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Reversibly Caged Glutamate: A Photochromic Agonist of Ionotropic Glutamate Receptors

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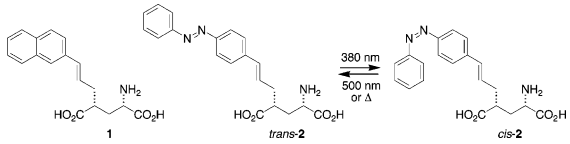
The ability to control the active concentration of neurotransmitters in a spatially and temporally precise manner has revolutionized the study of the central nervous system (CNS). In particular, caged glutamate has emerged as a tool for the dissection of both neural circuitry[1,2] and the fast kinetic events of channel activation.[3] Upon the irreversible photochemical cleavage of a protecting group,[2] released glutamate is free to bind ionotropic glutamate receptors (iGluRs), the major mediators of excitatory information transfer in the CNS.[4]

Photochromic ligands provide another opportunity for the control of neural excitability. First reported in the late 1960s in the form of a photoisomerizable inactivator of chymotrypsin,[5] photochromic ligands control the function of proteins through a reversible change in shape and/or polarity of an integral bistable photoswitch. In the case of photochromic agonists, one configuration functions as an activating ligand, while the other configuration is, ideally, inert toward the system of study.[1] While photochromic nicotinic acetylcholine receptor agonists,[6] enzyme inhibitors,[7] and regulatory peptides[8] have been reported, their benefits have been tempered by the difficulty of achieving perfect on/off activity between states.[9] Their successful implementation, however, avoids many disadvantages of caged systems, including precursor instability and photoproduct toxicity.[10]

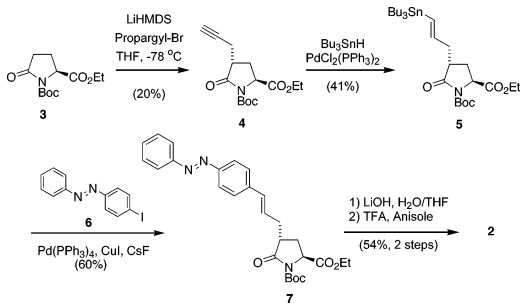
We recently developed a light-activated iGluR6 that is based on a photoisomerizable agonist tethered to an engineered cysteine.[11] Encouraged by this success, we investigated the possibility of a nontethered photochromic agonist that could function at wild-type receptors. Our design was based upon the report of a series of (2*S*,4*R*)-4-substituted glutamate analogues that are potent and selective iGluR5 and 6 kainate receptor (KAR) agonists.[12] In particular, we decided to replace the napthyl group of LY339434 (**1**) with an azobenzene to generate compound **2** (Scheme 1). We envisioned that the change in shape and polarity between *trans*-**2** and *cis*-**2** would generate differences in affinity to the receptor ligand binding domains.

The stereoselective synthesis of **2** was achieved starting from *N*-Boc protected ethyl pyroglutamate **3** (Scheme 2). Diastereoselective alkylation of **3** with propargyl bromide gave alkyne **4**, which underwent hydrostannation to afford vinyl stannane **5**. Palladium-catalyzed Stille coupling with iodoazobenzene **6**, obtained by the condensation of 4-iodoaniline with nitrosobenzene, then gave the *N*-Boc protected azobenzene pyroglutamate **7**. Finally, deprotection of **7** yielded **2** as the dihydrate, monosodium salt.

Scheme 1. Structures of (2*S*,4*R*)-4-Substituted Glutamate Analogues



Scheme 2. Synthesis of Photochromic Agonist **2**



NMR experiments with in situ illumination of the sample revealed photostationary states containing 76.3 ± 0.3% *cis*-**2** at 380 nm and 89 ± 1% *trans*-**2** at 500 nm (mean ± SEM, $n = 3$).[13] Furthermore, the half-life of thermal relaxation from *cis*- to *trans*-**2** in the dark was measured at 18 ± 3 h (mean ± SEM, $n = 3$).

Compound **2** was assayed with whole-cell voltage clamp recordings in HEK293 cells transiently expressing wild-type iGluR6(Q)[14] and pretreated with 0.3 mg/mL concanavalin A (ConA) to block desensitization.[15] Channel activation depended on the wavelength of irradiation, displaying increased inward currents in the *trans* relative to the *cis* state (Figure 1). Activity was competitively blocked by the non-NMDA receptor antagonist DNQX (Supporting Information).[16]

To investigate the *cis*/*trans* agonist profile of **2**, dose−response curves were generated with iGluR5(Q) and iGluR6(Q) under both 380 and 500 nm light (Figure 2). In agreement with previous reports of (2*S*,4*R*)-4-glutamic acid analogues,[12] **2** demonstrated high selectivity at iGluR5 over iGluR6 receptors, with approximately half-maximal efficacy with respect to a 300 μM glutamate evoked response. The apparent agonist affinity (EC_{50}) of iGluR5 was 9 μM under 500 nm illumination and reduced ~10-fold under 380 nm light. Due to the solubility constraints of **2**, we were unable to generate full titration curves at iGluR6. The AMPA receptor iGluR2 failed to produce inward currents in the presence of 250 μM **2**, demonstrating the subtype selectivity of this agonist.

Activation and deactivation proceeded at rates ($\tau_{on-500nm} = 310 \pm 15$ ms; $\tau_{off-380nm} = 250 \pm 14$ ms; mean ± SEM, $n = 3$) slower than the microsecond time scale of traditional uncaging experiments.[17] However, our studies were conducted at light intensities

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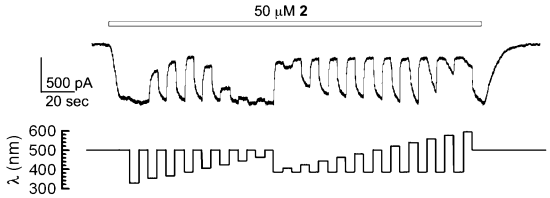

Figure 1. Whole-cell voltage clamp recording of iGluR6(Q) currents in HEK293 cells under UV−visible illumination.

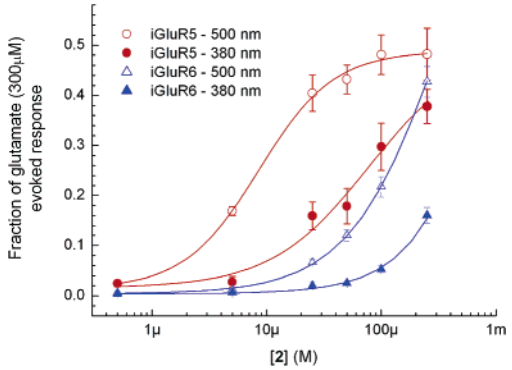

Figure 2. Dose−response curves for inward currents evoked by **2** at iGluR5 and iGluR6 expressing HEK293 cells under 380 and 500 nm illumination.

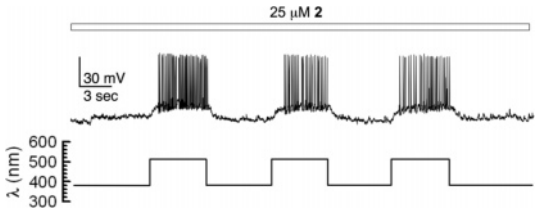

Figure 3. Whole-cell current clamp recording of cultured rat hippocampal neurons in the presence of 25 μM **2** under 380 and 500 nm illumination.

(4.8 mW/mm^2 at 500 nm and 1.8 mW/mm^2 at 380 nm) several orders of magnitude weaker than laser pulse-photolysis techniques. Much faster rates of isomerization are expected at comparable light intensities.[18]

We tested the ability of **2** to depolarize cultured rat hippocampal neurons, which are known to express the KAR subunits iGluR6 and KA2, but not iGluR5.[19] Neurons were exposed to a 25 μM concentration of **2** under 380 nm light and current clamped at −65 mV, without pretreatment with ConA. Switching wavelengths between 380 and 500 nm light was then sufficient to trigger, and extinguish, sustained trains of action potentials (Figure 3). While iGluR6 channels are only activated to a small extent at 25 μM (Figure 2), this can still produce significant depolarization. Given the large number of receptors in the cell, only a small fraction needs to be concurrently activated by glutamate release to trigger neuronal firing.

Taken together, these results describe a simple approach for obtaining remote control of iGluR activity and neuronal firing with a photochromic agonist. The active agonist is subtype-specific, possesses good efficacy and affinity, and can be conveniently controlled by the wavelength of light used. Future work will concern the synthesis of substituted analogues of **2** and in-depth studies profiling their selectivity, receptor desensitization, and utility in neuronal excitation. Ideally, these photochromic agonists will become a valuable complement to irreversibly caged neurotransmitters as well as other methods of remote neuronal control.[20−22]

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Supporting Information Available: Detailed synthetic protocols, relevant spectroscopic data, methods of receptor transfection, electrophysiological recordings, and illumination protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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