Cyclothiazide unMASKS AMPA-Evoked STIMULATION of [3H]-L-glutamate release FROM rat hippocampal synaptosomes

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The effect of α-amino-3-hydroxy-5-methylisoxazolepropionato (AMPA) on Ca2+-sensitive, tetrodotoxin (TTX)-insensitive K+ -stimulated [3H]-L-glutamate release from rat hippocampal synaptosomes was demonstrated. AMPA in the presence, but not in the absence of cyclothiazide, a drug which blocks AMPA receptor desensitization, elicited a dose-dependent increase in K+ -stimulated [3H]-L-glutamate release but had no effect on basal release. The AMPA/cyclothiazide stimulation was blocked by CNQX and by GYKI 52466, an antagonist at the cyclothiazide site. These results indicate that AMPA receptors are present on presynaptic terminals and suggest that they may play a role in the regulation of neurotransmitter release.

Keywords: AMPA; GYKI; cyclothiazide; CNQX; synaptic plasticity; glutamate release; autoreceptors

Introduction Ionotropic glutamate receptors can be subdivided into N-methyl-D-aspartate (NMDA) and non-NMDA (kainate and α-amino-3-hydroxy-5-methylisoxazolepropionate (AMPA)) subtypes and, to date, at least seven separate cDNAs encoding non-NMDA receptor subunits have been cloned and characterized. Among these GluR1-GluR4 are proposed as the constituent subunits of functional AMPA receptors (Wentholt et al., 1992).

AMPA receptors are important in a range of physiological (Barnes & Henley, 1992) and pathophysiological (Choi, 1992) processes including a long-term potentiation (LTP) in the rat hippocampus. LTP is of widespread interest since it has been proposed as an underlying molecular mechanism for learning and memory (Bliss & Collingridge, 1993). Nonetheless, while the involvement of postsynaptic AMPA receptors in LTP has been demonstrated (Bliss & Collingridge, 1993) the existence of presynaptic AMPA receptors has not been established. Here we present evidence for functionally relevant AMPA receptors on the presynaptic terminals in the rat hippocampus which regulate the release of neurotransmitter.

Methods The hippocampal were dissected from two female Wistar rats (100–170 g) per experiment and synaptosomes were prepared as described previously (McMahon et al., 1989). The synaptosomes were incubated in pre-gassed (95% O2; 5% CO2) Krebs buffer (mM; NaCl 118.0, KCl 4.75, KH2PO4 1.2, MgSO4 1.2, CaCl2 2.5, NaHCO3 25.0; glucose 11.0) plus [3H]-L-glutamate (25 nM; 50 Ci mmol-1) at 37°C for 7 min. The uptake reaction was terminated by two washes in 10 volumes of ice-cold Krebs buffer and the washed [3H]-L-glutamate-loaded synaptosomes were resuspended to 5 ml in fresh ice-cold Krebs.

Aliquots (0.1 ml) were added to 0.9 ml of either prewarmed Krebs buffer, high K+-Krebs buffer (containing an additional 20 mM KCl which gives approximately half-maximal stimulation of release) or Krebs buffer containing 50 μM 4-aminopyridine (4-AP) in the absence (control) or presence of drugs and incubated at 37°C for 2 min. Released [3H]-L-glutamate was separated from synaptosomal [3H]-L-glutamate by rapid filtration through GF/B filters followed by a 1 ml wash with ice-cold Krebs buffer. The data are expressed as percentage of control stimulated release as defined by the difference between the basal and K+ -stimulated [3H]-L-glutamate release in the absence of drugs (100%).

Greater than 75% of the K+-stimulated [3H]-L-glutamate release was Ca2+-dependent and K+-stimulated release was not blocked by the voltage-sensitive Na+ channel blocker tetrodotoxin (100 nM) indicating that release was from synaptosomes comprising presynaptic terminals. Analysis by high performance liquid chromatography (h.p.l.c.) confirmed that the tritium released was in the form of [3H]-L-glutamate rather than any 3H-labelled metabolic product (data not shown).

Results The pharmacological integrity of the synaptosomes was confirmed using the previously characterized ability of carbachol to inhibit endogenous and exogenously applied neurotransmitter release (Marchi et al., 1989) Consistent with those previous observations, in our assay system carbachol (100 μM) reduced K+-stimulated release by 40% and that inhibition was completely inhibited by 0.1 μM atropine (Figure 1).

AMPA alone had no effect on stimulated release but in the presence of cyclothiazide AMPA potentiated K+-stimulated but not basal [3H]-L-glutamate release (Figure 2a) in a concentration-dependent manner (Figure 2b). The maximum

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increase in K⁺-stimulated [³H]-l-glutamate release was ~220% with an EC₅₀ value of 0.3 μM. The AMPA/cyclothiazide response was blocked by CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and the 2,4-benzodiazepine GYKI 52466 (1-(4’-amino-phenyl)-3-acetyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5H-2,3-benzodiazepine) (Figure 2c).

Discussion The bell-shaped dose-response for AMPA (Figure 2b) can be attributed to 10 μM cyclothiazide failing to block 100 μM AMPA-evoked receptor desensitization since the decrease does not occur with 100 μM cyclothiazide. Consistent with the results presented here, cyclothiazide potentiation of AMPA-induced [³H]-noradrenaline release from rat hippocampal slices has also been observed recently but antagonism by GYKI 52466 was not reported (Desai et al., 1994).

The AMPA/cyclothiazide response was inhibited by CNQX and GYKI 52466. CNQX is a well characterized competitive antagonist at the AMPA binding site (Barnes & Henley, 1992) whereas GYKI 52466 is a highly selective non-competitive antagonist of AMPA-evoked responses (Donevan & Rogawski, 1993) and excitotoxicity (Zorumski et al., 1993) in rat CNS. The mechanism of inhibition by GYKI 52466 has been proposed as promoting the rate of desensitization of AMPA receptors by interaction at the cyclothiazide site. Thus GYKI 52466 is believed to be a competitive antagonist at the same allosteric site at which cyclothiazide is an agonist (Zorumski et al., 1993).

The results presented here are relevant to the debate over possible presynaptic mechanisms for the induction and maintenance of LTP. While the molecular mechanisms regulating neurotransmitter release in vivo remain to be determined, our data indicate that activation of presynaptic AMPA receptors, under conditions where desensitization is prevented, can increase release. Furthermore, we demonstrate that the recently available compounds cyclothiazide and GYKI 52466 and their subsequent derivatives will provide invaluable tools to study the properties of pre- and postsynaptic AMPA receptors.

We are grateful to Dr D. Schoepp (Eli Lilly, Indianapolis) for cyclothiazide, Dr Istvan Tarnawa (IDR, Budapest) for GYKI 52466 and Dr Ewart Davies (Birmingham) for help with the h.p.l.c. Supported by MRC and Wellcome Trust grants to JMH. K.D. is an MRC-Novo Nordisk collaborative PhD scholar.

References


(Received June 30, 1994
Accepted July 18, 1994)