stimulus intensities CNQX has a dual effect on the dorsal root evoked e.p.s.p., namely a reduction in the amplitude of the short latency e.p.s.p. with abolition of the spike and a reduction in the half-time to decay of the e.p.s.p. Under these conditions it is likely that both low and high threshold afferents are active in generating the long duration polysynaptic e.p.s.p. in ventral horn neurones. High threshold afferents such as A delta and C arising from muscle, joint and skin mechanoreceptors terminate largely in superficial dorsal horn and activate motoneurones via polysynaptic relays which generate the withdrawal reflex (Eccles & Lundberg, 1959). Intracellular studies *in vitro* show that natural and electrical stimulation of such afferents produce in rat lumbar motoneurones slowly decaying polysynaptic e.p.s.ps similar to the CNQX-sensitive synaptic responses described in this study (King *et al*., 1990; Thompson *et al*., 1990). It must be inferred from this then that such afferents mediate at least part of their postsynaptic effects onto ventral horn neurones including motoneurones via CNQX-sensitive receptors such as kainate or quisqualate/AMPA receptors. However the present data cannot discriminate between an effect of CNQX on the final synapse onto motoneurones or earlier synapses within the pathway. Previous studies using the NMDA antagonist D-AP5 have emphasized its selectivity against the long latency components of both the extracellularly recorded DR-VRR (Evans, 1989) and the polysynaptic e.p.s.p. (Fletcher *et al*., 1988). Such data has been collectively interpreted as indicating a major role for NMDA receptors within multisynaptic spinal pathways. The present study indicates that the contribution of non-NMDA receptor types to synaptic transmission within these pathways may have been considerably underestimated. A role for non-NMDA receptors in polysynaptic spinal pathways has been implicated by studies with non-selective antagonists such as kynurenate which attenuate all components of the DR-VRR (Long *et al*., 1988). Additionally, in rat substantia gelatinosa, A delta and C afferent-evoked monosynaptic e.p.s.ps are antagonized by CNQX and kynurenate (Yoshimura & Jessel, 1990).

Under appropriate conditions NMDA-mediated synaptic transmission in the central nervous system can be facilitated by glycine (Johnson & Ascher, 1987). This effect is mediated by an action of glycine at a strychnine-insensitive binding site which is allosterically linked to the NMDA receptor and is susceptible to antagonism by CNQX (Birch *et al*., 1988). Several factors in this study argue against antagonism of NMDA-mediated responses by an action at this site by CNQX as an explanation for the observed reduction of longer latency polysynaptic e.p.s.ps in ventral horn cells. The concentration of CNQX used in this study was 1–5 μM which is below the limit of 10 μM at which the effect on NMDA becomes significant (Birch *et al*., 1988; Hablitz & Sutor, 1990; Davies & Collingridge, 1990). Furthermore this concentration-range of CNQX antagonizes depolarizations to exogenous Quis with little effect on NMDA. More significantly, superfusion of D-serine which reduces the effects of CNQX on this glycine binding site (Birch *et al*., 1988) does not influence the profile of antagonism produced by CNQX in ventral horn neurones. In the rat spinal cord, glycine fails to augment the depolarization to NMDA (Birch *et al*., 1988) indicating that it may be present at saturating concentrations endogenously. Supporting this is the fact that in the ventral horn, unlike some other areas e.g. cortex (Thomson, 1990), CNQX discriminates effectively between NMDA and Quis even in the absence of glycine or D-serine.

The profile of antagonism produced both intracellularly and extracellularly for D-AP5 is similar to that described previously, namely a selective attenuation of longer latency synaptic components (Evans *et al*., 1982; Fletcher *et al*., 1988; Long *et al*., 1988). However, when high intensity dorsal root stimulation is employed, a modest reduction of the amplitude of the short latency e.p.s.p. is measured. The reason for this is unclear since others have reported no consistent effect of D-AP5 on this component (Flatman *et al*., 1987; Long *et al*., 1988). Such a result may be explained by postulating a minor NMDA receptor-mediated contribution to the synaptic activation of motoneurones by low threshold Group I and II afferents. A role for NMDA receptors in monosynaptic transmission has been demonstrated in vertebrate spinal cord (Corradetti *et al*., 1985; Dale & Roberts, 1985) and in cultured hippocampal and spinal neurones (Forsythe & Westbrook, 1988). In a population of dorsal horn neurones, D-AP5 reduced the peak amplitude of A delta-evoked monosynaptic e.p.s.ps by around 20% (Yoshimura & Jessel, 1990).

Co-application of CNQX and D-AP5 fails to abolish completely ventral horn synaptic potentials particularly under conditions of high intensity dorsal root stimulation. Similar findings have been reported for substantia gelatinosa neurones (Yoshimura & Jessel, 1990), vertebrate motoneurones (Alford & Grillner, 1990) and hippocampal neurones (Davies & Collingridge, 1989). These data can be interpreted in two ways; an involvement of receptors to putative transmitters other than amino acids or a high concentration of endogenous transmitter in the synaptic cleft overcoming competitive blockade. Further pharmacological studies will be required to differentiate between these two possibilities.

The use of a diverse range of amino acid receptor antagonists in the spinal cord has led to a generalized scheme whereby it is proposed that non-NMDA receptors mediate short latency, monosynaptic transmission whereas NMDA

receptors mediate predominantly longer latency polysynaptic transmission (Evans, 1989). The results of this study with CNQX do not contradict this but also suggest that it may be simplistic and that a significant component of polysynaptic transmission, at least onto ventral horn neurones, is relayed via non-NMDA receptors such as AMPA/quisqualate or kainate type. Similarly, on the basis of evidence presented here and other studies (Corradetti *et al.*, 1985; Dale & Roberts, 1985; Forsythe & Westbrook, 1988), a case can be made for involvement of NMDA receptors in short latency mono- or di-synaptic responses. Ultimately, independent classes of muscle or cutaneous afferents and the involvement of the different types of amino acid receptors in transmission onto second order dorsal or ventral horn neurones must be analysed separately rather than collectively since it is likely that differences exist. For example in the ventral but not dorsal horn, nociceptive sensory responses appear to be in part mediated by NMDA receptors (Headley *et al.*, 1987). One desirable way to achieve this would be to test selective amino acid antagonists against naturally evoked sensory responses (Headley *et al.*, 1987; Honore *et al.*, 1988; Salt & Eaton, 1989).

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