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AMPA RECEPTOR MODULATORS HAVE DIFFERENT IMPACT ON HIPPOCAMPAL PYRAMIDAL CELLS AND INTERNEURONS

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Abstract—Positive modulators of AMPA receptors enhance synaptic plasticity and memory encoding. Facilitation of AMPA receptor currents not only results in enhanced activation of excitatory neurons but also increases the activity of inhibitory interneurons by up-modulating their excitatory input. However, little is known about the effects of these modulators on cells other than pyramidal neurons and about their impact on local microcircuits. This study examined the effects of members from three subfamilies of modulators (mainly CX516, CX546 and cyclothiazide) on excitatory synaptic responses in four classes of hippocampal CA1 neurons and on excitatory and disynaptically induced inhibitory field potentials in hippocampal slices. Effects on excitatory postsynaptic currents (EPSCs) were examined in pyramidal cells, in two types of inhibitory interneurons located in stratum radiatum and oriens, and in stratum radiatum giant cells, a novel type of excitatory neuron. With CX516, increases in EPSC amplitude in pyramidal cells were two to three times larger than in interneurons and six times larger than in radiatum giant cells. The effects of CX546 on response duration similarly were largest in pyramidal cells. However, this drug also strongly differentiated between stratum oriens and radiatum interneurons with increases being four times larger in the latter. In contrast, cyclothiazide had similar effects on response duration in all cell types. In field recordings, CX516 was several times more potent in enhancing excitatory postsynaptic potentials (EPSPs) than feedback or feedforward circuits, as expected from its larger influence on pyramidal cells. In contrast, BDP-20, a CX546 analog, was more potent in enhancing feedforward inhibition than either EPSPs or feedback inhibition. This preference for feedforward over feedback circuits is probably related to its higher potency in stratum radiatum versus oriens interneurons. Taken together, AMPA receptor modulators differ substantially in their potency and/or efficacy across major classes of neurons which is likely to have consequences with regard to their impact on circuits and behavior. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: radiatum giant cells, CX516, CX546, BDP-20, cyclothiazide, 1-naphthyl acetyl spermine.

Positive modulation of AMPA type glutamate receptors by specific allosteric modulators has been shown to increase excitatory synaptic transmission (Ito et al., 1990; Arai et al., 1994), facilitate synaptic plasticity (Arai and Lynch, 1992; Staubli et al., 1994; Arai et al., 1996c, 2004), and enhance learning (Staubli et al., 1994; Larson et al., 1995). The latter effects are usually interpreted as being the result of a general increase in excitatory drive in the principal neurons of the hippocampus and elsewhere. However, one aspect that has perhaps not been sufficiently appreciated is that many GABAergic interneurons are driven by AMPA receptors and hence that the modulators are likely to enhance also the inhibitory output generated by them. As a result, enhancing excitatory afferent activity to a given neuron does not necessarily lead to an equivalent gain in depolarization and output of that cell since inhibitory afferents may also have increased in strength. This has been documented for CX516 and IDRA-21 in hippocampal neurons (Arai et al., 1996a,c) and for aniracetam and cyclothiazide in cortical pyramidal cells (Ling and Benardo, 2005). Effects of AMPA receptor modulators on signal processing through local circuits may accordingly be complex and depend on the balance of their impact at excitatory synapses in the different constituent neurons, as well as on the spatial and temporal patterning of the excitatory and inhibitory currents. To date there have been few efforts to assess such circuit level effects of the modulators. Moreover, attempts to characterize their influence at the neuronal level have mostly focused on principal neurons in the hippocampus. A limited number of studies have shown that these drugs are effective also in other regions such as cortex (Vandergriff et al., 2001; Baumbarger et al., 2001) and auditory brainstem (Gardner et al., 2001) and that they enhance synaptic responses in cortical interneurons (Rozov et al., 2001; Goldberg et al., 2003), but there have been no systematic efforts to compare drug effects across different classes of neurons. Interestingly, however, a recent comparison between thalamic neurons and CA1 pyramidal cells found that effects of some modulators on excitatory transmission can differ to unexpectedly large degrees (Xia et al., 2005). It is thus essential to evaluate further the impact of these modulators on different types of neurons.

Neurons in the hippocampal area CA1 are generally classified into pyramidal cells and interneurons. The former are excitatory and represent the majority of neurons (~80%), the latter provide feedforward and feedback inhibition to the principal cells through local synaptic interactions. Although smaller in number, the impact of the interneurons is immense in that a single cell can exert control over thousands of pyramidal neurons (for review, see Freund and Buzsaki, 1996). Interneurons are highly diverse morphologically and physiologically and efforts to

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Abbreviations: ACSF, artificial cerebrospinal fluid; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; LTP, long-term potentiation; Naspm, 1-naphthyl acetyl spermine/*N*-acetyl-spermine; SO, stratum oriens; SR, stratum radiatum.

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classify them have accordingly relied on multiple factors such as morphology, location of the soma, distribution of dendritic and axonal arborizations, membrane properties, local circuit interactions and co-localization with calcium-binding proteins/neuropeptides (Freund and Buzsaki, 1996; Parra et al., 1998). Some of these interneurons have their cell bodies in the stratum radiatum (SR) and stratum oriens/alveus area (SO). The former are mainly activated by Schaffer-commissural fibers which also synapse on pyramidal cell dendrites and they provide a feedforward inhibition by either shunting dendritic depolarization or suppressing action potential generation in the principal neurons. The interneurons in SO often receive collateral projections from pyramidal cells and provide feedback inhibition to the principal cells, thereby controlling the CA1 output. However, the SO interneurons may be involved in both feedforward and feedback inhibitory circuitry since they also receive input from Schaffer-commissural fibers (Lacaille et al., 1987).

Like pyramidal cells, interneurons are mostly driven by AMPA receptors but the subunit composition of these receptors and their biophysical and pharmacological properties vary. Studies using single-cell PCR and immunocytochemistry revealed that interneurons in general express the subunit GluR2 at lower levels than pyramidal cells and that GluR4 and GluR1 are more abundant (Geiger et al., 1995; Leranth et al., 1996). These variations in composition can potentially affect the action of AMPA receptor modulators. The present study in fact emerged from observations that some modulators had different potencies for enhancing inhibition versus excitation of primary neurons. In the first part of the study we accordingly compared the effects of some prototypical AMPA receptor modulators on excitatory input to pyramidal neurons with their ability to enhance disynaptically induced feedback and feedforward IPSPs (inhibitory postsynaptic potentials) in hippocampal area CA1. In the main part of the study we then used whole-cell recording to directly measure the effects of the modulators on excitatory postsynaptic currents (EPSCs) in pyramidal neurons and in two types of interneurons, namely the SR and SO interneurons mentioned above. Further included in this analysis were 'radiatum giant cells' which are non-pyramidal cells with a distinct triangular soma located in the SR. These cells have recently been discovered to represent a novel type of excitatory neuron in CA1 (Gulyas et al., 1998) and they resemble pyramidal neurons in several aspects. Thus they have similar dendritic arbors and their dendrites possess spines, they express calcineurin and CaMKII (Sik et al., 1998), and long-term potentiation (LTP) and long-term depression can be induced at their excitatory synapses in an NMDA receptor dependent manner (Maccaferri and McBain, 1996; Christie et al., 2000). On the other hand, giant cells have been found to project to the olfactory bulb (Van Groen and Wyss, 1990; Gulyas et al., 1998) and thus they appear to have long-distance axonal projections distinct from those of pyramidal neurons.

The prototypical modulators examined in this study are cyclothiazide, CX516 and CX546 (and a close relative of the latter, called BDP-20/CX554). These modulators were previously shown to differ in chemical structure and in their effects on AMPA receptor kinetics, excitatory synaptic transmission in pyramidal cells (Arai and Lynch, 1998; Arai et al., 2002) and LTP (Arai et al., 2004). In those studies, the main effect of CX546 on synaptic responses was to prolong the decay of EPSCs (Arai et al., 2002; Nagarajan et al., 2001). In contrast, CX516 at low concentrations primarily increased response amplitude and its effects on response duration became prominent only at millimolar concentrations (Arai et al., 1996a). It has been suggested that both compounds mainly influence channel gating and ligand binding/unbinding (Arai et al., 1996a,b, 2002; Nagarajan et al., 2001), with CX516 having a more prominent effect on channel opening and CX546 on channel closing (Arai et al., 2002). In contrast, the main effect of cyclothiazide is believed to be the blocking of receptor desensitization (Yamada and Tang, 1993; Partin et al., 1996). The present data show that enhancement of synaptic currents by these modulators differs substantially between classes of neurons, that the pattern of effects is distinct for each of these modulators, and that feedforward and feedback circuits may respond in markedly different ways as a result of this. Some of the data have been published in abstract form (Xia and Arai, 2003).

EXPERIMENTAL PROCEDURES

Slice preparation

Hippocampal slices (350–400 μm thick) were prepared from Sprague–Dawley rats (P14–P18) as previously described (Arai et al., 2004). The animals were anesthetized with halothane before decapitation. All experiments conformed to the SIU-SOM guidelines on the ethical use of animals and were carried out according to an institutionally approved protocol and in observation of the guidelines of the National Institutes of Health. All efforts were made to minimize the number of animals used and their suffering. The brain was rapidly removed from the skull and placed into ice-cold artificial cerebrospinal fluid (ACSF). After removing the cerebellum and anterior part of the brain, the brain block was glued onto a disk and slices were cut horizontally using a vibratome (Leica VT1000S, Leica Microsystems Inc., Bannockburn, IL, USA). For field recording, transverse hippocampal slices (450 μm) were cut with a McIlwain-type chopper.

Electrophysiology

After incubation in a holding chamber containing oxygenated ACSF for at least 1 h, slices were transferred to a recording chamber mounted on an upright microscope (Olympus BX50WI, Olympus USA, Melville, NY, USA), and continuously perfused at 0.5 ml/min with ACSF containing (in mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 5, $MgCl_2$ 1, $CaCl_2$ 2, D-glucose 10 and HEPES 10 (pH adjusted to 7.4 with NaOH). Neurons were visualized with infrared differential interference contrast (DIC) videomicroscopy and were initially identified by the location of the soma and the orientation of dendritic arborization. Whole-cell recordings were made from four different types of hippocampal CA1 neurons: (1) SO interneurons, (2) SR interneurons, (3) pyramidal cells, and (4) radiatum giant cells. Biocytin (0.5–1%) was routinely included in the recording pipette in order to identify the morphology of the neurons after the experiments (see below). Synaptic responses were evoked by electrical stimulation in an adjacent area using a twisted nichrome electrode. Whole-cell recordings were made