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New caged neurotransmitter analogs selective for glutamate receptor sub-types based on methoxynitroindoline and nitrophenylethoxycarbonyl caging groups

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ABSTRACT

Photolysis is widely used in experimental neuroscience to isolate post-synaptic receptor activation from presynaptic processes, to determine receptor mechanisms in situ, for pharmacological dissection of signaling pathways, or for photostimulation/inhibition in neural networks. We have evaluated new caged neuroactive amino acids that use 4-methoxy-7-nitroindolinyl- (MNI) or 1-(2-nitrophenyl)ethoxycarbonyl (NPEC) photoprotecting groups to make caged ligands specific for glutamate receptor subtypes. Each was tested for interference with synaptic transmission and excitability and for receptor-specific actions in slice preparations. No adverse effects were found at glutamate receptors. At high concentration, MNI-caged, but not NPEC-caged ligands, interfered with GABA-ergic transmission.

MNI-caged amino acids have sub-microsecond release times suitable for investigating mechanisms at fast synaptic receptors in situ. MNI-NMDA and MNI-kainate were synthesized and tested. MNI-NMDA showed stoichiometric release of chirally pure NMDA. Wide-field photolysis in cerebellar interneurons produced a fast-rising sustained activation of NMDA receptors, and localized laser photolysis gave a fast, transient response. Photolysis of MNI-kainate to release up to 4 μM kainate generated large inward currents at resting membrane potential in Purkinje neurons. Application of GYKI 53655 indicated that 40% of the current was due to AMPA receptor activation by kainate. Signaling via metabotropic glutamate receptors (mGluR) does not require fast release rates. NPEC cages are simpler to prepare but have slower photorelease. Photolysis of NPEC-ACPD or NPEC-DHPG in Purkinje neurons generated slow inward currents blocked by the mGluR type 1 antagonist CPCCOEt similar to the slow sEPSC seen with parallel fiber burst stimulation. NPEC-AMPA was also tested in Purkinje neurons and showed large sustained inward currents selective for AMPA receptors with little activation of kainate receptors. MNI-caged L-glutamate, NMDA and kainate inhibit GABA-A receptors with IC_{50} concentrations close to the maximum concentrations useful in receptor signaling experiments.

1. Introduction

Flash photolysis of inert photolabile precursors – 'caged' ligands – is an experimental tool that enables the release of ligands adjacent to their receptors to overcome slow diffusion in the extracellular or intracellular compartments that would otherwise determine the rates of receptor activation. Photolysis allows diffusional equilibration of the caged ligand in the extracellular or intracellular space adjacent to receptors before photorelease by a pulse of light from a laser, flashlamp or LED source. This is usually at near-UV wavelengths and combined with conventional or laser-scanning microscopy. The ligands can be applied on the same timescales and with similar spatial scales as in their physiological

Abbreviations: DHPG, (*S*)-3,5-Dihydroxyphenylglycine; ACDP, (1*S*,3*R*)-1-Aminocyclopentane-1,3-dicarboxylic acid; MCPG, (*S*)-α-Methyl-4-carboxyphenylglycine; AMPA, (*S*)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NBQX, 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide; NMDA, *N*-Methyl-D-aspartic acid; CPCCOEt, 7-(Hydroxyimino)-cyclopropa[*b*]chromen-1a-carboxylate ethyl ester; GYKI 53655, 1-(4-Aminophenyl)-3-methylcarbamyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride; QX314, *N*-(2,6-Dimethylphenylcarbamoylmethyl)triethylammonium; D-AP5, D-(−)-2-Amino-5-phosphonopentanoic acid; TTX, Octahydro-12-(hydroxymethyl)-2-imino-5,9:7,10a-dimethano-10a*H*-[1,3]dioxocino[6,5-*d*]pyrimidine-4,7,10,11,12-pentol; SR 95531, 6-Imino-3-(4-methoxyphenyl)-1(6*H*)-pyridazinebutanoic acid hydrobromide.

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context. The method is used in neuroscience to activate receptors at synapses in situ in brain slices, and intracellularly to study second messenger signaling pathways. Detailed kinetic investigations can be made of receptor mechanisms (Canepari and Ogden, 2006; DiGregorio et al., 2007) and intracellular signaling pathways (Khodakhah and Ogden, 1995). It can also be used to apply labile transmitters such as nitric oxide, cannabinoids or ATP (Murphy et al., 1994; Jabs et al., 2007; Heinbockel et al., 2005) at known concentration, minimizing effects of catabolism or uptake on the concentration reaching the receptors.

The application of photolysis in neuroscience depends mainly on the availability of caged ligands with suitable photochemical and pharmacological properties but the design, synthesis and testing of caged neurotransmitters and other caged receptor ligands has generally slowed the introduction of new reagents. Here we have applied photochemistry that is well-established in this field to generate caged ligands showing specificity for glutamate receptor sub-types. A significant issue in developing photolabile precursors of neuroactive amino acids has been their level of stability to hydrolysis. Reagents with poor resistance to hydrolysis can leak active neurotransmitter during equilibration with the biological preparation, causing receptor desensitization. 7-Nitroindolinyl- and 4-methoxy-7-nitroindolinyl-L-glutamate were introduced as reagents with good hydrolytic stability (Papageorgiou et al., 1999; Papageorgiou and Corrie, 2000) and have fast, sub-microsecond timescale photolysis reactions (Morrison et al., 2002). They have been widely used since in kinetic investigations of glutamate receptors and for photostimulation (for example Canepari et al., 2001a,b; Matsuzaki et al., 2001; Gasparini and Magee, 2006; DiGregorio et al., 2007; Trigo et al., 2009a). This approach has been adapted here to generate the receptor-specific caged ligands MNI-NMDA and MNI-kainate. For other glutamate receptor ligands we have used 2-nitrobenzyl-based photochemistry to produce stable protection of amino groups (Corrie et al., 1993). These NPEC-caged ligands have slower release rates upon photolysis, on a 50–100 ms timescale, and are suitable for studying G-protein coupled metabotropic receptor signaling. Here we report tests of the new reagents for use as experimental tools in neuroscience.

1.1. Photochemistry of NPEC- and MNI-caged ligands

Synthesis of the new caged ligands was based on published protocols for MNI- and NPEC-caged L-glutamate (Papageorgiou and Corrie, 2000, 2002) and (Corrie et al., 1993) respectively. Structures of the new compounds and outlines of the photochemical cleavage reactions are shown in Fig. 1. Spectroscopic data that verify the structures are given in Supplementary information.

NPEC-caged L-glutamate (*N*-1-(2-nitrophenyl)ethoxycarbonyl-L-glutamate) was introduced as an efficient caged L-glutamate that is resistant to hydrolysis. The synthesis, photochemical properties, mechanistic investigations and application as a caged neurotransmitter at the squid giant synapse were described by Corrie et al. (1993). The synthesis was adapted here to generate NPEC-ACDP, NPEC-DHPG and NPEC-AMPA (Fig. 1). The NPEC caging group has

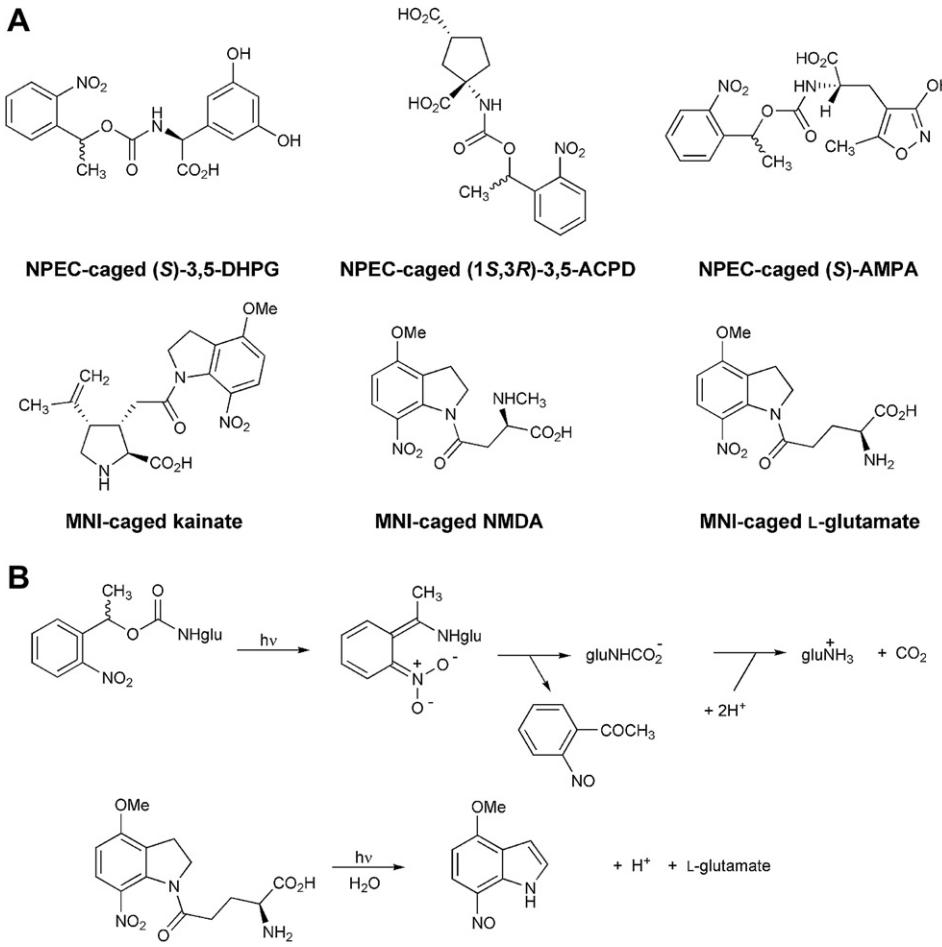

Fig. 1. Reactions and structures of the reagents tested here. Panel A: Structures of MNI- and NPEC-caged compounds. MNI-glutamate is also shown for reference. Panel B: Photolysis reactions for NPEC-glutamate (Corrie et al., 1993) and MNI-L-glutamate (Morrison et al., 2002) respectively.