L-Glutamate diethyl ester and deaminated analogues as excitatory amino acid antagonists in rat cerebral cortex

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1 The effects of L-glutamate diethyl ester (GDEE) HCl, glutarate diethyl ester (GlrDEE) and glutarate dimethyl ester (GlrDME) on depolarizing responses to α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate (Kain), N-methyl-D-aspartate (NMDA) and quisqualate (Quis), and spontaneous paroxysmal discharges (SPDs) were examined. Experiments were performed on slices of rat cingulate cortex using the *in vitro* grease gap recording technique in nominally Mg^{2+} -free Krebs medium.

2 GDEE HCl (3–14 mM) caused a concentration-dependent depolarization of the d.c. baseline potential. L-Glutamate (0.1–0.5 mM), HCl (15 mM) and sucrose (30 mM) also depolarized the baseline. GlrDEE (3–12 mM) and GlrDME (4–26 mM) had no consistent effect on baseline potential.

3 GDEE HCl (10 mM) had no effect on depolarizing responses to AMPA, Kain and NMDA, but caused potentiation of those to Quis with a dose-ratio of 0.53 (0.44–0.63) ($n = 4$). In two other experiments, where the depolarization of the baseline induced by GDEE HCl was large, a depression of Quis response amplitude was observed.

4 GlrDEE (10 mM) antagonized depolarizing responses to Kain, and to a lesser extent NMDA, with dose-ratios of 2.14 (1.92–2.38) and 1.61 (1.39–1.87), respectively. This concentration of GlrDEE had no effect on AMPA responses, but potentiated Quis responses, with a dose-ratio of 0.64 (0.58–0.71).

5 GlrDME (10 mM) antagonized depolarizing responses to Kain and to Quis, with dose-ratios of 1.66 (1.48–1.85) and 1.22 (1.15–1.29), respectively, and had no effect on responses to NMDA.

6 The SPDs were inhibited by GDEE HCl (IC_{50} 6.7 ± 0.37 mM), GlrDEE (IC_{50} 5.6 ± 0.38 mM) and GlrDME (IC_{50} 10.4 ± 0.73 mM).

7 In conclusion, there is little evidence that GDEE HCl is an antagonist of the postsynaptic excitatory amino acid receptors in the rat neocortex, and its effects may result from its contamination with L-glutamate and increased osmolarity of the bathing medium at high concentrations. The deaminated analogues of GDEE are very weak Kain antagonists.

Keywords: Amino acids; cerebral cortex; glutamate; synaptic receptors

Introduction

The electrophysiological study of excitatory amino acid (EAA) action has led to the proposal of three classes of EAA postsynaptic receptors, based on the action of specific or relatively selective antagonists (Watkins & Evans, 1981). These receptors have been designated α -amino-3-hydroxy-5-methylisoxazole-4-propionate/quisqualate (AMPA/Quis)-, kainate (Kain)- and N-methyl-D-aspartate (NMDA)-preferring receptors on the basis of their agonist specificity. The differentiation between NMDA and non-NMDA receptors is made more definitive by the specific blockade of NMDA receptors by antagonists. In addition, the quinoxaline-2,3-diones are potent and relatively selective antagonists of non-NMDA receptors (Honore *et al.*, 1988). The distinction between the non-NMDA receptors AMPA/Quis and Kain is, however, less clearly defined and is based upon the relative selectivity of antagonists such as γ -D-glutamylglycine and γ -D-glutamyl-amino-methylsulphonate (Davies & Watkins 1981; 1985). The original distinction was made with the observation of a selective blockade of microiontophoretically applied Quis and AMPA with L-glutamate diethyl ester (GDEE) in the spinal cord (McLennan & Lodge, 1979; Krogsgaard-Larsen *et al.*, 1980). This has resulted in the proposal of specific antagonist properties for GDEE against AMPA/Quis compared with Kain and NMDA (McLennan, 1983). However, GDEE has also been shown to suppress spontaneous activity and acetylcholine excitation in the spinal cord (MacDonald *et al.*, 1977; McLennan & Lodge, 1979), and to hyperpolarize spinal neurones (Zieglgänsberger & Pulil, 1973) indicating a more non-specific inhibitory action. In addition, a subsequent *in vitro*

study of GDEE in the frog spinal cord showed no antagonist activity at the three postsynaptic EAA receptors at concentrations up to 5 mM, and some apparently non-specific effects, with the inhibition of synaptic activity (Evans *et al.*, 1979). In the hippocampus, though, a more selective action of GDEE against EAAs over ACh has been observed (Segal, 1976; Spencer *et al.*, 1983).

The low potency of GDEE when bath-applied has limited its further use as a pharmacological tool in electrophysiological experiments. However, this has not prevented the use of GDEE in both biochemical and behavioural experiments as an antagonist of EAA-mediated action. GDEE has been shown to inhibit the release of [3H]-D-aspartate from, and the mobilization of intracellular Ca^{2+} in cultured cerebellar granule cells (Drejer *et al.*, 1986; Bouchelouche *et al.*, 1989). Behavioural studies have shown that GDEE and some deaminated analogues protect against seizures induced by DL-homocysteine thiolactone or Quis, but not seizures induced by pentylenetetrazole (Freed, 1986; Freed & Braun, 1988).

The aim of the present study was to evaluate GDEE HCl, and two deaminated analogues glutarate diethyl ester (GlrDEE) and glutarate dimethyl ester (GlrDME), against depolarizing responses to the EAA receptor agonists AMPA, Kain, NMDA and Quis, and the spontaneous paroxysmal discharges (SPDs) induced by the removal of Mg^{2+} from the bathing medium, in slices of the rat cingulate cortex *in vitro*.

Methods

All experiments were performed on the rat cingulate cortex preparation (Harrison & Simmonds, 1985). Male Wistar rats (200–300 g) were killed by decapitation and the whole brain removed. Once submerged in chilled Krebs medium containing 1 mM Mg^{2+} , serial transverse sections of forebrain (500 μ m)

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were made with a Vibroslice (Campden Instruments). Sections at the mid-line and lateral to the cingulate cortex resulted in wedge-shaped pieces of tissue containing cingulate cortex and underlying white matter. The slices were then incubated in Krebs medium and gassed for 1.5 h before being placed in two-compartment baths. The majority of the cortical tissue was in one compartment, with the remaining cortex and underlying white matter projecting through a greased slot in a two-piece barrier dividing the two compartments. Each compartment was perfused independently with Krebs medium at $22 \pm 1^\circ\text{C}$. The Krebs medium contained (mM): NaCl 118, KCl 2.1, KH_2PO_4 1.2, CaCl_2 2.5, NaHCO_3 25, MgSO_4 1.0 and glucose 11, and was gassed continuously with 95% O_2 and 5% CO_2 . However, the Krebs in the cortical compartment was modified so that it contained no Mg^{2+} . This allowed the appearance of SPD-like activity, probably through the removal of the voltage-dependent block of NMDA receptors by Mg^{2+} (Nowak *et al.*, 1984) and the increased efflux of L-glutamate from presynaptic terminals (Smith *et al.*, 1989). In those experiments where drug effects on responses to bath applied EAA agonists were examined, 0.1–0.2 μM tetrodotoxin (TTX) was present in the Krebs medium in the cortical compartment to suppress this SPD-like activity.

Drug solutions diluted in the Krebs medium were passed through the cortical compartment only. The potential difference between the two compartments was recorded continuously with a pen recorder, and negativity induced in the cortical compartment was interpreted as a depolarization of the cortical tissue. The recordings were made by use of amplifiers with low pass frequency filters, so that high frequency noise was eliminated. Although, this interfered with the frequency response of the recording system, limiting any potential conclusions about changes in amplitude and duration of high frequency events such as the SPDs, their frequency could be measured. Once the frequency of the SPD-like activity had stabilized, the control frequency was measured over a 20 min period, and then the prospective agents perfused at a range of increasing concentrations in the Krebs medium, for 60 min each. The frequency over the last 20 min period was then measured. At the end of the drug applications, the slices were returned to perfusion with control Krebs medium for at least 60 min to monitor recovery, and again the frequency was measured over a 20 min period. Any drug-induced changes were expressed as a percentage inhibition of control frequency. The results were fitted by non-linear regression analysis based on the logistic equation;

$$y = 100 \times (C^p / C^p + K^p)$$

where y = % inhibition, C = concentration, $K = \text{IC}_{50}$ and p = slope constant and for each experiment an IC_{50} value was calculated. All statistical analyses were performed using Student's t test.

In experiments examining the influence of GDEE HCl, GlrDEE and GlrDME on the depolarizing responses to EAAs, AMPA (RS- α -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid, Tocris), Kain (kainic acid, Sigma), NMDA (N-methyl-D-aspartic acid, Tocris) and Quis (quisqualic acid, Sigma) were routinely used as agonists. Concentrations on the approximately linear portion of the dose-response (DR) curves for these agonists were selected (AMPA 2.5–5 μM , Kain 5–20 μM , NMDA 10–40 μM and Quis 5–20 μM) and perfused for 2 min. Responses were measured at peak amplitude. The prospective agents were perfused for 30 min prior to and then during the re-determination of agonist responses bridging the control responses. From the parallel shift of the DR lines between these responses, dose-ratios for the EAA agonists were calculated from equipotent concentrations of the agonists in the absence and presence of GDEE and deaminated analogues. All the agonists were prepared as 10 mM solutions in Krebs medium, except tetrodotoxin (TTX, Sigma), which was prepared as a 1 mM solution in 0.01 M acetic acid, and diluted in the Krebs medium immediately before use. GDEE HCl (L-glutamate diethyl ester HCl salt >99% purity by thin

layer chromatography (t.l.c.), Sigma), GlrDEE (glutamate diethyl ester >99% purity by gas chromatography (g.c.), Sigma), GlrDME (glutamate dimethyl ester $\approx$ 98% purity by g.c., Sigma), L-glutamate Na, glutaric acid and sucrose (all Sigma) were dissolved directly into the Krebs medium. All base chemicals (Analar) were obtained from BDH.

Results

Baseline effects

GDEE HCl depolarized the cortical tissue in the absence and presence of TTX. At concentrations between 3 and 14 mM GDEE HCl, in the absence of TTX, there was an initial rapid depolarizing phase, which reached a plateau within 10–15 min (Figure 1a). This effect was concentration-dependent (Figure 2). There were additional ramp-like depolarizing shifts with 14 mM GDEE HCl, ranging between 0–0.15 mV h^{-1} , following the initial rapid depolarization. In the presence of TTX, 10 mM GDEE HCl produced a mean depolarization of 0.16 ± 0.022 , consistent with the depolarizations seen in the absence of TTX (Figure 2). In addition, where the tissue had been exposed to AMPA or Quis, 10 mM GDEE HCl also produced ramp-like depolarizing shifts with rates ranging between 0.1–0.18 mV h^{-1} and 0.09–0.48 mV h^{-1} for AMPA and Quis, respectively. The rates of the ramp-like depolarizing shifts for NMDA- and Kain-exposed slices never exceeded 0.05 mV h^{-1} in the presence of 10 mM GDEE HCl and TTX. In contrast, both GlrDEE (3–12 mM) and GlrDME (4–26 mM) had no concentration-dependent effects on baseline potential in the absence of TTX ($P > 0.5$ and $P > 0.05$, respectively, correlation analysis). In the case of GlrDEE, there were no significant changes ($P > 0.05$) in baseline potential at any individual concentrations. However, in the presence of TTX, 10 mM GlrDEE caused a small but significant hyperpolarization of baseline (0.08 ± 0.030 mV, $n = 20$). With GlrDME, in the absence of TTX, the effect of individual concentrations on baseline potential varied, with significant mean hyperpolarizing responses at some concentrations (4, 5.5, 13 and 16 mM, range of means 0.07–0.14 mV, $P < 0.05$) and not others (7.5, 8, 10, 14 and 26 mM). In the presence of TTX, 10 mM GlrDME produced a hyperpolarization of 0.14 ± 0.031 mV ($n = 17$). Although 2 and 6 mM HCl (in the absence of TTX) had no significant effect on the baseline d.c. potential ($P > 0.2$), the application of 15 mM HCl (Figure 1d) resulted in a small depolarization, which rapidly attained a plateau of 0.16 ± 0.03 mV ($n = 4$, $P < 0.05$). L-Glutamate (0.1 and 0.2 mM) produced small transient depolarization of the baseline when first applied, which rapidly returned towards the control level in the absence of TTX (Figure 3a). However, with 0.5 mM L-glutamate there was a sustained depolarization of 0.10 ± 0.014 mV ($n = 5$, $P < 0.005$) following the initial transient depolarization (Figure 3a). No effect on the baseline was observed with 5 mM glutaric acid (data not shown) and 10 mM sucrose (both $P > 0.2$), but 30 mM sucrose (Figure 3b) resulted in a small depolarization 0.07 ± 0.014 mV ($n = 6$, $P < 0.01$) in the absence of TTX.

Excitatory amino acid-induced depolarizations in the presence of tetrodotoxin

GDEE HCl (10 mM) had no significant effect on responses to AMPA, Kain and NMDA (Figure 4). Against Quis, however, there was a potentiation of the depolarizing responses producing leftward parallel shifts of the DR line with a mean of 0.28 log units ($n = 4$). In two other experiments where there was a relatively rapid rate of baseline depolarization by GDEE HCl, responses to Quis became progressively more depressed in amplitude, as the baseline depolarized (Figure 5). This effect was reversed after returning to control Krebs medium. No such effect was observed with AMPA responses.

Figure 1 Continuous chart recordings (d.c.) of the spontaneous paroxysmal discharge (SPD)-like activity in the absence of Mg^{2+} , with the effect of increasing concentrations of (a) L-glutamate diethyl ester (GDEE), (b) glutarate diethyl ester (GlrDEE), (c) glutarate dimethyl ester (GlrDME) and (d) HCl on the baseline potential and the frequency of SPDs, and the reversal upon return to control Krebs medium. Cortical negativity is upwards.

Figure 2 The concentration-effect data for the initial depolarization of baseline potential by L-glutamate diethyl ester (GDEE) HCl in the absence of tetrodotoxin (TTX). The points represent values from individual experiments ($n = 38$). Using linear regression analysis, a line was fitted with a slope of 0.25, and a correlation coefficient of 0.47 ($P < 0.003$). The filled square and vertical line represent the mean $\pm$ s.e. mean of the initial depolarization produced by 10 mM GDEE HCl in the presence of TTX ($n = 22$).

GlrDEE (10 mM) had a different spectrum of activity to GDEE, antagonizing responses to Kain and NMDA (Figures 6 and 4), with parallel rightward shifts of the DR lines. For three of the five experiments against Kain responses in Figure 4 recovery was monitored, with a complete reversal of the antagonist action observed in two, but not in the third. However, like GDEE, GlrDEE had no effect against responses to AMPA and potentiated those to Quis, producing parallel leftward shifts of the DR line with a mean of 0.20 log units.

GlrDME (10 mM) antagonized responses to Kain and to a much lesser extent to Quis, but had no effect on responses to NMDA (Figure 4). For two of the five experiments against responses to Kain recovery was monitored, with a complete reversal of the antagonism by GlrDME in both. GlrDME was not tested against AMPA.

Spontaneous paroxysmal discharge-like activity

GDEE HCl, GlrDEE and GlrDME all reduced the frequency of SPD-like activity in a concentration-dependent manner (Figures 1 and 7). The most potent was GlrDEE with an IC_{50} of 5.6 ± 0.38 mM ($n = 12$). The threshold concentration for this effect was about 4 mM with complete inhibition at 12 mM. Complete recovery of control frequency was observed within

Figure 3 Continuous chart recordings (d.c.) of the spontaneous paroxysmal discharge (SPD)-like activity in the absence of added Mg^{2+} , with the effect of increasing concentrations of (a) L-glutamate (L-Glu), and (b) sucrose (Suc) on baseline potential and the frequency of SPDs, and the reversal upon return to control Krebs medium. Cortical negativity is upwards.

40 min of returning slices to control Krebs medium. GDEE HCl was slightly less potent with an IC_{50} of 6.7 ± 0.37 mM ($n = 13$). A virtually complete inhibition was observed at about 14 mM. Recovery was again achieved after about 40 min on return to control medium. GlrDME was the least potent as an inhibitor of the SPDs, with an IC_{50} of 10.4 ± 0.73 mM ($n = 23$). The threshold concentration for the effect was about

4.5 mM, with complete inhibition occurring at 20 mM. Again complete recovery was observed within about 40 min of return to control Krebs medium.

In view of the high concentrations required, it is possible that the buffering capacity of the Krebs medium is compromised, so the effect of these compounds on the pH of the Krebs medium was investigated. Before the drug solutions were perfused, the pH of continuously gassed control Krebs medium was monitored. Drug was then added to the Krebs medium and the pH was continuously monitored until a new pH was obtained, stable for 10 min, while being continuously gassed. Monitoring continued in the perfusion reservoir prior to and during perfusion. The lag time between perfusion reservoir and the tissue bath was ≈ 30 s, and the pH increase between reservoir and bath was found to be the same with either control Krebs medium or that acidified with 15 mM HCl (0.16–0.21 and 0.16–0.20 respectively, $n = 7$). Neither GlrDEE nor GlrDME had any effects on pH, up to concentrations of 30 mM (increase in pH < 0.05 pH units). However, the addition of GDEE HCl to the Krebs medium resulted in acidification, such that at 10 mM and 30 mM concentrations, there were decreases in pH of 0.12 and 0.34 pH units, respectively.

In a few experiments the effect of decreasing pH on the SPDs was examined by the addition of HCl solution to the Krebs medium. The effect of HCl on the pH of the Krebs medium was monitored in the same way as that with GDEE HCl, GlrDEE and GlrDME. The acidified Krebs medium was then perfused for 60 min, as with the drug applications. Again any change in the frequency of the SPDs was measured as a percentage inhibition of control frequency. The addition of HCl to give final concentrations of 2 mM and 6 mM produced decreases in pH of < 0.05 and 0.10 pH units, respectively, but

Figure 4 The effect of L-glutamate diethyl ester (GDEE), glutarate diethyl ester (GlrDEE) and glutarate dimethyl ester (GlrDME) (all 10 mM) on the dose-response lines for α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate (Kain), N-methyl-D-aspartate (NMDA), and quisqualate (Quis) in the presence of tetrodotoxin. Lateral leftward shifts or potentiation and rightward shifts or antagonism were measured as log dose-ratios. The horizontal lines represent the s.e. mean and the bars the means of $n = 5$ experiments, except those for AMPA and Quis in GDEE, $n = 4$. * $P < 0.05$, ** $P < 0.01$.

Figure 5 A continuous recording of d.c. potential in the presence of tetrodotoxin (TTX) showing the depolarizing responses to quisqualate (Quis, Q), in the absence and presence of 10 mM L-glutamate diethyl ester (GDEE). It illustrates the large ramp-like depolarization of baseline (0.48 mV h^{-1}) coincident with occlusion of Quis responses seen in two experiments. The reversal of this depression is illustrated on the lowest trace at +100 min and +200 min after re-application of control Krebs medium.

had no significant effect ($P > 0.1$) on the frequency of SPDs (both $n = 6$). However, a final concentration of 15 mM HCl, producing a decrease in pH of 0.40 pH units, significantly ($P < 0.05$) reduced SPD frequency by $38.9 \pm 8.5\%$ ($n = 4$).

In view of the fact that these compounds possibly contain up to 1–2% impurities such as L-glutamate or glutaric acid, the effects of these compounds were examined. L-Glutamate (0.1–0.5 mM), although producing transient increases in frequency of SPDs at the onset of application, had no significant effect ($P > 0.05$) on the SPD frequency when measured 60 min after application ($n = 4$). However, there was a tendency towards a decrease in frequency, and a reduction in SPD amplitude, the reduction in SPD amplitude not being reversed 60 min after wash out. The application of 5 mM glutaric acid had no significant effect ($P > 0.05$) on the frequency of SPDs ($n = 6$). L-Glutamate (0.1–3 mM) and 0.1 and 0.3 mM glutaric acid had no effect on pH, whilst 1 and 5 mM glutaric acid reduced pH by 0.07 and 0.20 pH units, respectively. Increasing the osmolarity of the Krebs medium with concentrations of 10 and 30 mM sucrose resulted in no effect at 10 mM, but a significant depression of SPD frequency at 30 mM by $32.0 \pm 2.72\%$ ($n = 5$, $P < 0.001$).

Discussion

L-Glutamate diethyl ester

The results from the present study indicate that GDEE HCl has no antagonist activity on postsynaptic EAA ionophore-receptors, consistent with the observations in the spinal cord

Figure 6 A continuous recording of d.c. potential showing the depolarizing responses to $20 \mu\text{M}$ N-methyl-D-aspartate (NMDA, N), $5 \mu\text{M}$ α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA, A) $20 \mu\text{M}$ quisqualate (Quis Q) and $20 \mu\text{M}$ kainate (Kain, K), from the cortical wedge preparation in the absence (a) and the presence (b) of 10 mM glutamate diethyl ester (GlrDEE). Responses to NMDA and Kain were depressed, that to Quis was augmented, and AMPA little affected. The recovery (c) of NMDA, Kain and Quis response amplitude occurred after a period of wash with control medium.

(Evans *et al.*, 1979), and *Xenopus* oocyte expression studies (Hirono *et al.*, 1988). Since responses to AMPA are unaffected by GDEE HCl, the potentiation of responses to Quis is also not due to a direct action on the AMPA/Quis receptor. There

Figure 7 Dose-effect curves for L-glutamate diethyl ester (◆) and deaminated analogues glutarate diethyl ester (■) and glutarate dimethyl ester (▲) showing the inhibition of spontaneous paroxysmal discharge (SPD) frequency observed in 0 Mg^{2+} Krebs medium. The vertical lines represent the s.e.mean and the points the means of 4–10 values for the inhibition of SPD frequency expressed as a percentage of control.

is some evidence that GDEE displaces one of the components of Cl^- -dependent [^3H]-L-glutamate binding (Werling *et al.*, 1983), which probably represents Cl^- -dependent L-glutamate uptake, in view of its comparable pharmacological profile to [^3H]-AP4 binding (Butcher *et al.*, 1983) and its sensitivity to freeze-thawing. Since Quis is much more potent than AMPA in displacing this binding and Quis is a known substrate of Cl^- -dependent uptake (Zaczek *et al.*, 1987), a block of this uptake would explain these results. Another consequence of uptake blockade is that it could lead to an enhanced tonic activation of EAA receptors by endogenous L-glutamate, and hence the depolarization of baseline observed with GDEE HCl. However, the contamination of GDEE HCl by up to 1% L-glutamate, which probably explains its weak agonist activity in *Xenopus* oocyte expression studies (Hirono *et al.*, 1988), means that the concentrations of GDEE HCl used in this study would contain 0.03–0.14 mM L-glutamate. The application of 0.1–0.5 mM L-glutamate did not greatly affect baseline potential, so the depolarizing action of the L-glutamate *per se*, does not explain the depolarizing action of GDEE HCl. Recent evidence indicates that the main component of the depolarizing action of bath applied L-glutamate in brain slices is due to the electrogenic nature of Na^+ -dependent L-glutamate uptake (Frenguelli *et al.*, 1991). Therefore, it is possible that the depolarizing action of GDEE HCl is the result of the blockade of Cl^- -dependent L-glutamate uptake combined with the enhanced electrogenic Na^+ -dependent uptake of endogenous and contaminating L-glutamate. The reason for the greater depolarizing action of GDEE HCl on slices exposed to AMPA and Quis remains unclear, unless the activation of the AMPA/Quis receptor has a long term effect on L-glutamate uptake mechanisms. The reduction of Quis responses seen with very large depolarizing shifts may simply reflect occlusion by the rising baseline.

The depression of the frequency of the SPDs by GDEE HCl observed in 0Mg^{2+} Krebs is consistent with the depression of synaptic activity seen in the spinal cord. Previous study has shown that the SPDs are mediated via the action of released EAAs on postsynaptic NMDA receptors, since the frequency of SPDs is very sensitive to NMDA receptor antagonists (Horne *et al.*, 1986), but not the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (Fletcher *et al.*, 1988). Therefore, the lack of any EAA antagonist activity for GDEE HCl, indicates that some other mechanism is involved. In addition, this lack of EAA antagonist activity means that the decrease in extracellular pH produced by GDEE HCl is insufficient to affect NMDA receptors, which have been shown to be very sensitive to changes in pH in the physiological range (Treynalis & Cull-Candy, 1990). Indeed, concentrations of HCl and glutaric acid which produced changes in pH of the Krebs medium that would be effective in producing some reduction in the level of NMDA receptors activation, caused little or modest change in SPD frequency. However, the increase in pH between perfusion reservoir and tissue bath is probably sufficient to account for this discrepancy. Certainly a previous study indicates that SPDs in the cortical wedge preparation are very sensitive to changes in extracellular pH produced by manipulation of the CO_2 concentration in the Krebs medium (Aram & Lodge, 1987). However, it is unlikely that a decrease in extracellular pH is the major contributory factor to the inhibition of the SPDs by GDEE HCl in this study.

It is possible that the osmolarity of the Krebs medium is affected by the high concentrations of GDEE HCl used in this present study. Attempts to increase osmolarity with sucrose resulted in inhibition of the SPDs, consistent with the fact that increased osmolarity has been shown to decrease neuronal excitability (Rosen & Andrew, 1990). However, this effect was a partial inhibition at a concentration in excess of concentrations of GDEE HCl producing near maximal inhibition. This would suggest that increased osmolarity and certainly increased molarity are only contributory non-specific factors in the inhibitory action of GDEE HCl on synaptic activity.

Indeed, since GDEE suppresses the release of [^3H]-D-aspartate from cerebellar granule cells (Drejer *et al.*, 1986), GDEE HCl could block the presynaptic release of EAAs, inhibiting the appearance of SPD-like activity. In addition, non-specific effects such as membrane stabilization and blockade of other ion channels cannot be ruled out as mediators of the inhibitory action of GDEE.

Glutarate diethyl ester and glutarate dimethyl ester

In contrast to GDEE HCl, GlrDEE and GlrDME have some EAA antagonist activity, indicating that deamination of the L-glutamate diester structure confers a different activity on these compounds. In the case of GlrDEE it has both NMDA and Kain, but not AMPA receptor antagonist activity, indicating a separation of AMPA and Kain receptors. Much electrophysiological evidence indicates that the actions of Kain are mediated via the AMPA/Quis receptor expressed in the *Xenopus* oocyte (Verdoorn *et al.*, 1989) and isolated hippocampal neurones (Kiskin *et al.*, 1986), and this is supported by antagonist studies in the cortical wedge preparation (Fletcher *et al.*, 1988). However, another expression study in the *Xenopus* oocyte has revealed the presence of a Kain receptor not activated by AMPA (Umbach & Gundersen, 1989), indicating the possibility of postsynaptic ionophore-receptors for Kain distinct from the AMPA/Quis receptors. GlrDEE also potentiated responses to Quis, indicating there may also be an interaction of GlrDEE with Cl^- -dependent L-glutamate uptake. Although the NMDA antagonist activity exhibited by GlrDEE would explain the inhibition of SPDs, it is more likely that a non-specific mechanism similar to that for GDEE is involved, with the NMDA antagonism contributing to the increased potency compared to GDEE HCl. GlrDME showed some antagonist activity against both Kain and Quis, and not NMDA, at a concentration that is not near maximal for the inhibition of the SPDs. Therefore, the action of GlrDME on SPDs cannot be ascribed to any one EAA receptor, and probably results from a non-specific mechanism of action as well.

As with GDEE HCl, the contamination of the deaminated analogues of GDEE must be considered. With these compounds the most probable contaminant is glutaric acid, which is likely to be as much as 1 and 2%, i.e. concentrations in this study of 0.03–0.12 and 0.08–0.52 mM, for GlrDEE and GlrDME, respectively. Since glutaric acid at these concentrations, and GlrDEE and GlrDME all had little effect on the pH of the Krebs medium, it is unlikely that acidification of the Krebs medium leading to NMDA receptor blockade is responsible for the antagonism of NMDA responses by GlrDEE or the inhibition of SPDs. As with GDEE HCl, the underlying mechanism may involve, in part at least, the decrease in neuronal excitability resulting from the increase in extracellular osmolarity (when high concentrations are used).

These results, together with a previous study (Evans *et al.*, 1979), provide little evidence for the action of GDEE as an antagonist of postsynaptic ionophore-receptors in the CNS, when bath applied. The fact that GDEE was effective as a selective AMPA/Quis antagonist in iontophoretic experiments remains unresolved. As a result, when GDEE is applied by a method other than iontophoresis, it is impossible to determine whether GDEE is acting as a selective AMPA/Quis antagonist or via some less specific mechanism. Therefore, in behavioural studies where seizures induced by EAA have been blocked by GDEE (Freed, 1986; Freed & Braun, 1988), the antagonism of seizures cannot necessarily be attributed to the antagonism of EAA postsynaptic ionophore-receptors, although in the case of the deaminated analogues of GDEE this may contribute. Finally, it is clear from this study that the lack of AMPA/Quis receptor antagonist action for GDEE in the rat neocortex prevents its use as a tool in receptor classification in these types of experiment.

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