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Interactions of 2,3-benzodiazepines and cyclothiazide at AMPA receptors: patch clamp recordings in cultured neurones and area CA1 in hippocampal slices

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1 The 2,3-benzodiazepines GYKI 52466, GYKI 53405 and GYKI 53655 antagonized AMPA-induced currents in cultured superior colliculus neurones in a non use-dependent manner (steady state IC_{50}s: GYKI 52466 9.8±0.6 μM; GYKI 53405 3.1±0.6 μM; GYKI 53655 0.8±0.1 μM).

2 Higher concentrations of all three antagonists slowed the onset kinetics and quickened the offset kinetics of AMPA-induced currents indicative of an allosteric interaction with the AMPA recognition site.

3 Cyclothiazide (3–300 μM) dramatically slowed desensitization of AMPA-induced currents and potentiated steady state currents (EC_{50} 10.0±2.5 μM) to a much greater degree than peak currents. Both τ_{on} and τ_{off} were also increased by cyclothiazide in a concentration-dependent manner (EC_{50}: τ_{on} 42.1±4.5 μM; τ_{off} 31.6±6.6 μM).

4 Cyclothiazide (10–100 μM) shifted the concentration-response curves of the 2,3-benzodiazepines to the right. For example, with 10 μM cyclothiazide the IC_{50}s of GYKI 52466 and GYKI 53405 on steady-state AMPA-induced currents were 57.9±9.5 and 41.6±1.5 μM, respectively.

5 GYKI 53405 and GYKI 52466 concentration-dependently reversed the effects of cyclothiazide (100 μM) on offset kinetics (GYKI 53405 IC_{50} 16.6±4.2 μM). However, the 2,3-benzodiazepines were unable to reintroduce desensitization in the presence of cyclothiazide and even concentration-dependently slowed the onset kinetics of AMPA responses further (GYKI 53405 EC_{50} 8.0±2.8 μM).

6 GYKI 52466 decreased the peak amplitude of hippocampal area CA1 AMPA receptor-mediated excitatory postsynaptic currents (e.p.s.cs) (IC_{50} 10.8±0.8 μM) with no apparent effect on response kinetics. Cyclothiazide prolonged the decay time constant of AMPA receptor-mediated e.p.s.cs (EC_{50} 35.7±6.5 μM) with less pronounced effects in slowing e.p.s.c. onset kinetics and increasing e.p.s.c. amplitude.

7 Cyclothiazide (330 μM) shifted the concentration-response curve for the effects of GYKI 52466 on AMPA receptor-mediated e.p.s.c. peak amplitude to the right (GYKI 52466 IC_{50} 26.9±9.4 μM). Likewise, GYKI 52466 (30–100 μM)) shifted the concentration-response curve for the effects of cyclothiazide on AMPA receptor-mediated e.p.s.c. decay time constants to the right.

8 In conclusion, cyclothiazide and the 2,3-benzodiazepines seem to bind to different sites on AMPA receptors but exert strong allosteric interactions with one another and with other domains such as the agonist recognition site. The interactions of GYKI 52466 and cyclothiazide on AMPA receptor-mediated e.p.s.cs in area CA1 of hippocampal slices provide evidence that the decay time constant of these synaptic events are not governed by desensitization.

Keywords: AMPA receptor-mediated e.p.s.cs; AMPA-induced currents; GYKI 52466; GYKI 53405; GYKI 53655; cyclothiazide; hippocampal slice; superior colliculus culture; kinetics; allosteric interactions

Introduction

Glutamate is probably the major fast excitatory transmitter in the mammalian central nervous system (see Danysz *et al.*, 1995a for review). As such, there is a great deal of interest in the biological actions of glutamate receptor antagonists. Most research effort has concentrated on the N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptor due to the early availability of a variety of selective antagonists for this receptor. The discovery of the potent and selective competitive (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo-(F)quinoxaline (NBQX, Sheardown *et al.*, 1990; Parsons *et al.*, 1994), was therefore followed rapidly by extensive research on its actions in preclinical models. The newest compounds to have gained considerable scientific attention are 2,3-benzodiazepines, such as 1-(4-aminophenyl)-4-methyl-7,8-methyl-endioxyl-5H-2,3-benzodiazepine (GYKI 52466), which are relatively selective, non-competitive AMPA receptor antagonists (Quardouz & Durand, 1991; Donevan & Rogawski, 1993; Szabo & Henley, 1993; Tarnawa *et al.*, 1993; Donevan *et al.*, 1994; Chizh *et al.*, 1994; Parsons *et al.*, 1994; Wilding & Huettner, 1995) and interfere with AMPA receptor-mediated neurotransmission both *in vitro* and *in vivo* (Tarnawa *et al.*, 1992; Engberg *et al.*, 1993; Cumberbatch *et al.*, 1994; Quardouz & Durand, 1994). Like NBQX, the 2,3-benzodiazepines also reduce AMPA receptor-mediated toxicity *in vitro* (May & Robison, 1993a) and *in vivo* (Moncada *et al.*, 1991; Bisaga *et*

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al., 1993) and are effective in animal models of focal ischaemia (Smith & Meldrum, 1992) and epilepsy (Chapman et al., 1991, 1993; Smith et al., 1991; Yamaguchi et al., 1993; Löscher & Hönack, 1994; see Rogawski, 1993 for review).

It is now clear that NBQX and the 2,3-benzodiazepines have very different mechanisms of antagonistic action at AMPA receptors i.e. competitive and non-competitive respectively (Donevan & Rogawski, 1993; Rogawski, 1993; Parsons et al., 1994). The 2,3-benzodiazepines seem to interact with a novel modulatory site and may therefore represent promising leads for the development of therapeutic agents directed at AMPA receptors due to their non-competitive nature and, as a consequence, different effects on AMPA response kinetics (Rogwaski, 1993; Parsons et al., 1994). The other side of the coin is represented by positive modulators of AMPA receptors such as aniracetam which facilitate the actions of agonists at AMPA receptors by decreasing desensitization, as such an effect has been proposed to represent a promising approach for cognitive enhancement (e.g. Ito et al., 1990; Staubli et al., 1992; Lee & Benfield, 1994; see Danysz et al., 1995b for review). The diuretic agent cyclothiazide (3-bicyclo-[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide) and derivatives thereof also inhibit desensitization of AMPA receptors and enhance long-term potentiation (LTP) *in vitro* but are much more potent and effective in this regard (Bertolino et al., 1993; Livsey et al., 1993; Partin et al., 1993; 1994a,b; Patneau et al., 1993; Yamada & Tang, 1993; Zivkovic et al., 1995).

In view of the contrasting modulatory effects of the 2,3-benzodiazepines and cyclothiazide and its derivatives, it seems pertinent to test whether these compounds are acting in opposing ways at the same recognition site on AMPA receptors. This question has indeed been addressed previously in biochemical studies (Barnes et al., 1994; Desai et al., 1994; 1995; Hoyt et al., 1995) and electrophysiological experiments on field potentials in cortical wedges (Palmer & Lodge, 1993) and whole cell voltage-clamp recordings in *Xenopus* oocytes (Sharp et al., 1994). However, the effects of both classes of agent on receptor kinetics and synaptic transmission cannot be addressed with such experiments (see however, Zorumski et al., 1993). The present study utilised patch and concentration-clamp techniques to test for interactions between 2,3-benzodiazepines and cyclothiazide on AMPA receptor-mediated currents in cultured superior colliculus neurones and AMPA receptor-mediated e.p.s.cs in hippocampal slices. Preliminary data have been presented in abstract form and as a short paper (Rammes et al., 1994a, b).

Methods

Cell culture

Superior colliculi were obtained from rat embryos (E20 to E21) and were then transferred to calcium- and magnesium-free Hank's buffered salt solution (Gibco) on ice. Cells were mechanically dissociated in 0.05% DNAase/0.3% ovomucoid (Sigma) following an 8 min pre-incubation with 0.66% trypsin/0.1% DNAase (Sigma). The dissociated cells were then centrifuged at 18*g* for 10 min, re-suspended in minimum essential medium (Gibco) and plated at a density of 300,000 cells cm^{-2} onto poly-D,L-ornithine (Sigma)/laminin (Gibco) - precoated plastic petri dishes (Falcon). The cells were nourished with $NaHCO_3$/HEPES-buffered minimum essential medium supplemented with 5% foetal calf serum and 5% horse serum (Gibco) and incubated at 37°C with 5% CO_2 (95% humidity). The medium was exchanged completely following inhibition of further glial mitosis with cytosine-β-D-arabinofuranoside (20 μM Sigma) after about 7 days *in vitro*.

Concentration clamp recordings from cultured superior colliculus neurones

Whole cell recordings were made from cultured superior collicular neurones (10 to 14 days *in vitro*) at room temperature (20–22°C) with the aid of an EPC-7 amplifier (List). Most recordings were made at a membrane potential of −70 mV. Patch clamp electrodes were pulled and polished with a horizontal puller (DMZ) and had an internal tip diameter of about 1 μm and a tip resistance of 4 to 10 MΩ. Cells were continuously superfused via one of eight channels of a custom designed fast superfusion system with a common outflow. Test substances then were applied by rapidly switching channels. Complete exchange of the superfused solution was achieved within 10 ms. The application of solutions and the synchronized on-line electronic acquisition of data were controlled by the programme PCLAMP for IBM-PC. Subsequently, AUTESP for IBM-PC (Garching Instruments, Munich) was used to analyse the data semi-automatically off-line. Only results from stable cells were accepted for inclusion in the final analysis i.e. following recovery of responses to AMPA by at least 75% of their depression or enhancement by the agents tested.

The contents of the intracellular (electrode) solution were as follows (mM): CsCl 120, TEACl 20, EGTA 10, $MgCl_2$ 1, $CaCl_2$ 0.2, glucose 10, ATP 2, cyclic AMP 0.25. The extracellular solutions had the following basic composition (mM): NaCl 140, KCl 3, glucose 10, HEPES 10, $CaCl_2$ 0.2, sucrose 4.5, glycine 0.001. Neurones were pharmacologically isolated from one another by the inclusion of tetrodotoxin (0.3 μM, TTX) to block voltage-activated sodium currents. Test substances were added to this solution in concentrations detailed in results and pH corrected, when necessary, to 7.35.

AMPA receptor-mediated e.p.s.cs in area CA1 of hippocampal slices

Transverse hippocampal slices (400 μm thick) were obtained from male Wistar rats (140–200 g) which were anaesthetized with ether before decapitation. The brain was removed rapidly and slices were prepared in ice-cold artificial cerebrospinal fluid (aCSF) with a Campden vibroslicer. All slices were placed in a holding chamber for at least 90 min before being transferred to an immersion chamber for recordings. The flow rate of the aCSF through the recording chamber was 1.5 ml min^{-1}. The composition of the aCSF was (mM): NaCl 124, KCl 3, $NaHCO_3$ 26, $CaCl_2$ 2, $MgSO_4$ 1, D-glucose 10, NaH_2PO_4 1.25. At all stages, the aCSF was bubbled with 95% O_2/5% CO_2 and had a final pH of 7.3.

Patch clamp recordings were made from pyramidal-neurones in stratum pyramidale of area CA1. The mean input resistance of the cells was 188 ± 14 MΩ, (n = 40). Glass electrodes (4.5–5 MΩ) contained (mM): $CsMeO_4$ 130, HEPES 5, NaCl 1, $MgCl_2$ 1, $CaCl_2$ 0.035, EGTA 0.05 and QX-314 5-20. Currents were recorded with a switched voltage clamp amplifier (SEC 1L, NPI electronic; Tamm/Germany) using the 'blind' whole-cell recording technique (Boulton et al., 1994). Switching frequencies of 20–25 kHz (25% duty cycle) were used. Series resistance was monitored continuously and frequently compensated in bridge mode (for details see Misgeld et al., 1989; Swandulla & Misgeld, 1990). Test stimuli (0.066 Hz, 5–12 mA) were delivered via bipolar tungsten electrodes insulated to the tip (5 μm tip diameter) positioned in the Schaffer collateral-commissural pathway (Sccp). To obtain pure AMPA receptor-mediated excitatory postsynaptic currents (e.p.s.cs), slices were perfused with D-2-amino-5-phosphonopentanoic acid (D-AP5, 50 μM), picrotoxin (50 μM) and CGP 35348 (200 μM) to block NMDA, γ-aminobutyric acid ($GABA)_A$ and $GABA_B$ receptors respectively. Voltage-activated sodium channels and K^+-channels were blocked intracellularly by QX-314 and Cs^+. All experiments were performed at room temperature at a holding potential of −70 mV. E.p.s.cs were amplified, filtered (667 Hz) and digitized (2k Hz) using a laboratory interface (ITC-16 Computer Interface, Instrutech

Corp.). Digitized current responses were stored to disk on a Macintosh Quadra 700 computer with the acquisition programme Pulse v. 7.21 (Heka electronic GmbH, Lambrecht/Germany. AMPA receptor-mediated e.p.s.cs were well-fitted by single exponential functions to obtain the time constants of response kinetics.

Drugs

The following pharmacological compounds (with sources) were used: glycine (Sigma), AMPA (Tocris), D-AP5 and QX-314 (Lignocaine N-ethyl bromide; RBI), CGP 35348 (3-amino-propyl(diethoxymethyl)-phosphonic acid; Ciba Geigy). GYKI 52466 (kind gift from Dr Tarnawa, Institute for Drug Research, Budapest, Hungary), GYKI 53405 (1-(4-aminophenyl)-3-acetyl-4-methyl-3,4-dihydro-7,8-methyl-endioxyl-5H-2,3-benzodiazepine), GYKI 53655 (1-(4-aminophenyl)-3-methyl-carbamyl-4-methyl-3,4-dihydro-7,8-methyl-endioxyl-5H-2,3-benzodiazepine and cyclothiazide were kind gifts from Dr Leander, Ely Lilly, U.S.A.

For stock solutions, cyclothiazide (50 mM) was normally dissolved in dimethyl sulphoxide (DMSO). Although DMSO alone (0.66% in aCSF) had no effects on AMPA-induced currents this vehicle did produce a variable increase in AMPA receptor-mediated e.p.s.c. amplitude (48±19%) without affecting response kinetics or cell input characteristics. As such, in slice experiments with cyclothiazide, DMSO (0.66%) was present continuously in the aCSF. Moreover, similar results were obtained in later experiments where cyclothiazide stock solutions were made without DMSO by adjusting pH to >13.

Results

Superior colliculus cultures

2,3-Benzodiazepines Control inward currents to AMPA (100 μM) rose rapidly to a peak and then desensitized somewhat less rapidly to a steady-state of around 20% of this maximum (Figure 1, e.g. controls in experiments with GYKI 53405: peak 894±38 pA, steady-state 204±19 pA, τ_{on}=7.6±0.3 ms, τ desensitization = 32.2±1.1 ms, τ_{off}=174.1±7.9 ms, n=58). The 2,3-benzodiazepines concentration-dependently antagonized both peak and steady-state components of current responses to AMPA (100 μM) but were somewhat more potent against steady-state currents at concentrations less than their respective IC_{50}s (Figures 1 and 2). The most potent 2,3-benzodiazepine was GYKI 53655 (steady-state IC_{50}=0.8±0.1 μM), followed by GYKI 53405 (steady-state IC_{50}=3.1±0.6 μM) and then GYKI 52466

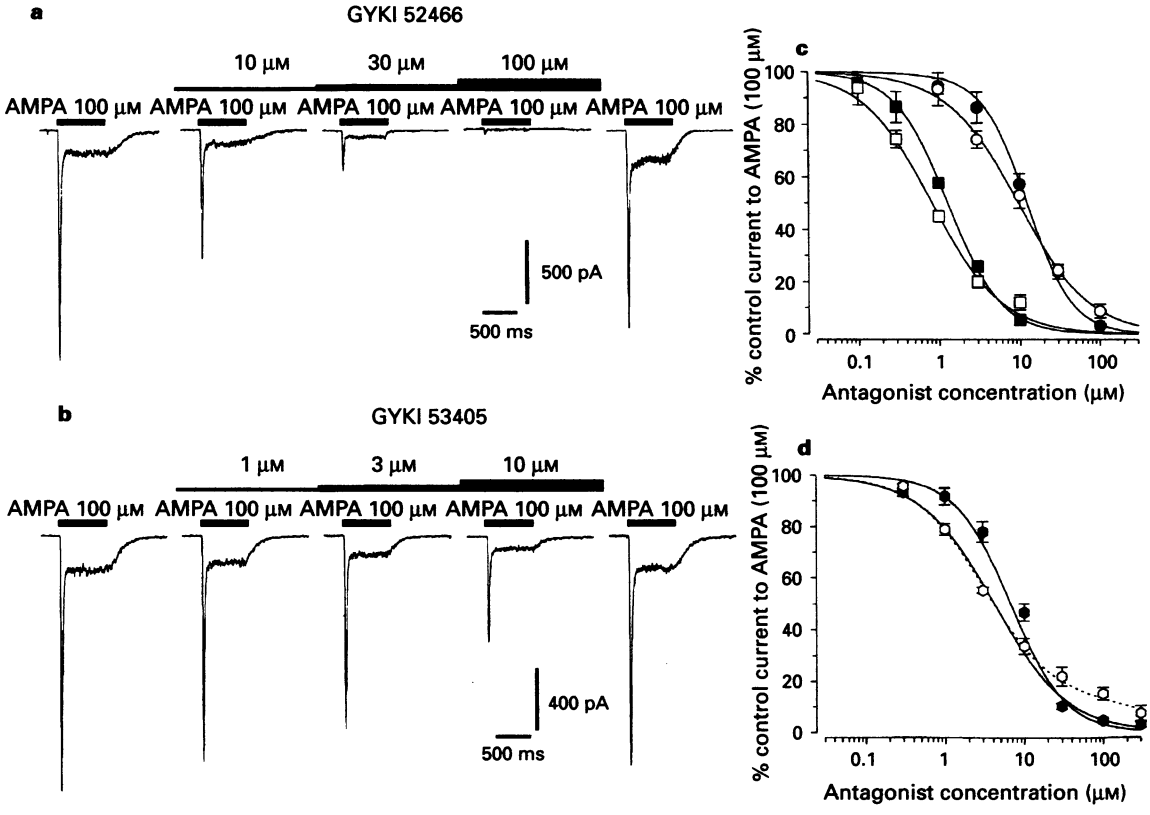

Figure 1 The 2,3-benzodiazepenes antagonize AMPA-induced inward currents in cultured superior colliculus neurones. AMPA (100 μM) was applied for 700 ms every 15 s at −70 mV. (a) The left and right panels show control and recovery responses respectively. The middle three panels show equilibrium responses in the continuous presence of GYKI 52466 10, 30 and 100 μM respectively. (b) The middle three panels show equilibrium responses in the continuous presence of GYKI 53405 1, 3 and 10 μM respectively. (c d) Pooled responses were quantified as peak and steady-state (plateau) currents after subtraction of any leak current and plotted, after normalization to control, as means±s.e. mean against antagonist concentration (logarithmic). At least 6 cells were tested at each concentration of antagonist. In (c); (●) GYKI 52466 peak; (○) GYKI 52466 plateau; (■) GYKI 53655 peak; (□) GYKI 53655 plateau. In (d); (●) GYKI 53405 peak; (○) GYKI 53405 plateau. The 4 parameter logistic equation was used to fit the data (solid curves) and to calculate the IC_{50}s for the 2,3-benzodiazepines. These values were as follows. GYKI 52466: peak IC_{50}=12.4±1.1 μM (Hill Coeff.=1.56±0.11); steady-state IC_{50}=9.76±0.55 μM (Hill Coeff.=0.99±0.04). GYKI 53655: peak IC_{50}=1.29±0.05 μM (Hill Coeff.=1.34±0.05); steady-state IC_{50}=0.83±0.09 μM (Hill Coeff.=1.02±0.08). GYKI 53405: peak IC_{50}=6.68±1.22 μM (Hill Coeff.=1.24±0.15); steady-state IC_{50}=4.16±0.59 μM (Hill Coeff.=0.93±0.13). However, the inhibition of steady state currents by GYKI 53405 was better fit by a two site model (dotted curve) with the following parameters: $IC_{50}1$=3.07±0.59 μM (capacity 87.4%); $IC_{50}2$=517±218 μM.

(steady-state $IC_{50}=9.8\pm0.6$ μM). IC_{50}s against peak currents were 1.3 ± 0.1 μM, 6.7 ± 1.2 μM and 12.4 ± 1.1 μM respectively.

Higher concentrations of all three antagonists slowed onset kinetics and speeded offset kinetics, indicative of an allosteric interaction with the AMPA recognition site (e.g. with GYKI 53405 (100 μM) τ_{on} was 163.5 ± 27.7 ms and τ_{off} was 119.5 ± 8.1 ms, $n=9$, Figure 5a). This effect probably accounts for the steeper Hill coefficients against peak currents (Figure 1c and d). It was not possible to calculate accurately the potency of the 2,3-benzodiazepines in mediating such effects due to the small amplitude of currents recorded at higher concentrations of antagonist. However, the IC_{50} of GYKI 53405 on τ_{off} was estimated to be about 40 μM. In contrast, none of the three antagonists had any effect on desensitization kinetics at any concentration where such an effect would have been detectable (Figures 1 and 5a).

Currents in the presence of GYKI 53655 showed some degree of outward rectification above +30 mV (Figure 2b). Such minor voltage-dependent effects were not accompanied by any detectable change in response kinetics or use-dependency (data not shown) which argues against open channel blockade as a possible, contributory, mode of action for their antagonistic effects.

Cyclothiazide Cyclothiazide (3–300 μM) concentration-dependently potentiated steady-state AMPA-induced inward currents ($EC_{50}10.0\pm2.5$ μM) to a greater degree than peak currents (Figure 3c). For example, peak and steady-state currents were potentiated by cyclothiazide (100 μM) to $290\pm29\%$ and $1271\pm137\%$ of control respectively ($n=14$). The effects of cyclothiazide were slow in onset and very slow in offset (Figure 3b). This slow offset was almost certainly not a problem of residual cyclothiazide in the perfusion pipette but rather seems to reflect the necessity of agonist binding to its recognition site for cyclothiazide to dissociate i.e. use-dependent unbinding. In support of this assumption was the finding that recovery was twice as quick in experiments where AMPA was applied every 7.5 s rather than every 15 s as normal (data not shown). The effects of cyclothiazide on current amplitude were accompanied by a slowing of AMPA response kinetics (Figures 3a and 5b). Desensitization of AMPA-induced currents was slowed dramatically by cyclothiazide (3–300 μM) in a concentration-dependent manner, with pronounced effects even at the lowest concentration tested (e.g. control $\tau=21.8\pm1.1$ ms, $n=35$; cyclothiazide 10 μM $\tau=991\pm232$ ms, $n=13$). Indeed, the very slow residual desensitization was so prolonged and variable (Figure 5b), that it probably partially reflects recruitment of additional underlying mechanisms such as run-down or the involvement of other, ion-activated channels in carrying a component of the potentiated current. These factors prevented the calculation of an EC_{50} for cyclothiazide in mediating this effect. Both onset and offset rates were slowed by cyclothiazide in a concentration-dependent manner (Figure 5b), this effect being most apparent with the highest concentration tested, namely 300 μM (control $\tau_{on}=4.7\pm0.2$ ms, cyclothiazide $\tau_{on}=27.5\pm6.1$ ms; control $\tau_{off}=127.3\pm9.0$ ms, cyclothiazide $\tau_{off}=227.8\pm46.3$ ms, $n=6$). Estimation of the potency of cyclothiazide in slowing τ_{on} and τ_{off} using the four parameter logistic equation gave EC_{50}s of 42.1 ± 4.5 μM and 31.6 ± 6.6 μM respectively.

Interactions between 2,3-benzodiazepines and cyclothiazide - Cyclothiazide concentration-dependently shifted the antagonist concentration-response curves of GYKI 52466 and GYKI 53405 to the right in a manner suggestive of a common site of action for these substances (Figure 4). e.g. with cyclothiazide (10 μM) the IC_{50}s of GYKI 52466 and GYKI 53405 on steady-state AMPA-induced currents were 57.9 ± 9.5 and

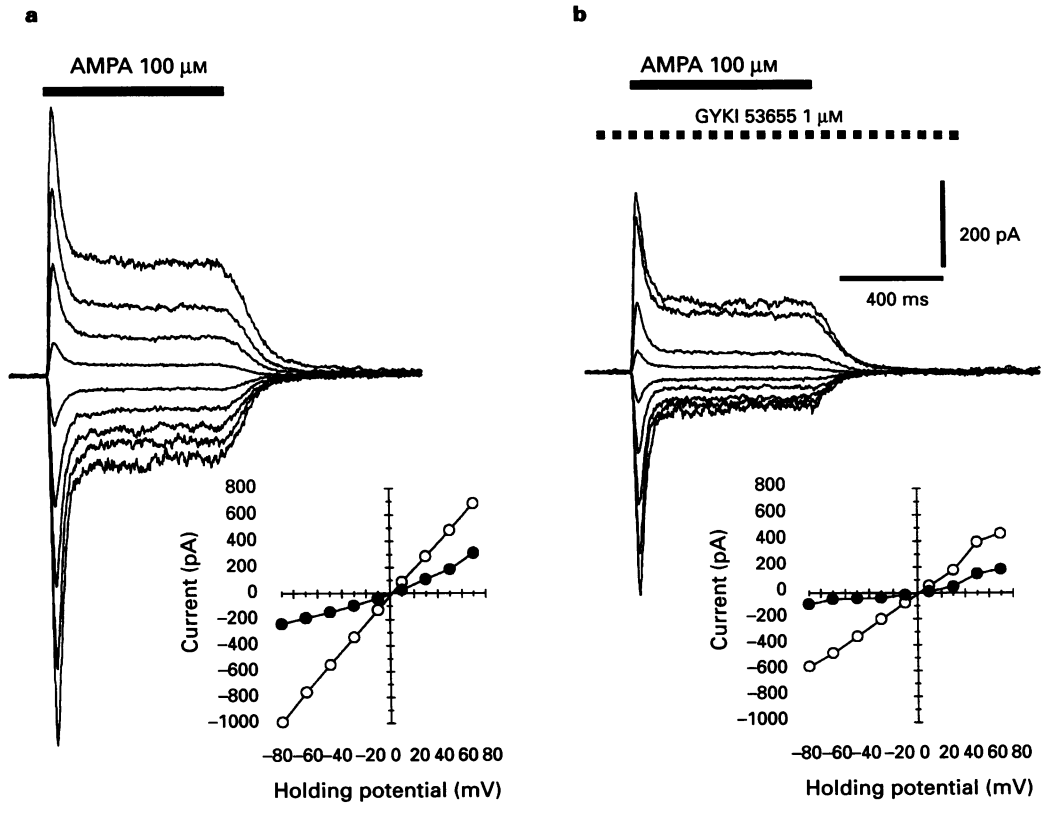

Figure 2 Voltage-dependency of the AMPA receptor antagonistic effects of GYKI 53655. (a) Control current responses to AMPA (100 μM) were recorded at various membrane potentials (−90 to +70 mV). (b) AMPA responses of the same neurone in the continuous presence of GYKI 53655 (1 μM) were recorded at the same membrane potentials. Insets show the i.v. curves for peak (open symbols) and steady state (plateau) (solid symbols) currents in the absence and presence of GYKI 53655 (1 μM).

41.6±1.5 μM respectively. This is illustrated for original data with GYKI 52466 in Figure 4a where it can clearly be seen that cyclothiazide (10 μM) potentiated steady-state AMPA-induced inward currents and at the same time decreased the affinity of GYKI 52466 on the same neurone. Cyclothiazide (100 μM) was able to shift the concentration-response curve of GYKI 53405 against AMPA-induced inward currents even further to the right (IC_{50} = 102.3±11.6 μM; Figure 4c).

In the presence of cyclothiazide (100 μM), GYKI 53405 was unable to reintroduce desensitization and even concentration-dependently slowed the onset kinetics of AMPA responses further (Figure 5c and d, EC_{50} 8.8±2.8 μM; e.g. with GYKI 53405 (100 μM) τ_{on} was increased from 24.1±1.3 ms to 86.4±6.6 ms, n=6). In contrast, GYKI 53405 was able to reverse concentration-dependently the effects of cyclothiazide on offset kinetics (Figure 5c, IC_{50}16.6±4.2 μM; e.g. with GYKI 53405 (100 μM) τ_{off} was reduced from 192.1±3.0 ms to 117.5±6.1 ms, n=6). Similar effects were seen with GYKI 52466 in the presence of cyclothiazide (10 μM) where e.g. GYKI 52466 (100 μM) was able to decrease τ_{off} from 173.4±11.9 ms to 77.8±11.9 ms (IC_{50} 81.3±21.3 μM) but slowed τ_{on} further from 5.9±0.4 to 27.1±1.9 ms, n=7).

Hippocampal area CA1 AMPA receptor-mediated e.p.s.cs

GYKI 52466 In agreement with our previous data (Rammes *et al.*, 1994a), GYKI 52466 concentration-dependently decreased the peak amplitude of area CA1 AMPA receptor-mediated e.p.s.cs (IC_{50} = 10.8±0.8 μM) without having any substantial effect on response kinetics (Figures 6 and 8, Table 1A). The slow access of the compound to the slice (Figure 6c) made it difficult to test concentrations below 3 μM with stable recording conditions.

Cyclothiazide Cyclothiazide concentration-dependently enhanced the peak amplitudes of AMPA receptor-mediated e.p.s.cs (Figure 7a, Table 1B). However, the magnitude of this effect was relatively small and highly variable, which prohibited calculation of a realistic value for its potency in this regard. In contrast, the effects of cyclothiazide in prolonging the decay time constant of AMPA receptor-mediated e.p.s.cs was much clearer and concentration-dependent (EC_{50} 35.7±6.5 μM, Figures 7 and 9). Cyclothiazide had a less pronounced effect in slowing onset kinetics of AMPA receptor-mediated e.p.s.cs than that seen on AMPA-induced currents in cultured neurones (Figures 7b, c, Table 1B).

Interactions between GYKI 52466 and cyclothiazide In agreement with our data from cultured superior colliculus neurones, cyclothiazide and GYKI 52466 showed allosteric interactions on area CA1 hippocampal neurones. Cyclothiazide (330 μM) shifted the concentration-response curve for the effects of GYKI 52466 on AMPA receptor-mediated e.p.s.c. peak amplitude to the right (GYKI 52466 IC_{50} 26.9±9.4 μM, Figure 8). Likewise, GYKI 52466 (30–100 μM) shifted the concentration-response curve for the effects of cyclothiazide on AMPA receptor-mediated e.p.s.cs decay time constants to the

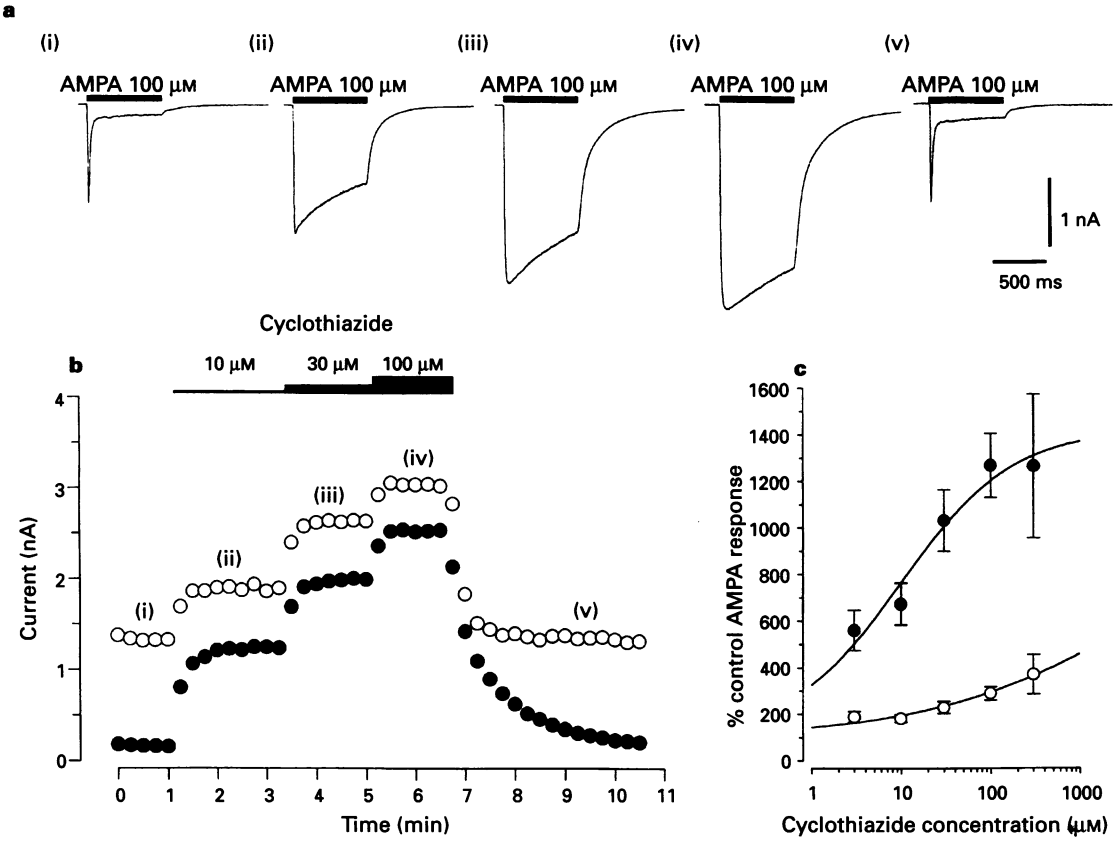

Figure 3 Cyclothiazide potentiates AMPA-induced inward currents by reducing desensitization. AMPA (100 μM) was applied as in Figure 1. (a) The left and right panels show control and recovery responses respectively. The middle three panels show equilibrium responses in the continuous presence of cyclothiazide 10, 30 and 100 μM respectively, recorded at the time points indicated by the number ((i)–(v)) in graph b. Note the relatively rapid recovery of peak AMPA-induced currents (○) to control levels and the much slower time course for recovery of steady-state (plateau) (●) currents. (c) Pooled responses were quantified as in Figure 1 c and d and plotted against log concentration of cyclothiazide (n=6–12 per concentration). The 4 parameter logistic equation was used to fit the data and to calculate the EC_{50}s for cyclothiazide on steady state currents of 10.0±2.5 μM.

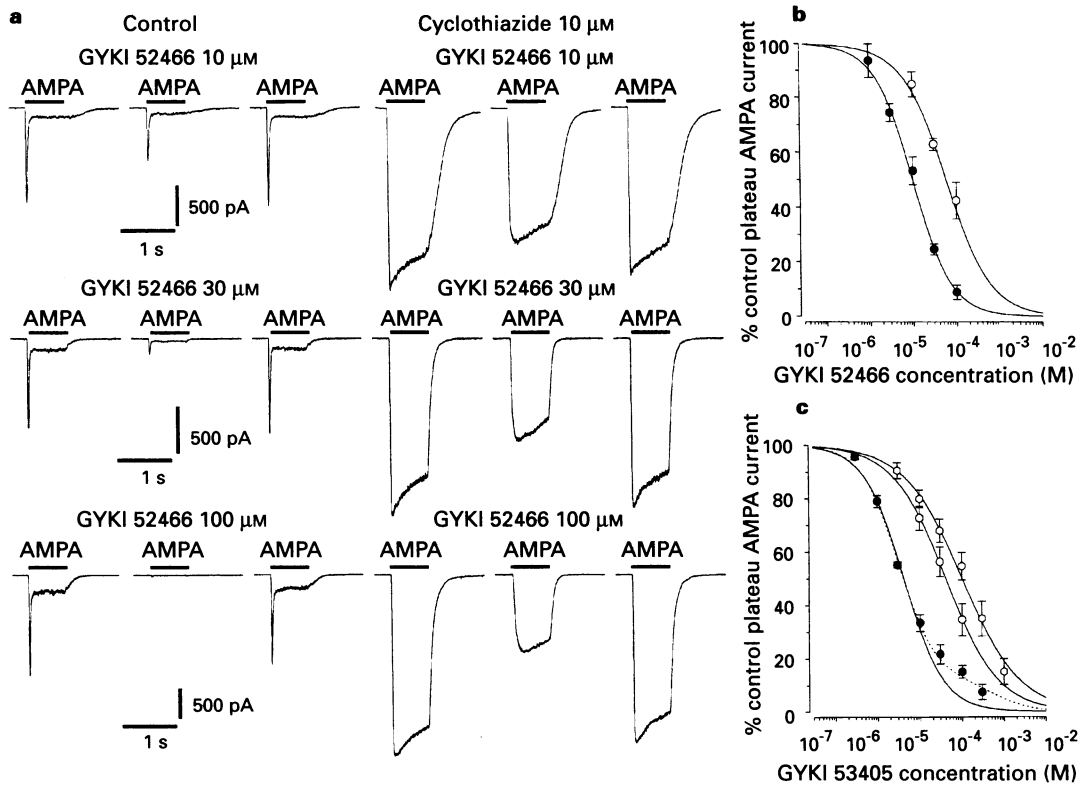

Figure 4 Cyclothiazide counteracts the antagonistic effects of GYKI 52466 and GYKI 53405 on AMPA-induced currents. (a) AMPA (100 μM) was applied as in Figure 1. The first and third traces show control and recovery responses respectively whereas the second trace shows equilibrium responses in the presence of the GYKI 52466 (10 μM) top, (30 μM) middle or (100 μM) bottom. NB: different neurones. The right side of (a) is a similar presentation of the effects of GYKI 52466 on the same neurones in the presence of cyclothiazide (10 μM). (b and c) AMPA (100 μM) was applied in the absence (●) or continuous presence of cyclothiazide, (○) 10 μM; (◯) 100 μM: Pooled steady-state responses were quantified as in Figure 1 and plotted against antagonist concentration (logarithmic) ($n = 7-14$ per concentration). The 4 parameter logistic equation was used to fit the data (solid curves) and to calculate the IC_{50}s for the 2,3 benzodiazepines. GYKI 52466 with cyclothiazide (10 μM); $IC_{50} = 57.9 \pm 9.5$ μM (Hill Coeff. $= 0.85 \pm 0.17$). GYKI 53405 with cyclothiazide (10 μM); $IC_{50} = 41.6 \pm 1.5$ μM (Hill Coeff. $= 0.69 \pm 0.03$). GYKI 53405 with cyclothiazide (100 μM); $IC_{50} = 102.3 \pm 11.6$ μM (Hill Coeff. $= 0.63 \pm 0.04$). NB: the inhibition of control steady state currents by GYKI 53405 was better fitted by a two site model (dotted curve), see Figure 1.

right (Figure 9) and had somewhat less pronounced effects in slowing τ_{on} further (Table 1C). These findings contrast with our own interpretation of preliminary data on AMPA receptor-mediated e.p.s.cs in hippocampal slices and highlight the need for cautious interpretation of results based on 'one-hit' pharmacology (Rammes *et al.*, 1994a).

Discussion

Effects of the 2,3-benzodiazepines on AMPA-induced currents

The AMPA receptor antagonistic effects of the 2,3-benzodiazepines observed in the present study in cultured superior colliculus neurones are largely in line with those previously reported for cultured hippocampal (Donevan & Rogawski, 1992; Donevan *et al.*, 1994) and cortical neurones (Wilding & Huettner, 1995). Although previous reports indicate that the 2,3-benzodiazepines are simple non-competitive AMPA receptor antagonists (Donevan & Rogawski, 1992; Donevan *et al.*, 1994) the present data indicate that these compounds also allosterically influence the affinity of the agonist recognition site (see also Parsons *et al.*, 1994). This was reflected in the relative persistence of steady-state AMPA-induced currents at higher concentrations of the 2,3-benzodiazepines, accompanied by slower onset and faster offset kinetics. The relatively greater inhibition of peak responses by higher concentrations of the 2,3-benzodiazepines may be due to the inherent lower affinity of AMPA receptors for agonists in the non-desensitized state i.e. at the peak of the response (Kiskin *et al.*, 1986; Trussell & Fischbach, 1989; Patneau & Mayer, 1990; 1991; Patneau *et al.*, 1992; Hall *et al.*, 1993; Hennegriff *et al.*, 1994; Parsons *et al.*, 1994). A decrease in agonist-induced desensitization, similar to the effects of low concentrations of NBQX, might also contribute to the relative persistence of steady-state currents at higher antagonist concentrations (Parsons *et al.*, 1994).

It seems unlikely that open channel blockade contributes to the AMPA receptor antagonistic effects of the 2,3-benzodiazepines. Thus, in contrast to the data of Zorumski *et al.* (1983), there was no evidence of use-dependency for any of the three compounds tested in the present study or in the studies of Donevan & Rogawski, (1993) and Parsons *et al.* (1994). Moreover, whilst the effects of GYKI 53655 showed some degree of voltage-dependency (see also Otis *et al.*, 1994), it should be noted that even control AMPA-induced currents showed relatively weak outward rectification (see also Verdoon *et al.*, 1991; Jonas & Sakmann, 1992; Zhang & Trussell, 1994).

Effects of cyclothiazide on AMPA-induced currents

Cyclothiazide concentration-dependently potentiated the steady-state component of AMPA-induced inward currents in cultured superior colliculus neurones to a much greater degree

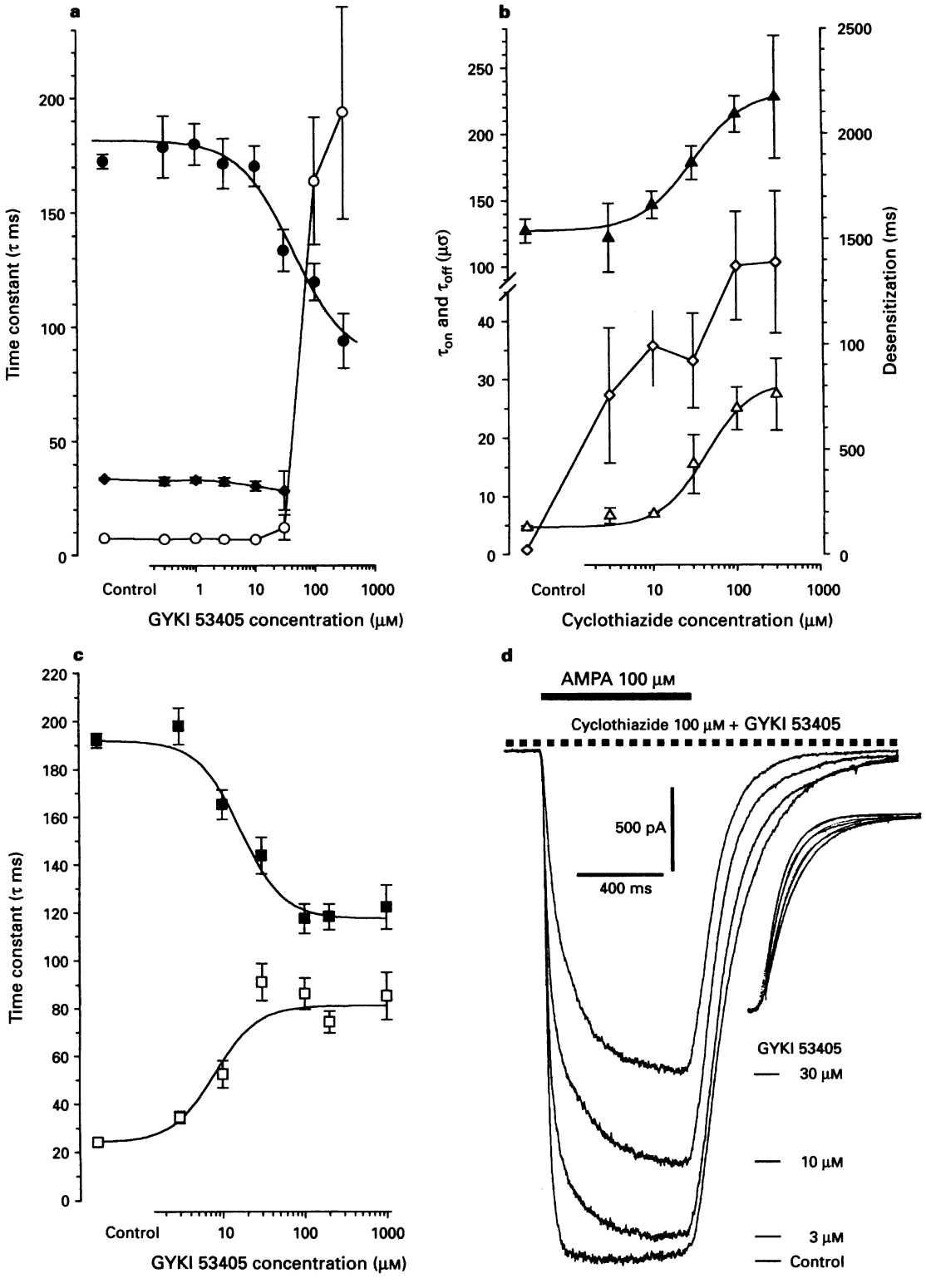

Figure 5 Influence of GYKI 53405 and cyclothiazide on the kinetics of AMPA-induced inward currents. (a) Effects of GYKI 53405 alone. Concentrations of GYKI 53405 above 10 μM slowed the onset kinetics (τ_{on}) and quickened the offset kinetics (τ_{off}) of AMPA-induced inward currents. Desensitization kinetics were not affected at concentrations of GYKI 53405 up to 30 μM; little desensitization was evident in the small currents recorded at higher concentrations of GYKI 53405. τ_{on} (○), τ_{off} (●) and desensitization (◆) have been plotted against GYKI 53405 concentration (logarithmic). (b) Effects of cyclothiazide alone. Cyclothiazide slowed both τ_{on} and τ_{off} of AMPA-induced inward currents in a concentration-dependent manner. Desensitization was greatly reduced at even the lowest concentration of cyclothiazide tested (3 μM). τ_{on} (△), τ_{off} (▲) (1st ordinate) and desensitization (◇) (2nd ordinate) have been plotted against cyclothiazide concentration (logarithmic). The EC_{50} for cyclothiazide on τ_{on} was 42.1 ± 4.5 μM (Hill Coeff. − 1.6 ± 0.33). The EC_{50} for cyclothiazide on τ_{off} was 31.6 ± 6.6 μM (Hill Coeff. − 1.37 ± 0.23). NB: the first ordinate has also been subdivided into two ranges (0–45 ms and 100–280 ms). (c) Interactions of GYKI 53405 and cyclothiazide. GYKI 53405 concentration-dependently reversed the effects of cyclothiazide (100 μM) on τ_{off} (■), IC_{50} = 16.6 ± 4.2 μM, Hill Coeff. 1.7 ± 0.6) but slowed τ_{on} further (□, GYKI 53405 EC_{50} = 8.8 ± 2.8 μM; Hill Coeff. − 1.7 ± 0.7) (d) Original data showing that GYKI 53405 (3–30 μM) concentration-dependently decreased τ_{off} but slowed τ_{on} in the presence of cyclothiazide (100 μM). Inset shows the effects on offset kinetics after normalization of responses to 'control' steady-state levels ('control' τ_{off} 196 ms, GYKI 53405 3 μM τ_{off} 163 ms, GYKI 53405 10 μM τ_{off} 133 ms, GYKI 53405 30 μM τ_{off} 119 ms). NB: GYKI 53405 was unable to reintroduce desensitization in the presence of cyclothiazide (100 μM).

than peak currents. This effect was accompanied by a pronounced reduction of desensitization and a moderate slowing in both the onset and offset kinetics of AMPA responses. Again, these findings are in line with previous reports in cultured hippocampal neurones and indicate that the AMPA receptors expressed in the two systems are similar (Yamada & Tang, 1993; Trussel *et al.*, 1993; Patneau *et al.*, 1993; Wong & Mayer, 1993). The slowing of AMPA offset kinetic by cyclothiazide might be taken as evidence that the affinity of AMPA was also increased by cyclothiazide; a conclusion that would be supported by biochemical data from Desai *et al.* (1994). However, the fact that the onset kinetics of AMPA responses were also slower in the presence of cyclothiazide is hard to reconcile with an increase in AMPA affinity alone.

Interactions between cyclothiazide and the 2,3-benzodiazepines on AMPA-induced currents

Cyclothiazide shifted the GYKI 52466 and GYKI 53405 concentration-response curves to the right in a manner suggestive of a common site of action for these two classes of compound. Although, GYKI 53405 and GYKI 52466 concentration-dependently reversed the effects of cyclothiazide on offset kinetics, these 2,3-benzodiazepines were unable to reintroduce desensitization in the presence of cyclothiazide and actually concentration-dependently slowed the onset kinetics of AMPA responses further. This observation is similar to the effects of the 2,3-benzodiazepines on AMPA response kinetics *per se* and could be taken to imply that the slowing of offset kinetics by cyclothiazide and subsequent restoration to near control levels by the addition of the 2,3-benzodiazepines, purely reflect opposing actions of the two classes of drug on the apparent affinity of AMPA. Likewise, in a preliminary report, Yamada & Turetsky, (1994) have shown that the AMPA receptor antagonistic effects of both GYKI 52466 and NBQX are weakened to a similar degree by cyclothiazide. In contrast, our own provisional data indicate that the allosteric interaction between NBQX and cyclothiazide is far less pronounced (Rammes *et al.*, 1995).

Whatever the underlying mechanism, such data are incompatible with cyclothiazide acting directly at the 2,3-benzodiazepine site (Zorumski *et al.*, 1993) but are still supportive of allosteric interactions between the two classes of compound. Similar interactions have been reported for 2,3-benzodiazepines and cyclothiazide on AMPA-induced depolarization in cortical slices (Palmer & Lodge, 1993) and AMPA-induced increases in free intracellular Ca^{2+} in cultured neurones (Hoyt *et al.*, 1995). In contrast, no such interaction was seen on AMPA- or kainate-induced noradrenaline release in rat hippocampal slices (Desai *et al.*, 1995). The reason for this later discrepancy is unclear but could be related to the slow association kinetics of cyclothiazide, which are even more pronounced in tissue slices (see Figure 7d).

Effects of cyclothiazide and the 2,3-benzodiazepines on AMPA receptor-mediated e.p.s.cs

Support for the specificity and relevance of the present concentration-clamp data on interactions between cyclothiazide

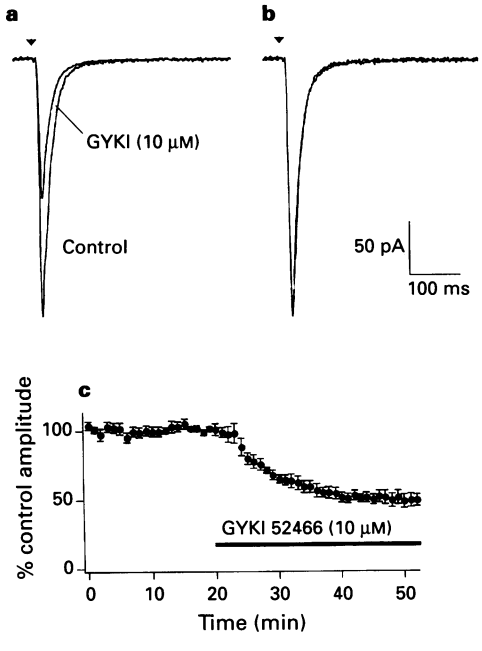

Figure 6 GYKI 52466 reduces the amplitude of AMPA receptor-mediated e.p.s.cs without affecting response kinetics. (a) E.p.s.cs were recorded in area CA1 pyramidal neurones in hippocampal slices before (control) and after equilibration in the presence of GYKI 52466 10 μM (GYKI) traces are averages of 50 responses recorded over 12.5 min. (b) The same signal averages have been normalised to show the lack of effect of GYKI 52466 on response kinetics. (c) Time course of the effects of GYKI 52466 (10 μM): e.p.s.cs were averaged in groups of 4 consecutive responses (4 × 15 s = 1 min) and were then normalized with respect to the grouped average e.p.s.c. amplitude during the 20 min prior to GYKI 52466 application and have been plotted as means ± s.e.mean against time ($n = 5$). GYKI 52466 (10 μM) was present for 33 min as indicated by the bar. GYKI 52466 had no effect on the mean cell input resistance (control 182 ± 8 MΩ, GYKI 52466 170 ± 20 MΩ).

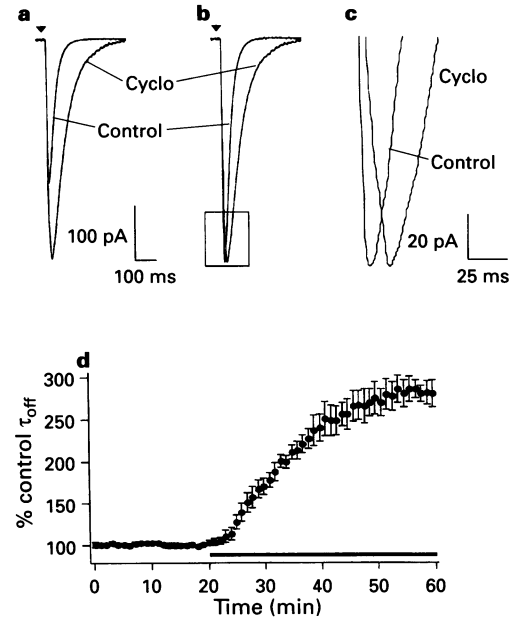

Figure 7 Cyclothiazide prolongs the decay of AMPA receptor-mediated e.p.s.cs. (a) E.p.s.cs were recorded in area CA1 pyramidal neurones in hippocampal slices before (control) and after equilibration in the presence of cyclothiazide (330 μM, Cyclo), traces are averages of 50 responses recorded over 12.5 min. (b) The same signal averages have been normalized to show the effect of cyclothiazide on AMPA receptor-mediated e.p.s.c. kinetics. (c) The boxed area of responses shown in (b) has been expanded to illustrate the small effect of cyclothiazide on AMPA receptor-mediated e.p.s.c. onset kinetics. (d) Time course of the effects of cyclothiazide (330 μM, $n = 9$). Presentation as in Figure 6. Cyclothiazide (330 μM) was present for 40 min as indicated by the bar. Cyclothiazide had no effect on the mean cell input resistance (control 266 ± 29 MΩ, cyclothiazide 259 ± 48 MΩ).

and the 2,3-benzodiazepines at AMPA receptors was provided by similar interactions between GYKI 52466 and cyclothiazide on AMPA receptor-mediated e.p.s.cs in hippocampal slices. In agreement with data from Donevan & Rogawski, (1993), GYKI 52466 antagonized the peak amplitude of AMPA receptor-mediated e.p.s.cs in a concentration-dependent manner with no apparent effect on response kinetics. The failure to see any obvious effect of GYKI 52466 *per se* on AMPA receptor-mediated e.p.s.c. response kinetics is probably partially due to the relatively low inherent affinity of the 2,3-benzodiazepines in mediating such allosteric interactions i.e. changes in τ_{on} and τ_{off} would first have been expected at concentrations of GYKI 52466 above 30 μM where AMPA receptor-mediated e.p.s.cs were probably too small to assess such effects accurately. Cyclothiazide potentiated AMPA receptor-mediated e.p.s.c. amplitude and slowed onset kinetics to a variable degree, but had a more pronounced, concentration-dependent effect on AMPA receptor-mediated e.p.s.c. decay time constants, a finding which is also in close agreement with previous data (Trussell *et al.*, 1993; Pelletier & Hablitz, 1994).

Cyclothiazide was able to shift the GYKI 52466 concentration-response curve on AMPA receptor-mediated e.p.s.c. peak amplitudes to the right. Likewise, GYKI 52466 was able to shift the cyclothiazide concentration-response curve on AMPA receptor-mediated e.p.s.c. decay time constants to the right. As in the concentration-clamp experiments, GYKI 52466 also slowed onset kinetics and fastened offset kinetics of AMPA receptor-mediated e.p.s.cs in the presence of cyclothiazide (330 μM).

Evidence that offset kinetics govern e.p.s.c. decay

Two points are worth mentioning at this juncture. Firstly, the maximal effects of cyclothiazide (330 μM) amounted to only a 2.8 fold slowing in AMPA receptor-mediated e.p.s.c. decay time constants. The magnitude of this effect is compatible with that observed for cyclothiazide on the offset kinetics of AMPA-induced currents but is hard to reconcile with the pronounced effects of cyclothiazide on AMPA receptor desensitization. Likewise, the potency of cyclothiazide in slowing both τ_{off} in cultured neurones and AMPA receptor-mediated e.p.s.c. decay time constants was similar, but lower than that for the potentiation of steady-state AMPA-induced currents. Secondly, GYKI 52466 was able to reverse the effect of cyclothiazide on AMPA receptor-mediated e.p.s.c. decay time constants. Again, this interaction is compatible with that of cyclothiazide and 2,3-benzodiazepines on AMPA response

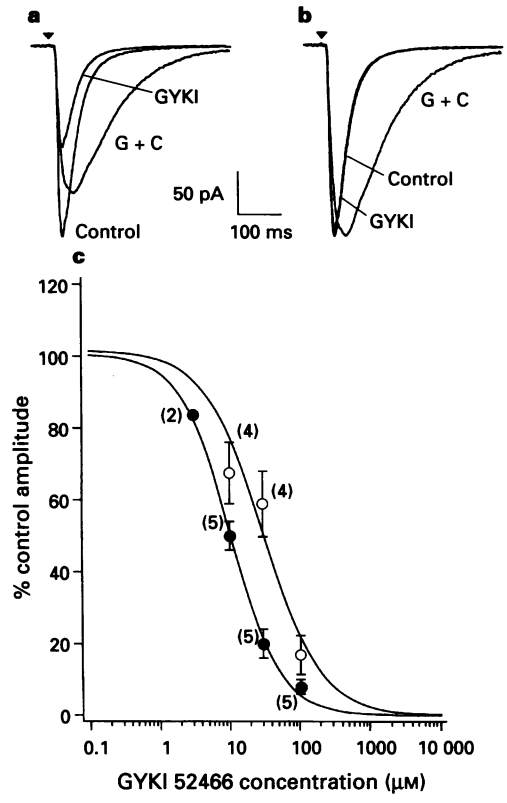

Figure 8 Cyclothiazide (330 μM) weakens the antagonistic effects of GYKI 52466 on AMPA receptor-mediated e.p.s.c. amplitude. (a) E.p.s.cs were recorded in area CA1 pyramidal neurones in hippocampal slices before (control) and after equilibration in the presence of GYKI 52466 (10 μM, GYKI). Additional application of cyclothiazide (330 μM) increased e.p.s.c. amplitude (G+C). Traces are averages of 80 responses recorded over 20 min. (b) Responses have been normalized to show the lack of effect of GYKI 52466 alone on response kinetics but evident effects of the combination of GYKI 52466 and cyclothiazide in slowing both onset kinetics and decay time constants. (c) Concentration-response curves for GYKI 52466 in the absence (●) and presence (○) of cyclothiazide (330 μM): e.p.s.c. amplitudes were normalized with respect to control and have been plotted as means ± s.e.mean against GYKI 52466 concentration (μM). Curves were fitted according to the 4 parameter logistic equation. GYKI 52466 $IC_{50} = 10.8 \pm 0.8$ μM; GYKI 52466 IC_{50} with cyclothiazide (330 μM) $= 26.9 \pm 9.4$ μM. The number of cells at each concentration are given in parentheses.

Table 1 Effects of GYKI 52466 and cyclothiazide on AMPA receptor-mediated e.p.s.c. kinetics

A *GYKI 52466 alone*						
GYKI 52466 (μM)	*Peak*	*s.e. mean*	*Onset (20–80%)*	*s.e. mean*	*Decay (τ)*	*s.e. mean*
3	83.7	–	86.5	–	97.3	–
10	50.0	4.2	105.8	10.1	118.0	6.0
30	20.0	4.4	90.4	11.5	103.1	10.0
100	8.0	2.3	91.7	17.1	82.4	9.3
B *Cyclothiazide alone*						
10	96.3	5.2	121.8	5.4	145.0	4.4
30	110.7	19.7	120.2	13.4	182.2	8.4
100	125.6	16.8	153.5	8.3	258.2	31.0
330	130.0	17.3	139.4	12.5	279.4	22.1
C *GYKI 52466 with cyclothiazide* (330 μM)						
0	100.0	–	139.4	12.5	279.4	22.1
10	67.4	8.4	157.5	23.2	263.8	25.6
30	59.0	9.2	177.4	14.9	219.5	18.4
100	16.9	5.4	218.3	65.0	197.7	12.3

All values were normalized to control e.p.s.cs and are presented as mean % of control with s.e.mean. Onset (20–80%) represents the rise time of AMPA receptor-mediated e.p.s.cs between 20% and 80% of their maximal amplitude whereas decay (τ) was a true exponential fit of decay kinetics. Control e.p.s.cs had onset kinetics of 45.7 ± 5.7 pA ms^{-1} and decay kinetics (τ) of 30.4 ± 2.2 ms ($n = 40$).

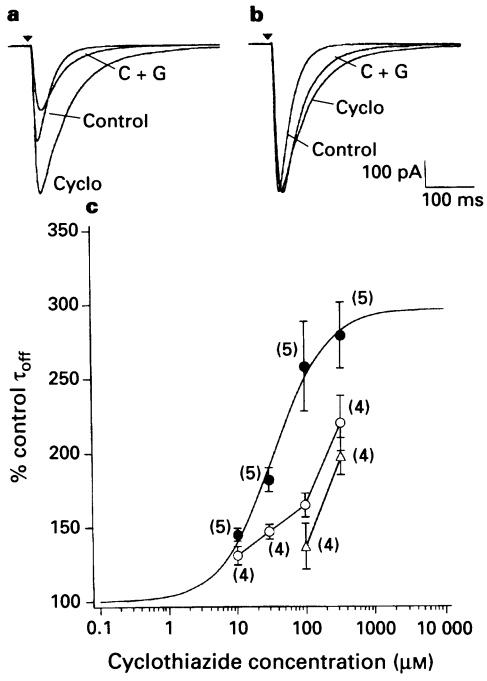

Figure 9 GYKI 52466 weakens the effects of cyclothiazide on AMPA receptor-mediated e.p.s.c. decay time constants. (a) e.p.s.cs were recorded before (control, 80 responses) and after equilibration in the presence of cyclothiazide (330 μM) (Cyclo, 40 responses). Additional application of GYKI 52466 (30 μM) reduced e.p.s.c. amplitude (C+G, 24 responses). (b) Responses depicted in (a) have been normalized with respect to peak e.p.s.c. amplitude to illustrate the slowing of decay time constants by cyclothiazide and the partial reversal of this effect by GYKI 52466 (30 μM). (c) Concentration-response curves for cyclothiazide in the absence and presence of GYKI 52466 (30–100 μM): e.p.s.c. decay kinetics were normalised with respect to control and have been plotted as means ± s.e.mean against cyclothiazide concentration (μM). The 4 parameter logistic equation was used to calculate the EC_{50} for cyclothiazide alone (●) of 35.7 ± 6.5 μM (NB: the Hill Coeff. was fixed at 1.0 due to the inability to test the high concentrations of cyclothiazide necessary for an accurate assessment of the maximal effect). An equivalent fit could not be made with the available data for cyclothiazide in the presence of GYKI 52466 (30 (○) and 100 μM (△)). As such data points have been joined to illustrate the trend for a rightward shift of the cyclothiazide concentration-response curve. The number of cells at each concentration is given in parentheses.

offset kinetics, but cannot be accounted for by interactions at the level of AMPA receptor desensitization. It therefore seems likely that AMPA receptor-mediated e.p.s.c. decay time constants in area CA1 hippocampal neurones are not governed by receptor desensitization. The data are more consistent with the possibility that the kinetics of AMPA receptor-mediated e.p.s.cs are determined by AMPA receptor affinity and, in turn, agonist dissociation kinetics. Indeed, Clements *et al.* (1992) and Hestrin, (1992) came to a similar conclusion on the basis of their comparative experiments on AMPA receptor-mediated e.p.s.cs and responses of outside-out patches to a short pulse of glutamate. In this regard, previous interpretations that the prolongation of AMPA receptor-mediated e.p.s.cs by cyclothiazide and aniracetam can be taken to provide evidence that the time course of AMPA receptor-mediated synaptic transmission is governed largely by desensitization should be considered with caution (Isaacson & Nicoll, 1991; Trussell *et al.*, 1993; Pelletier & Hablitz, 1994; Raman *et al.*, 1994).

However, there are also some obvious caveats in our interpretation of the present data. Firstly, macroscopic desensitization can be influenced both by effects on the rate of entry into the desensitized state and by effects on the rate constant for ion channel closure (Vyklicky *et al.*, 1991). Secondly, τ_{off} of AMPA-induced currents was assessed in the desensitized state. According to our interpretation, the kinetics of AMPA receptor-mediated e.p.s.cs may be governed by the dissociation kinetics of glutamate from non-desensitized AMPA receptors. In this regard, it would certainly have been pertinent to determine the interactions of cyclothiazide and 2,3-benzodiazepines on AMPA-induced currents following very short i.e. 1–2 ms applications in outside-out patches. We were not able to perform such experiments with cyclothiazide and the 2,3-benzodiazepines with our perfusion system. However, aniracetam has been tested in this manner and was found to slow both desensitization and offset kinetics of glutamate-induced currents to a similar degree as its prolongation of e.p.s.c. duration (Tang *et al.*, 1991; Hestrin, 1992). Finally, the possibility that the interactions of the 2,3-benzodiazepines and cyclothiazide on e.p.s.c. kinetics are indirect via effects on glutamate release mediated at presynaptic AMPA receptors cannot be excluded (Trussel *et al.*, 1993; Barnes *et al.*, 1994).

Therapeutic implications

In contrast to the effects of cyclothiazide on AMPA receptor-mediated e.p.s.cs discussed above, decreased desensitization is indeed more likely to underlie the potentiation of AMPA-induced excitotoxicity by cyclothiazide in neuronal cultures (May & Robison, 1993b; Moudy *et al.*, 1994). As such, it is perhaps less surprising that the neuroprotective affinity of GYKI 52466 was not observed to be different in the presence of cyclothiazide (Rock & Campbell, 1993). It is at present not known if an endogenous agonist at the cyclothiazide recognition site is present in the brain. If such a substance were to be released under excitotoxic conditions, it might be expected to exacerbate the toxic activation of AMPA receptors by glutamate. If this were indeed to occur, the results of the present study and that of Rock & Campbell (1993) might indicate that the 2,3-benzodiazepines would, if anything, be less potent under such conditions. Similar neurotoxic effects are also seen with other positive modulators of AMPA receptors that decrease desensitization. For example, whilst therapeutically relevant i.e. nM concentrations of aniracetam exert neuroprotective activity *in vitro* via stimulation of phosphatidyl inositol (PI) hydrolysis, the mM concentrations required to enhance synaptic transmission and LTP *in vitro* (Ito *et al.*, 1990; Mayersohn *et al.*, 1993) also potentiate AMPA receptor-mediated toxicity *in vitro* (Pizzi *et al.*, 1993).

As such, the development of agents aimed at decreasing AMPA receptor desensitization may represent the wrong approach to improve cognitive processes (see Danysz *et al.*, 1995b for review). Thus, the present data indicate that desensitization does not play the major role in determining the kinetics of AMPA receptor-mediated e.p.s.cs. In contrast, desensitization is indeed more likely to be an endogenous mechanism to counteract the pathological effects of tonic activation of AMPA receptors by sustained, albeit relatively moderate, increases in extracellular glutamate during e.g. ischaemia (e.g. Globus *et al.*, 1991) or in chronic neurodegenerative diseases (Greenamyre *et al.*, 1988; Palmer & Gershon, 1990, for review see Danysz *et al.*, 1995a).

In this regard, the AMPAkines may have a more promising profile than agents such as cyclothiazide as they may have been reported to enhance peak AMPA-induced currents more than cyclothiazide but steady-state currents less than cyclothiazide (Arai *et al.*, 1994). This may indicate that they have a greater effect on AMPA receptor affinity than on desensitization. Such properties could also underlie their promising preclinical profile in models of long-term potentiation (LTP) and learning in normal animals (Granger *et al.*, 1993; Staubli *et al.*, 1994a, b; Coogan *et al.*, 1994). However, a further caveat to this approach is the fact that positive modulation of AMPA receptors would be expected to affect most glutamatergic synapses in the

brain and not just those where synaptic plasticity is desired i.e. they might be predicted to have effects reminiscent of central stimulants like amphetamine.

Conclusions

Cyclothiazide and the 2,3-benzodiazepines seem to bind to different sites on the AMPA receptor but exert relatively strong allosteric interactions with one another and with other domains such as the AMPA recognition site. The interactions of GYKI 52466 and cyclothiazide on AMPA receptor-mediated e.p.s.cs in area CA1 of hippocampal slices provide evidence that the decay time constants of these synaptic events may be partially governed by the offset kinetics of glutamate at AMPA receptors and not by desensitization.

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