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Evidence for a role of presynaptic AMPA receptors in the control of neuronal glutamate release in the rat forebrain

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Abstract

The role of presynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in controlling the neuronal release of excitatory amino acids has been investigated. Stimulation of presynaptic AMPA receptors by the endogenous agonist L-glutamate, or by (*R*,*S*)-AMPA, dose-dependently enhanced the Ca^{2+}-dependent, tetrodotoxin-insensitive, electrically-stimulated release of [^{3}H]D-aspartate from rat forebrain slices. This AMPA receptor-mediated response showed marked stereoselectivity with the activity residing solely in the (*S*)-isomer. (*R*)-AMPA was inactive in this respect. AMPA-evoked responses were significantly enhanced in the presence of the AMPA receptor desensitization inhibitor, cyclothiazide (10 μM). Moreover, responses to both AMPA and glutamate were inhibited by competitive (NBQX) and non-competitive (GYKI 52466) AMPA receptor-selective antagonists in a dose-dependent manner. These results provide strong support for the existence of presynaptic AMPA receptors acting to enhance the synaptic release of excitatory amino acids in the mammalian forebrain. Such a positive feedback system may play an important functional role in physiological (e.g., long-term potentiation) and/or pathological (e.g., epileptogenesis) processes in the mammalian central nervous system. AMPA-type autoreceptors may provide new targets for drug action. © 1997 Elsevier Science B.V.

Keywords: Excitatory amino acids; AMPA receptor; Glutamate; [^{3}H]D-Aspartate release; Autoreceptor

1. Introduction

In addition to serving important roles in energy metabolism, protein synthesis and ammonia detoxification, L-glutamate is the major excitatory amino acid in the mammalian central nervous system, acting at ionotropic and metabotropic glutamate receptor sub-types (Fonnum, 1984; Mayer and Westbrook, 1987; Monaghan et al., 1989). These receptors are classified according to their selective agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainic acid and (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) (Bettler and Mulle, 1995; Pin and Duvoisin, 1995). The latter class is usually referred to as the 'metabotropic' sub-type. There is now a wealth of evidence for postsynaptic glutamate receptor involvement in a range of central processes including fast excitatory transmission and the induction of synaptic plasticity, and in pathological events such as epileptogenesis and ischaemic cell damage (Mayer and Westbrook, 1987; Croucher et al., 1988; Croucher and Bradford, 1989; Bliss and Collingridge, 1993). However, little is known about the functional importance and pharmacological properties of glutamate receptors at the presynaptic nerve terminals.

The release of many central neurotransmitters, including acetylcholine (Szerb, 1979), dopamine (Carlsson, 1975), noradrenaline (Alberts et al., 1981) and γ-aminobutyrate (Baumann et al., 1990) is known to be regulated by activation of presynaptic autoreceptors by the synaptically released transmitters. Conversely, although the excitatory amino acids (including glutamate and aspartate) are the principal excitatory transmitters in the brain, the potential role of autoreceptors, particularly of the ionotropic sub-type, in the control of their neuronal release is at present obscure. Ionotropic glutamate receptors are divided into NMDA and non-NMDA (kainic acid and AMPA) sub-types. To date nine subunits of non-NMDA receptors have been cloned, each sharing approximately 70% amino acid sequence identity (Keinänen et al., 1990; Sommer and Seeburg, 1992) and evidence suggests that these various subunit proteins combine in the CNS to form receptors with differential distribution and various pharmacological properties. The receptors formed from the combination of

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GluR1–GluR4 (also commonly termed GluRA–GluRD) subunits have been shown to have a high affinity for AMPA and to undergo a rapid desensitization in response to prolonged AMPA application (Trussell and Fischbach, 1989). The diuretic compound cyclothiazide has recently been reported to inhibit this AMPA receptor desensitization and to strongly enhance AMPA-mediated cytotoxicity (Patneau et al., 1993; Yamada and Tang, 1993; Zorumski et al., 1993).

In the present study we provide evidence for the existence of presynaptic AMPA receptors in the rat central nervous system. Stimulation of this receptor subpopulation enhances Ca^{2+}-dependent, tetrodotoxin-insensitive, electrically-stimulated release of the non-metabolized excitatory amino acid 'marker' [^{3}H]D-aspartate from rat forebrain slices, indicating a role for these receptors in the positive feedback control of synaptically released excitatory amino acids. These receptors show marked stereoselectivity with the activity of racemic (*R*,*S*)-AMPA residing solely in the (*S*)-isomer. The response to receptor stimulation is potentiated by cyclothiazide and inhibited by competitive and non-competitive AMPA receptor antagonists. It is proposed that this AMPA receptor-mediated positive feedback system may play an important functional role in physiological and pathological processes in the mammalian central nervous system. Preliminary accounts of some of this work have been published (Patel and Croucher, 1995a,b; Patel et al., 1995).

2. Materials and methods

2.1. Preparation of brain slices

Male Wistar rats (240 ± 5 g) were killed by decapitation, the brains were removed rapidly and a modified brain slicer was used to cut 3 serial forebrain coronal slices of 500 μM thickness, sectioning from the anterior hippocampus posteriorly (Patel and Turnbull, 1981). The slices were placed in Krebs solution at room temperature and divided down the mid-sagittal plane providing 6 hemisections per animal. Each hemisection was preincubated individually for 45 min in 5 ml of brain modified Krebs bicarbonate buffer, pH 7.4 at 37°C and constantly oxygenated with 95% O_2/5% CO_2. The composition of the brain modified Krebs solution was (mM): NaCl 120, KCl 4.75, KH_2PO_4 1.2, $CaCl_2$ 2.6, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 10.

2.2. 'Loading' of brain slices with [^{3}H]D-aspartate and measurement of efflux

The tissue was 'loaded' with [^{3}H]D-aspartate by incubating for a further 45 min in buffer containing 40 nM [^{3}H]D-aspartate (specific activity 25 Ci/mmol). Individual hemisections were then transferred to gold microelectrode superfusion chambers which were placed in a waterbath at 37°C and superfused with oxygenated Krebs solution at a rate of 0.4 ml/min using a non-pulsatile pump (Ismatec IPN, Switzerland) for 45 min to wash off excess radiolabel. Six basal superfusate samples were then collected using a multiple fraction collector (Retriever IV, ISCO, USA) prior to each of 2 periods of electrical stimulation (S_1 and S_2), given 32 min apart. The stimulations comprised 5 min trains of biphasic rectangular pulses (36 mA, 2 ms) at a frequency of 20 Hz applied through the gold microelectrodes and delivered from an HSE Type 215/T stimulator. The stimulator operated in a constant current mode with pulses monitored concurrently on an oscilloscope. Preliminary studies confirmed that these stimulation parameters evoked a just-significant increase in efflux of label permitting the detection of both positive and negative modulation of release (data not shown). Superfusate fractions were collected every 2 min up to 74 min, and at the end of the experiment the slice sections were removed from the electrode chambers and solubilized in tissue solubilizer (NCS-II; Amersham International, UK). Scintillation fluid (Picofluor 40, Canberra Packard, USA) was added to the superfusates and solubilized tissue samples and the radioactivity content was quantified by liquid scintillation spectrometry (LKB Wallac RackBeta II; 65% counting efficiency).

In several experiments, 100 nM tetrodotoxin was added to the incubation medium 10 min prior to S_2 stimulation to determine the dependency of the response on axonal action potential propagation. The Ca^{2+}-dependency of the response was also studied by superfusing slices with Ca^{2+}-free Krebs solution, supplemented with 1 mM EGTA, during both the washout period and subsequent superfusion. All other drugs were introduced in the superfusate 10 min prior to S_2 and their influence on basal and stimulated efflux of [^{3}H]D-aspartate was examined. Exposure to agonists was terminated 10 min after S_2 with the antagonists remaining in the superfusate throughout the experiment.

Mean basal efflux levels (B_1 and B_2) were calculated as the mean level of radioactivity in the 3 samples immediately preceding the respective periods of electrical stimulation. Responses to electrical stimulation (S_1 and S_2) were calculated as the total levels of stimulated efflux minus mean basal levels. Ratios of basal and electrically-stimulated efflux of label before and after drug application (B_2/B_1 and S_2/S_1, respectively) were calculated to assess the influence of the drugs and other treatments on basal and stimulated release. Previous studies from other groups (Butcher et al., 1986, 1987; Martin et al., 1991; Barnes et al., 1994) have confirmed that the calculation of ratios in this manner provides a consistent measure of release against which the effects of drugs can be monitored.

2.3. Analysis of results

Results are presented as the mean ± S.E.M. of *n* independent observations. Statistical significance of differences

in responses was determined using Student's *t*-test for independent groups. Values were considered to be significantly different from control if $P < 0.05$.

2.4. Drugs

The following drugs were used (sources in parentheses): glutamic acid, kainic acid, γ-aminobutyric acid (GABA) and tetrodotoxin (Sigma, UK), (*R,S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), (*R*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ((*R*)-AMPA), (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ((*S*)-AMPA), (*R,S*)-3-amino-2-(4-chlorophenyl)-propylphosphonic acid (phaclofen) and cyclothiazide (Tocris Cookson, UK). 6-Nitro-7-sulphamoyl-benzo(*f*)quinoxaline-2,3-dione (NBQX) and 1-(aminophenyl)-4-methyl-7,8-methylendioxy-5H-2,3-benzodiazepine (GYKI 52466) were kind gifts from Dr. L. Nordholm (Novo Nordisk A/S) and Dr. I. Tarnawa (Institute for Drug Research, Budapest), respectively. D-[2,3-^{3}H]Aspartic acid, specific activity 25 Ci/mmol, was supplied by Amersham International (Amersham).

3. Results

3.1. Basal and electrically-stimulated efflux of [^{3}H]D-aspartate

In forebrain slices preloaded with [^{3}H]D-aspartate, in the absence of drug treatment, basal efflux of label represented $0.16 \pm 0.09\%$ ($n = 12$) of the total label present in the slice at the time of sampling. In these control experiments a mean B_2/B_1 ratio of 0.96 ± 0.15 ($n = 12$) was obtained, indicating that the basal efflux of label remained essentially constant during the course of the experiment. Electrical stimulation of the slices evoked significant increases in the efflux of label during both S_1 and S_2. Typical values were $1.13 \pm 0.18\%$ and $0.75 \pm 0.14\%$ ($n = 12$) of total label present in the slice for S_1 and S_2, respectively, resulting in a control S_2/S_1 ratio of approximately unity.

3.2. Tetrodotoxin- and Ca^{2+}-dependency of stimulated [^{3}H]D-aspartate efflux

To establish that the efflux of [^{3}H]D-aspartate evoked by electrical stimulation was due principally to direct stimulation of presynaptic nerve terminals within the slices and did not require axonal propagation of action potentials, the sensitivity of the response to tetrodotoxin was examined. The presence of the voltage-sensitive Na^+-channel blocker tetrodotoxin, 100 nM, did not significantly influence either basal or electrically-stimulated efflux of label in any experiment (Fig. 1), indicating that action potential propagation was not a requirement of these responses, i.e., that they were evoked principally at the presynaptic nerve terminal.

The Ca^{2+}-dependency of the stimulated efflux was also determined. In Ca^{2+}-free buffer containing 1 mM EGTA, electrically-simulated efflux was reduced by 54.9% ($n = 6$; $P < 0.01$) confirming that the released label originated largely from presynaptic neuronal stores. The basal release of label was unaffected under these conditions (data not shown).

Evidence suggests that $GABA_B$ receptors are localized on glutamatergic nerve terminals in the central nervous system and that stimulation of these receptors inhibits the synaptic release of glutamate (Pende et al., 1993; Waldmeier et al., 1994). Consistent with this concept, in the present study GABA, 50 μM, was shown to cause a significant inhibition of electrically-stimulated [^{3}H]D-aspartate efflux (Fig. 1). This effect was fully blocked by the selective $GABA_B$ receptor antagonist phaclofen, 300 μM, confirming that the response was $GABA_B$ receptor-mediated. Phaclofen, 300 μM, alone produced a small but statistically insignificant rise in electrically-stimulated efflux (Fig. 1).

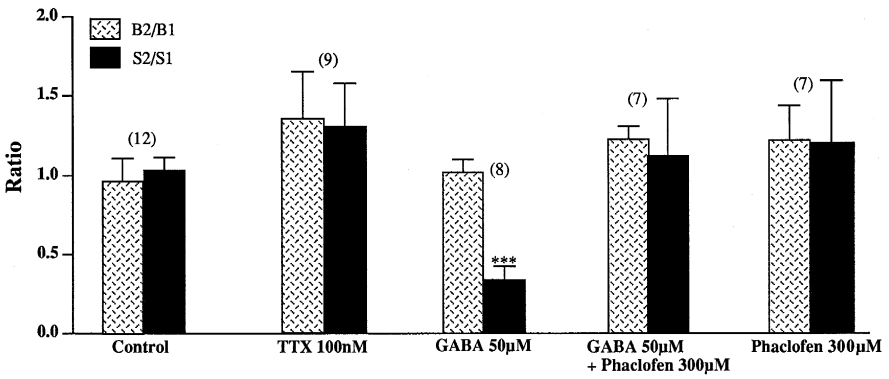

Fig. 1. The influence of tetrodotoxin and the inhibitory amino acid γ-aminobutyric acid on basal and electrically-stimulated [^{3}H]D-aspartate release from rat forebrain slices. Slices were preloaded with [^{3}H]D-aspartate and stimulated twice (S_1 and S_2), at an interval of 32 min, as described in Section 2. Ratios of basal (B_2/B_1) and stimulated (S_2/S_1) efflux of [^{3}H]D-aspartate were calculated as described in the text and used to assess the influence of drugs on the release of label. Each column represents the mean value (±S.E.M.) of *n* (in parentheses) independent observations. *** $P < 0.001$ versus electrical stimulation alone (Student's *t*-test for independent groups). TTX = tetrodotoxin; GABA = γ-aminobutyric acid.

Table 1
Effects of L-glutamate on basal and electrically-stimulated release of [^{3}H]D-aspartate from rat forebrain slices and inhibition by AMPA receptor antagonists

Treatment		Efflux ratio		*n*
Agonist	Antagonist	B_2/B_1	S_2/S_1	
Electrical stimulation alone	—	0.96 ± 0.15	1.03 ± 0.08	12
L-glutamate 1 μM	—	1.14 ± 0.15	0.86 ± 0.18	8
L-glutamate 10 μM	—	1.25 ± 0.12	3.45 ± 0.76 [a]	8
L-glutamate 100 μM	—	1.29 ± 0.24	3.93 ± 1.12 [a]	7
L-glutamate 1000 μM	—	2.54 ± 1.04	6.24 ± 1.76 [a]	6
L-glutamate 100 μM	NBQX 100 μM	1.25 ± 0.16	2.05 ± 0.89 [b]	8
L-glutamate 100 μM	GYKI 100 μM	1.06 ± 0.17	3.02 ± 1.29	6
—	NBQX 100 μM	0.90 ± 0.04	0.64 ± 0.28	9
—	GYKI 100 μM	0.99 ± 0.08	0.54 ± 0.22	8

Preloaded rat forebrain slices were electrically stimulated twice (S_1 and S_2) at an interval of 32 min. Ratios of overflow rate for basal (B_2/B_1) and stimulated (S_2/S_1) efflux were calculated, with agonists and/or antagonists present only during the B_2 and S_2 periods (see Section 2).
Results are expressed as mean ± S.E.M. of *n* rats, with significant differences from relevant controls: [a] $P < 0.05$ versus electrical stimulation alone; [b] $P < 0.05$ versus glutamate-induced response. GYKI = GYKI 52466.

3.3. Effects of L-glutamate on [^{3}H]D-aspartate efflux

L-Glutamate, 1–1000 μM, dose-dependently enhanced electrically-stimulated efflux of [^{3}H]-D-aspartate, with a maximum 6.2-fold enhancement at the highest dose (Table 1). L-Glutamate, 1–100 μM, did not influence the basal efflux of label, although at the highest concentration studied (1000 μM) a marked, although just statistically insignificant increase was evident (Table 1).

3.4. Effects of AMPA on [^{3}H]D-aspartate efflux

The racemic mixture of AMPA, over a concentration range of 1–1000 μM, evoked a similar dose-dependent potentiation of electrically stimulated efflux to L-glutamate, although with a lower maximal response (2.4-fold enhancement) (Fig. 2). Again, no significant increase in basal efflux was evident following any dose of the agonist.

3.5. Potentiation of AMPA-evoked responses by cyclothiazide

In the presence of a low (10 μM) concentration of the AMPA receptor desensitization inhibitor cyclothiazide, all AMPA-evoked responses were significantly increased, producing a parallel displacement of the log dose–response curve, with a new maximum 6.6-fold enhancement of the electrically-stimulated response (Figs. 2 and 3). Again, there was no influence of AMPA, this time in the presence of cyclothiazide, on the basal efflux of label. In addition, cyclothiazide, 10 μM, alone showed no effect on basal or stimulated efflux (Table 2).

The activity of the racemic (*R*,*S*)-AMPA appeared to reside solely in the (*S*)-isomer with the (*R*)-isomer being inactive (Fig. 2).

3.6. Inhibition of AMPA-evoked responses by competitive and non-competitive AMPA receptor antagonists

The actions of the competitive and non-competitive AMPA receptor antagonists NBQX and GYKI 52466, respectively, on AMPA-evoked responses were investigated using a submaximal concentration of AMPA in the presence of cyclothiazide. NBQX, 1 μM, did not influence the response to AMPA, 100 μM plus cyclothiazide, 10 μM (Table 2). However, NBQX, 10–100 μM, fully inhibited AMPA/cyclothiazide-evoked responses (Table 2). In addition, NBQX, 100 μM, produced a significant inhibi-

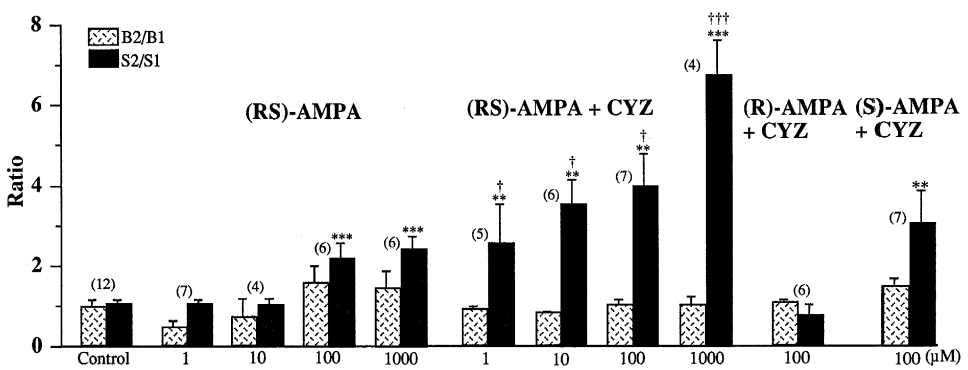

Fig. 2. The influence of cyclothiazide on AMPA-induced enhancement of electrically-stimulated [^{3}H]D-aspartate release from rat forebrain slices. Legend as for Fig. 1. ** $P < 0.01$, *** $P < 0.001$ versus electrical stimulation alone; † $P < 0.05$, ††† $P < 0.001$ versus AMPA-evoked responses (Student's *t*-test for independent groups). CYZ = cyclothiazide (10 μM).

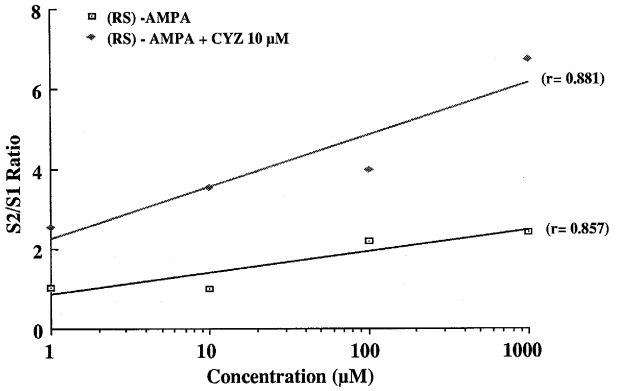

Fig. 3. Log dose–response curves for (*R,S*)-AMPA in the presence (filled diamond) or absence (dotted square) of the AMPA receptor desensitization inhibitor, cyclothiazide (10 μM). Curves were fitted by linear regression analysis (r = correlation coefficient). The mean enhancement of the AMPA-evoked response by cyclothiazide (10 μM) across this concentration range was 2.5-fold. CYZ = cyclothiazide.

tion of the response to glutamate, 100 μM (Table 1). Neither cyclothiazide, 10 μM, nor NBQX, 100 μM, alone (Table 1), significantly influenced basal or electrically-stimulated efflux.

A similar profile of response was seen following GYKI 52466, a non-competitive antagonist which is thought to act at the cyclothiazide binding site on the AMPA receptor (Zorumski et al., 1993). Thus, whilst GYKI 52466, 1 μM, was without effect on the response to AMPA, 100 μM plus cyclothizide, 10 μM, higher concentrations produced a dose-dependent inhibition with GYKI 52466, 100 μM, fully inhibiting the response (Table 3). At this concentration GYKI 52466 also inhibited the response to glutamate, 100 μM, by approximately 31% (i.e., the difference between the responses to L-glutamate in the presence and absence of GYKI 52466 and the response to electrical stimulation alone; Table 1). As with NBQX, GYKI 52466, 100 μM, alone did not significantly influence basal or electrically-stimulated efflux (Table 1).

4. Discussion

Relatively little is known about the presynaptic regulation of excitatory amino acid release, although there is indirect evidence to support a role for inhibitory autoreceptors, principally of the group III metabotropic sub-type, at various synapses in the central nervous system (McBean and Roberts, 1981; Koerner and Cotman, 1981; Gannon et al., 1989; Forsythe and Clements, 1990; Trombley and Westbrook, 1992; Attwell et al., 1995). The results of the present study suggest that AMPA-type autoreceptors, located on presynaptic glutamatergic and/or aspartatergic nerve terminals in the rat forebrain, provide a positive feedback system of control for synaptic release of these

Table 2
Effects of the competitive AMPA receptor antagonist NBQX on basal and electrically-stimulated efflux of [^{3}H]D-aspartate from rat forebrain slices in the presence of AMPA and cyclothiazide

Treatment		Efflux ratio		*n*
Agonist	Antagonist	B_2/B_1	S_2/S_1	
Electrical stimulation alone	—	0.96 ± 0.15	1.03 ± 0.08	12
AMPA 100 μM + CYZ 10 μM	—	1.02 ± 0.14	4.00 ± 0.80 [a]	7
AMPA 100 μM + CYZ 10 μM	NBQX 1 μM	1.32 ± 0.06	3.73 ± 0.69 [a]	6
AMPA 100 μM + CYZ 10 μM	NBQX 10 μM	0.93 ± 0.07	0.64 ± 0.23 [b]	5
AMPA 100 μM + CYZ 10 μM	NBQX 100 μM	1.08 ± 0.19	0.79 ± 0.30 [b]	6
CYZ 10 μM	—	1.15 ± 0.14	0.97 ± 0.18	11

Legend as for Table 1. CYZ = cyclothiazide.
[a] $P < 0.01$ versus electrical stimulation alone.
[b] $P < 0.01$ versus AMPA/CYZ-evoked responses.

Table 3
Effects of the non-competitive AMPA receptor antagonist GYKI 52466 on basal and stimulated efflux of [^{3}H]D-aspartate from rat forebrain slices in the presence of AMPA and cyclothiazide

Treatment		Efflux ratio		*n*
Agonist	Antagonist	B_2/B_1	S_2/S_1	
Electrical stimulation alone	—	0.96 ± 0.15	1.03 ± 0.08	12
AMPA 100 μM + CYZ 10 μM	—	1.02 ± 0.14	4.00 ± 0.80 [a]	7
AMPA 100 μM + CYZ 10 μM	GYKI 1 μM	1.22 ± 0.39	4.44 ± 0.90 [a]	6
AMPA 100 μM + CYZ 10 μM	GYKI 10 μM	1.01 ± 0.09	2.63 ± 0.57 [a]	11
AMPA 100 μM + CYZ 10 μM	GYKI 100 μM	0.83 ± 0.09	0.40 ± 0.24 [b]	7
CYZ 10 μM	—	1.15 ± 0.14	0.97 ± 0.18	11

Legend as for Table 1. CYZ = cyclothiazide; GYKI = GYKI 52466.
[a] $P < 0.01$ versus electrical stimulation alone.
[b] $P < 0.01$ versus AMPA/CYZ-evoked responses.

transmitters. The inhibition of release of [^{3}H]D-aspartate by GABA in the initial experiments of the present study, and its blockade by the selective $GABA_B$ receptor antagonist phaclofen, is consistent with evoked release of the label from glutamatergic nerve terminals as these are known to have inhibitory $GABA_B$-type heteroreceptors located on their terminal membranes (Huston et al., 1993; Pende et al., 1993; Waldmeier et al., 1994). Indeed, in agreement with Waldmeier et al. (1994), our results support a role for phaclofen-sensitive $GABA_B$ receptors in inhibiting neuronal glutamate release. Others have suggested that these $GABA_B$ receptors may be of a novel phaclofen-insensitive sub-type (Pende et al., 1993).

It is clearly important to establish the location of the receptors mediating the AMPA-evoked responses in the present experiments. That the positive modulatory responses to AMPA are also mediated by presynaptic and not postsynaptic receptors, is supported by the tetrodotoxin-insensitivity of the release of label (Fig. 1) and by the absence of effect of AMPA, in the presence or absence of cyclothiazide, on the basal release of radioactivity (Fig. 2). Moreover, the present results are consistent with the findings of Barnes et al. (1994) who recently demonstrated AMPA receptor-mediated enhancement of potassium-stimulated [^{3}H]L-glutamate release from rat hippocampal synaptosomes, a preparation rich in glutamatergic nerve terminals. Using the technique of intracerebral microdialysis we have also recently demonstrated presynaptic AMPA receptor-mediated enhancement of [^{3}H]L-glutamate release in the rat striatum in vivo (Patel et al., 1997). Taken together these observations strongly support a presynaptic location of the AMPA receptors mediating enhanced efflux of [^{3}H]D-aspartate in the rat forebrain slice preparation.

[^{3}H]D-aspartate has been widely used as a non-metabolized marker for neuronally released endogenous excitatory amino acids (Balcar and Johnston, 1972; Davies and Johnston, 1976) in a range of in vitro preparations, including brain slices (McBean and Roberts, 1981; Kerwin and Meldrum, 1983; Lombardi et al., 1993, 1994, 1996), synaptosomes (Attwell et al., 1995) and cell cultures (Meier et al., 1984; Gallo et al., 1992; Cousin et al., 1996). Despite recent evidence that [^{3}H]D-aspartate penetrates poorly into synaptic vesicles (see McMahon and Nicholls, 1991), evoked release of this labelled amino acid is considered to provide a valid gross index of synaptic excitatory amino acid release enabling the investigation of combined exocytotic and non-exocytotic events (Potashner, 1978; Notman et al., 1984; Arqueros et al., 1995). Indeed, it has been shown, using cultured neurones, that [^{3}H]D-aspartate is released, at least in part, from synaptic vesicles by an exocytotic mechanism (Gallo et al., 1992; Cousin et al., 1996). The clear Ca^{2+}-dependency of [^{3}H]D-aspartate release under our experimental conditions, and the consistency of the effects reported here with those on [^{3}H]L-glutamate release both in vitro and in vivo (Barnes et al., 1994; Patel et al., 1997), strongly suggest that we are studying effects on the depolarization-induced output of synaptic transmitter. In addition, a close correlation between [^{3}H]D-aspartate output and the release of [^{3}H]L-glutamate neosynthesized from [^{3}H]glutamine and of endogenous glutamate has also been recently demonstrated in striatal and cerebrocortical slices (Palmer and Reiter, 1994; Lombardi et al., 1996). However, the possibility that presynaptic AMPA receptor stimulation also affects the cytoplasmic output of glutamate and/or aspartate cannot be ruled out by the present results. Moreover, the possibility of selective regulation of neuronal glutamate or aspartate release, as previously reported in the hippocampus (Zhou et al., 1995), cannot be determined from [^{3}H]D-aspartate efflux. Studies of the influence of presynaptic AMPA receptor stimulation on endogenous amino acid release in vitro and in vivo are currently in progress to address these issues.

In the present study, the selective glutamate receptor agonist AMPA dose-dependently enhanced the electrically stimulated release of [^{3}H]D-aspartate, in the absence of effects on basal efflux. This lack of effect on basal efflux, also reported by Barnes et al. (1994) in hippocampal synaptosomes, suggests that the AMPA receptor-mediated response is activity-dependent and may involve intracellular mechanisms which interact synergistically with the depolarization-evoked, exocytotic release. That this action is mediated by presynaptic receptors, and not via interaction with the high affinity uptake systems for dicarboxylic amino acids (i.e., inhibition of re-uptake of [^{3}H]D-aspartate) (see Kanai et al., 1993), is suggested by the observation that cyclothiazide, a benzothiadiazide diuretic which selectively inhibits AMPA receptor desensitization (Partin et al., 1993), markedly potentiated the action of AMPA on stimulated release (Figs. 2 and 3). This action of AMPA also showed marked stereoselectivity with the activity residing solely in the (*S*)-isomer of the agonist (Fig. 2). The lack of effect of cyclothiazide alone, on either basal or stimulated release (Table 2), indicates a lack of tonic activation of presynaptic AMPA receptors by the presumed endogenous agonist glutamate under resting conditions or during the just-suprathreshold level of electrical stimulation employed in the present study. A similar lack of effect of cyclothiazide alone has also been reported in studies of basal and potassium-stimulated release of glutamate from rat hippocampal synaptosomes (Barnes et al., 1994). Further support for a receptor-mediated mode of action of AMPA in enhancing transmitter release is provided by the inhibitory effects of the selective AMPA receptor antagonists. Thus, both competitive (NBQX) (Sheardown et al., 1990) and non-competitive (GYKI 52466) (Tarnawa et al., 1990, 1993; Paternain et al., 1995) AMPA receptor antagonists dose-dependently inhibited the action of AMPA on depolarization-evoked release (Tables 2 and 3). NBQX acts as a classical competitive antagonist, competing with AMPA at the agonist binding site of the

receptor-channel complex (Sheardown et al., 1990). GYKI 52466, on the other hand, binds to a different site to inhibit AMPA receptor activity. This may be the same site as cyclothiazide (Palmer and Lodge, 1993; Zorumski et al., 1993) or a separate site which interacts allosterically with the cyclothiazide binding site to control AMPA receptor function (Donevan and Rogawski, 1993; Desai et al., 1995; Rammes et al., 1996; Yamada and Turetsky, 1996). Although the results of the present study, using a single concentration of cyclothiazide, cannot discriminate between these sites, they do demonstrate a greater (approximately 10-fold) potency of NBQX compared to GYKI 52466 in blocking the presynaptic AMPA receptor-mediated response (Tables 2 and 3). This is in agreement with the results of Parsons et al. (1994) who reported a 6-fold greater activity of NBQX compared with GYKI 52466 against AMPA-evoked responses in patch-clamped hippocampal neurones and supports a common pharmacology of presynaptic and postsynaptic AMPA receptors in respect of antagonist sensitivity. However, recent results from our laboratory (Thomas et al., 1997) have demonstrated marked differences in the agonist activity profiles of these AMPA receptor sub-types.

Although there appears to be a trend in reduction of stimulated release following NBQX and GYKI 52466, neither of the antagonists, when given alone, significantly influenced basal or electrically stimulated outflow of [^{3}H]D-aspartate (Table 1). This is consistent with the lack of activity of cyclothiazide when given alone and further suggests that positive modulation of glutamate release mediated by AMPA autoreceptors is only operative at high levels of neuronal activity, i.e., those resulting in substantial rises in synaptic glutamate. Such a system may permit the maintenance of 'supply on demand' for neuronal transmitter release, thus ensuring a maximal postsynaptic response to particularly intense presynaptic fibre activity.

Although inhibition of re-uptake of [^{3}H]D-aspartate by AMPA as a contributory factor in the observed responses cannot be ruled out by the present experiments, this seems unlikely due to the lack of significant effect of AMPA on basal efflux of label, coupled with the pharmacological profile of the response, as described above. To our knowledge there is no evidence in the literature for an inhibitory action of AMPA on excitatory amino acid re-uptake systems. Glutamate receptors have also been shown to be present on glial cells and activation of these in the brain slice preparation could contribute to the observed responses either directly, or indirectly via an increase in extracellular potassium. However, the close comparability of our own results to those obtained from studies using hippocampal synaptosomes (Barnes et al., 1994), a preparation containing more purified neuronal terminals, strongly implies that the presynaptic AMPA receptors on glutamate neurone terminals are the principal mediators of these responses.

Initial studies using glutamate itself showed a similar dose-dependent potentiation of electrically stimulated release of [^{3}H]D-aspartate, with accompanying increases in basal release at the highest doses (100–1000 μM) (Table 1). These responses, at least at the highest doses, may result in part from competition by glutamate with [^{3}H]D-aspartate for the glutamate uptake carriers. However, the finding that both NBQX and GYKI 52466 also caused marked inhibition of glutamate-evoked effects (Table 1) suggests that a significant component of these responses results from stimulation of presynaptic AMPA receptors.

Although the cellular mechanism of enhancement of glutamate release by presynaptic AMPA receptor stimulation is at present undefined, it is known that sub-types of AMPA receptors which are devoid of the GluR2 sub-unit show greater permeability to Ca^{2+} ions than their GluR2-containing counterparts (Keinänen et al., 1990; Hume et al., 1991; Burnashev et al., 1992; Bettler and Mulle, 1995). Stimulation of presynaptically located, Ca^{2+}-permeable AMPA receptors by glutamate, or other AMPA receptor agonists, could clearly cause an increase in nerve terminal intracellular Ca^{2+} concentration and result in enhanced exocytosis of neurotransmitter. The elevated intracellular Ca^{2+} may directly stimulate transmitter release or may act via signal transduction cascades, such as Ca^{2+}-dependent protein phosphorylation pathways that are known to stimulate transmitter release (Wang et al., 1988). An increase in intracellular Ca^{2+} concentration consistent with the above hypothesis has recently been demonstrated in hippocampal synaptosomes following presynaptic AMPA receptor stimulation (Malva et al., 1994).

Presynaptic AMPA receptors acting to enhance the neuronal release of excitatory amino acids such as glutamate may play a role in the aetiology of pathological processes such as epileptogenesis, ischaemic brain damage and neurodegenerative disorders. These processes are well known to be associated with abnormally high levels of extracellular glutamate in the brain. Indeed, using intracerebral microdialysis of electrically kindled animals, we have recently demonstrated marked elevations in extracellular glutamate levels in vivo both prior to and during epileptiform seizure activity (Kaura et al., 1995). This raises the interesting possibility that the anticonvulsant activity of AMPA receptor antagonists, first demonstrated by Croucher et al. (1984), may be due in part to inhibition of presynaptic AMPA receptor-mediated positive feedback on synaptic glutamate release. Presynaptic receptors controlling neurotransmitter release, including the AMPA autoreceptors described in the present study, may therefore provide new targets for drug development. We have recently shown that agents (e.g., 1*S*,3*S*-ACPD) acting at presynaptic metabotropic glutamate receptors to suppress glutamate release, show powerful anticonvulsant activity against electrically kindled seizures (Attwell et al., 1995). Antagonists acting selectively at presynaptic AMPA receptors on glutamate neurone terminals are likely to show similar, potentially therapeutically useful properties. Cur-

rent studies in our laboratory are investigating regional differences in presynaptic AMPA receptor-mediated responses and are defining the pharmacological profiles of these receptors in comparison with their postsynaptic counterparts. Initial indications of differences in pharmacological profiles (Thomas et al., 1997) support the hypothesis that it will be possible to develop novel, therapeutically useful agents acting selectively at presynaptic AMPA-type autoreceptors.

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