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Comparative analysis of inhibitory effects of caged ligands for the NMDA receptor

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Abstract

Photolytic release of neurotransmitters from caged precursors is a useful method to study synaptic processes with high temporal and spatial resolution. At present, the two most widely used classes of caged precursors for studies on glutamate receptors are based on derivatives of the 2-nitrobenzyl caging group (α-carboxy-2-nitrobenzyl, CNB) and the nitroindoline caging group (7-nitroindoline, NI, and 4-methoxy-7-nitroindoline, MNI). Besides NI- and MNI-caged amino acids being thermally more stable than the CNB-caged amino acids, there have been no other major advantages reported of using compounds from either of these two classes. Here, we show inhibitory effects of CNB-glutamate and a number of other CNB-caged agonists on *N*-methyl-D-aspartate (NMDA) receptors at non-saturating concentrations of the co-agonist glycine. In contrast, NI- and MNI-glutamate and most other NI-/MNI-caged agonists that we tested were inert under these conditions. Furthermore, we demonstrate that carboxynitroindoline-caged glycine (CNI-glycine), which was previously found to inhibit glycine receptors, has no such effect on NMDA receptors. Together, these findings underline the usefulness of NI- and MNI-caged ligands and show that CNB-caged compounds should be avoided in studies involving NMDA receptors.

Keywords: Flash photolysis; Caged compound; CNB; NI; Glutamate; NMDA receptor; Inhibition

1. Introduction

Photolytic release from caged precursors allows both rapid and spatially restricted application of neurotransmitters (Adams and Tsien, 1993; Corrie and Trentham, 1993), which makes this technique a powerful tool for studying kinetics of neurotransmitter-mediated processes, e.g. receptor activation, as well as for investigating spatial differences of these processes, e.g. effects of activating specific synapses in brain slices. However, a serious limitation of photolytic neurotransmitter release is its dependence on the availability of suitable caged precursors of the neurotransmitters in question. As previously pointed out (Canepari et al., 2001a), requirements for caged precursors include a fast and efficient photorelease as well as thermal stability, i.e. resistance to spontaneous hydrolysis. Most importantly, however, the precursor and the by-products of its photolysis must be biologically inert with respect to the system being studied. For studies on glutamate receptors, two major classes of caged ligands have previously been reported to fulfil these necessary criteria. These are caged ligands based on derivatives of the 2-nitrobenzyl group (α-carboxy-2-nitrobenzyl, CNB) and the nitroindoline group (7-nitroindoline, NI). CNB-caged glutamate (γ-*O*-(α-carboxy-2-nitrobenzyl) glutamate) has been used by different groups (e.g. Sawatari and Callaway, 2000; Yabuta et al., 2001; Dodt et al., 1998) to study synaptic processes involving the α-amino-3-hydroxy-5-methyl-

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4-isoxazole-propionate (AMPA) and *N*-methyl-D-aspartate (NMDA) receptor subclasses of ionotropic glutamate receptors (iGluRs). Similar to other CNB-caged compounds, CNB-glutamate is reasonably stable to thermal hydrolysis and the photorelease of glutamate is sufficiently rapid ($t_{1/2}$ = 21 μs) to make it useful in kinetic studies (Wieboldt et al., 1994; Cheng et al., 2002). Recently, a series of publications explicitly showed that CNB-glutamate is inert with respect to the AMPA receptors GluR1 and GluR2 as well as to the kainate receptor GluR6 (Li et al., 2003a,b; Li and Niu, 2004). CNB-caged NMDA has also been synthesized, but was found to inhibit NMDA receptors on rat hippocampal neurons (Gee et al., 1995). This problem was later solved by using the 2,2′-dinitrobenzhydryl-caged NMDA (DNBH-NMDA), which was reported to be inert at NMDA receptors (Gee et al., 1999). However, DNBH-caged NMDA is poorly water soluble, with maximum achievable concentrations of about 250–300 μM in aqueous solution (Gee et al., 1999).

The NI- and the closely related 4-methoxy-7-nitroindoline (MNI) caging groups have been described recently (Papageorgiou et al., 1999; Papageorgiou and Corrie, 2000) and NI/MNI-caged glutamate have been reported to be suitable for studies on metabotropic glutamate receptors (Canepari et al., 2001b) as well as on AMPA and NMDA receptors (Canepari et al., 2001a). Both NI- and MNI-caged amino acids are much more resistant to thermal hydrolysis than their CNB-caged counterparts, with hydrolysis being negligibly slow at physiological pH and room temperature, and photorelease being very fast ($t_{1/2} \sim 150$ ns measured for a model NI-caged acetate, Morrison et al., 2002). The NI and MNI compounds show the same photochemistry and the release rates of other aliphatic carboxylates such as glutamate, aspartate and glycine can confidently be predicted to be similar to that for the model compound. MNI-caged amino acids have a two- to three-fold higher photorelease efficiency than the NI-caged compounds (Papageorgiou et al., 2000).

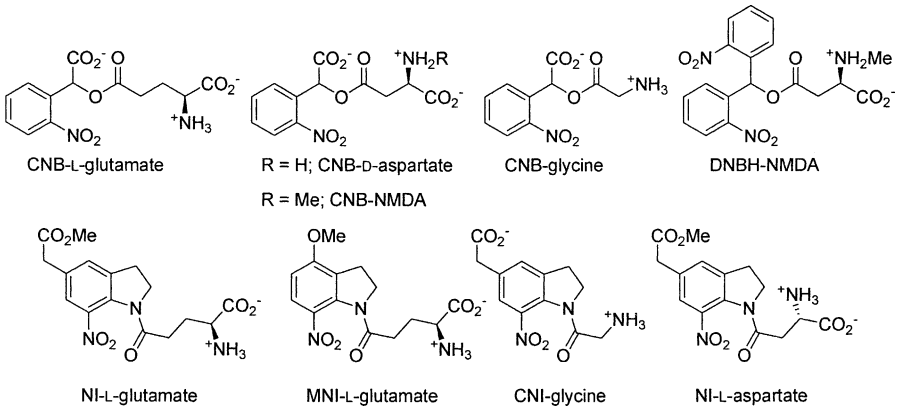
NMDA receptors are unique among iGluRs in that, in addition to glutamate, they require glycine as co-agonist for activation. The glycine-binding site, located on the NR1 subunit of these receptors, shares homology with the glutamate-binding site on the NR2 subunit (Laube et al., 1997), but is unrelated to the ligand-binding site of strychnine-sensitive glycine receptors (GlyRs, Laube et al., 2002). Although several caged glycine derivatives, including CNB- (Grewer et al., 2000) and carboxynitroindoline (CNI)-caged glycine (Canepari et al., 2001a), have been developed, they have only been tested and used on GlyRs, and it therefore remains unclear whether they are useful for studies on NMDA receptors.

In this study, we compare CNB- and NI/MNI-caged ligands for the NMDA receptor and their effects on heterologously expressed receptors. We describe a previously unrecognized inhibitory effect of CNB-glutamate and other CNB-caged ligands on NMDA receptors, which is not observed with the NI- and MNI-precursors. Furthermore, we show that CNI-glycine, which is known to inhibit GlyRs, is inert at the NMDA receptor, making it a promising compound for study of the glycine-binding site of this receptor.

2. Methods

2.1. Cell culture and transfection

HEK293 cells were cultured at 37 °C and 5% CO_2 in DMEM supplemented with 10% fetal calf serum. Twenty-four hours prior to transfection, cells were plated onto fibronectin-coated glass coverslips. Cotransfection of green fluorescent protein (GFP, in the pGreenLantern vector, Gibco) and the NMDA receptor subunits NR1 and either NR2A or NR2B (all in the pCis vector, Gorman et al., 1990) was carried out by using the Effectene transfection kit (Qiagen). The ratio of transfected DNA was 1:10:30 (GFP:NR1:NR2A) or 1:10:10 (GFP:NR1:NR2B). Transfected cells were incubated in the presence of 2 μM MDL (Tocris) along with 200 μM (for transfections with NR2A) or 1 mM (when NR2B was used) D-AP-5 (Tocris) for 24–48 h before recordings.



Scheme 1. Structures of CNB-, NI-, CNI-, DNBH-, and MNI-caged compounds used in this work.