A Protecting Group for Carboxylic Acids That Can Be Photolyzed by Visible Light †

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A Protecting Group for Carboxylic Acids That Can Be Photolyzed by Visible Light†

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ABSTRACT: We report on a photolabile protecting (caging) group that is new for carboxylic acids. Unlike previously used caging groups for carboxylic acids, it can be photolyzed rapidly and efficiently in the visible wavelength region. The caging group 7-*N*,*N*-diethyl aminocoumarin (DECM) was used to cage the γ-carboxyl group of glutamic acid, which is also a neurotransmitter. The caged compound has a major absorption band with a maximum at 390 nm ($\epsilon_{390} = 13651\ M^{-1}\ cm^{-1}$). Experiments are performed at 400 nm ($\epsilon_{400} = 12232\ M^{-1}\ cm^{-1}$) and longer wavelengths. DECM-caged glutamate is water soluble and stable at pH 7.4 and 22 °C. It photolyzes rapidly in aqueous solution to release glutamic acid within 3 μs with a quantum yield of 0.11 ± 0.008 in the visible region. In whole-cell current-recording experiments, using HEK-293 cells expressing glutamate receptors and visible light for photolysis, DECM-caged glutamate and its photolytic byproducts were found to be biologically inert. Neurotransmitter receptors that are activated by various carboxyl-group-containing compounds play a central role in signal transmission between $\sim 10^{12}$ neurons of the nervous system. Caged neurotransmitters have become an essential tool in transient kinetic investigations of the mechanism of action of neurotransmitter receptors. Previously uncaging the compounds suitable for transient kinetic investigations required ultraviolet light and expensive lasers, and, therefore, special precautions. The availability of caged neurotransmitters suitable for transient kinetic investigations that can be photolyzed by visible light allows the use of simple-to-use, readily available inexpensive light sources, thereby opening up this important field to an increasing number of investigators.

Photolabile protecting groups for functional groups commonly found in biologically important compounds have been developed during the last 40 years (*1*−*4*). To solve biological problems, they were first used by Kaplan et al. (*5*) to rapidly generate significant and known concentrations of biologically active compounds. The rapid release of biologically active compounds from photolabile precursors has become a very useful technique in transient kinetic investigations of neurotransmitter receptors. These proteins must be studied on cell surfaces, where rapid mixing techniques do not have the time resolution required for the investigation of the fast (microsecond to millisecond) processes involved in activation and inhibition of neurotransmitters receptors (*6*−*9*). This problem is overcome by equilibrating the cell surface receptors with a biologically inactive caged neurotransmitter. The reaction to be investigated is initiated in the μs time domain by photolysis of the caged compound (reviewed, *10*). The α-carboxy-2-nitrobenzyl photolabile protecting group (*11*) was successfully used in such investigations to cage the different neurotransmitters that activate different receptors, carbamoylcholine (a stable analogue of acetylcholine) (*11*), *N*-methyl-D-aspartic acid (*12*), kainate (*13*), γ-aminobutyric acid (*14*, *15*), glutamate (*16*), glycine (*17*), and serotonin (*18*). All these compounds are photolyzed in the μs − ms time region and with adequate quantum yield (reviewed, *19*, *20*). This and other caging groups for neurotransmitters (*21*−*24*) absorb only in the UV wavelength region and require expensive lasers and special rooms in which the instruments must be housed for safety reasons. Because of potential damage to the receptor-containing cells by prolonged exposure to UV light, only a few measurements can be made with each cell. Compounds that are photolyzed in the UV wavelength region can also be photolyzed by multiphoton excitation in the visible wavelength region (*25*, *26*). Uncaging, however, occurs only at the focus of the laser beam (*25*, *26*). The technique is, therefore, suitable for locating the position of a particular receptor, but not for activating sufficient receptors for transient kinetic measurements (*19*). All these problems can be avoided by use of a photolabile protecting group for neurotransmitters that can be photolyzed in the visible wavelength region.

Many organic molecules suitable for caging absorb in the visible wavelength region (*27*−*32*). However, they have negligible or no solubility in aqueous medium (*29*−*32*). Recently, the substituted 2-nitroveratrole group (*33*), nitroindolines (*34*), the phenacyl group (*35*), and substituted

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coumarins (*31*, *36*−*39*) have been studied as caging groups that can be deprotected by visible light. However, many of these compounds have undesirable properties limiting their usefulness, such as low solubility in aqueous medium and/or slow photolysis (*39*), and are, therefore, unsuitable for transient kinetic investigations of membrane-bound proteins on cell surfaces. In the case of the 2-nitroveratrole group the rate of release of the bioactive compound (*40*) is slow, in the minutes region. Various coumarin derivatives are stable only in DMSO solutions (*38*). Aqueous solubility is required to investigate membrane-bound proteins. The stability of caged compounds in aqueous medium is also important, because contamination of the receptors with free neurotransmitter while they equilibrate with the caged compound interferes with the kinetic measurements.

All the following characteristics are required for the caged compound to be useful in transient kinetic investigation of neurotransmitter receptors on cell surfaces.

(1) The compound must be soluble and stable in water at physiological pH.

(2) The release of bioactive compound should be sufficiently fast (in the microsecond time scale) so that release of neurotransmitter does not become the rate-determining step.

(3) The photolysis process should have sufficient quantum yield so that the bioactive compound is released in high enough concentration for kinetic measurements.

(4) Both the caged compound and the photolysis byproducts must be biologically inert.

(5) The caged compound should be photolyzed in the visible wavelength region in order to allow more measurements to be made with each cell because photodamage to the cell or the receptors is avoided. Much less expensive and simple-to-use light sources are available for experiments in the visible wavelength region, thereby opening up this important field to an increasing number of investigators.

Transient kinetic measurements with each cell require two control experiments. A standard concentration of neurotransmitter is applied to the cell at the beginning of the experiment, using the cell-flow technique (*6*). Although the cell-flow technique has a considerably poorer time resolution (∼10 ms) than the laser-pulse photolysis technique (∼50 μs) (*11*, *19*), it can be used to calculate the amount of neurotransmitter that is released from the caged compound in the kinetic measurements (*6*). This measurement, with the same standard concentration of the neurotransmitter, is repeated at the end of the laser-pulse photolysis measurement to assess possible damage to the receptors or the cell during the experiment. Additionally, obtaining a good seal between the recording electrode and a cell is time-consuming. The ability to make many kinetic measurements with each cell has, therefore, many advantages, including a reduction in the experimental error. It is, therefore, a necessity that the caged neurotransmitter is stable in water during the time it takes to make the measurements with a cell.

By virtue of the listed characteristics, the coumarin caging group for carboxyl groups, reported in this paper, is suitable for investigating the fast biological reactions mediated by cell surface receptors.

7-*N*,*N*-Diethylamino-4-hydroxymethyl coumarin (DECM)[1] has been used to cage nucleotides such as ADP and ATP. The DECM-caged nucleotides were photolyzed in the 300−400 nm wavelength region (*41*). Here we ask the questions: Can this caging group also be used with carboxylic acids, and is the quantum yield and rate of photolysis in the visible wavelength region sufficient (*19*) for transient kinetic measurements? Here we describe the synthesis of 7-*N*,*N*-diethylamino-4-hydroxymethyl coumarin caged glutamate. We report that the DECM-caged glutamic acid is water soluble, thermally stable, and biologically inert when tested with HEK 293 cells expressing the GluR6 glutamic acid receptor. It is photolyzed rapidly, within μs, and has a quantum yield of 0.11 ± 0.008, sufficient for transient kinetic investigations (*19*) in the visible region. These properties make it suitable for transient kinetic investigations of the glutamate receptor and, most probably, all the other neurotransmitter receptors that are activated by carboxyl-group-containing neurotransmitters.

MATERIALS AND METHODS

All the starting materials were procured from Aldrich.

Synthesis. (See the reactions in Scheme 1.)

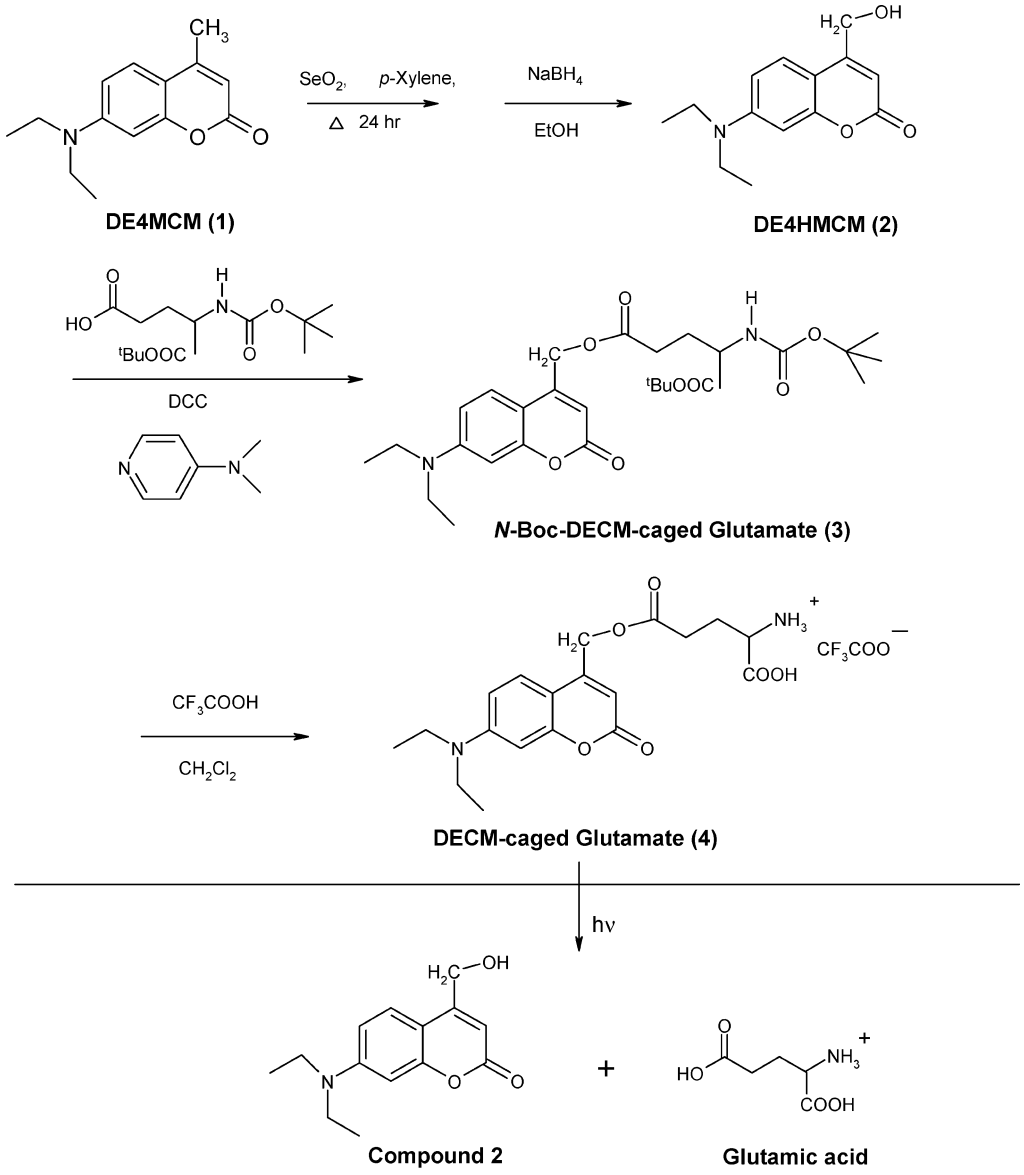
7-N,N-Diethylamino-4-hydroxymethylcoumarin (2). This was synthesized as previously described (*41*). To a solution of 4-methyl-7-*N*,*N*-diethylaminocoumarin **1** (2.32 g, 10.0 mmol) in *p*-xylene (60 mL), selenium dioxide (1.66 g, 15.0 mmol) was added. This reaction mixture was heated under reflux with vigorous stirring. After 24 h, the mixture was filtered and concentrated under reduced pressure. The dark brown residual oil was dissolved in ethanol (65 mL), sodium borohydride (190 mg, 5.0 mmol) was added, and the solution was stirred for 4 h at room temperature. The suspension was carefully hydrolyzed with 1 M HCl (10 mL), diluted with H_2O, and extracted three times with 20 mL CH_2Cl_2. The organic phase was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. By flash chromatography (FC) (CH_2Cl_2/acetone 5:1) 1.20 g (4.48 mmol, 50%) of alcohol **2** was obtained as a yellow solid: R_f 0.27 (hexane/EtOAc 1:2).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (d, $J = 9.0$ Hz, 1H), 6.55 (dd, $J = 9.0$, 2.6 Hz, 1H), 6.46 (d, $J = 2.6$ Hz, 1H), 6.27 (t, $J = 1.3$ Hz, 1H), 4.82 (d, $J = 1.3$ Hz, 2H), 3.39 (q, $J = 7.1$ Hz, 4H), 3.12 (s, 1H), 1.19 (t, $J = 7.1$ Hz, 6H).

(7-N,N-Diethylaminocoumarin-4-yl)-methyl-N-t-BOC-glutamate (3). *N*-*t*-BOC-L-glutamic acid-γ-butyl ester (2.751 g, 9.069 mmol), 4-dimethylaminopyridine (DMAP) (93.48 mg, 0.765 mmol), and 1,3-dicyclohexylcarbodiimide (DCC) (2.047 g, 9.92 mmol) in 100 mL of CH_2Cl_2 were stirred at room temperature for about 10 min. 0.7 g (2.834 mmol) of **2** was added to the reaction mixture, and the resulting mixture was stirred at room temperature in the dark for about 30 min. The reaction mixture was filtered and the solvent evaporated to give a yellow solid. The crude

[1] Abbreviations: HEK 293, human embryonic kidney cells; LaPP, laser-pulse photolysis; DE4MCM, 7-*N*,*N*-diethylamino-4-methyl coumarin; DE4HMCM, 7-*N*,*N*-diethylamino-4-hydroxymethyl coumarin; *N*-Boc-DECM, *N*-Butyloxycarbonyl 7-*N*,*N*-diethylamino coumarin; DECM, 7-*N*,*N*-diethylamino coumarin; FC, Flash chromatography; DCC, 1,3-dicyclohexylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; α-CNB, α-carboxyl-2-nitrobenzyl.

Scheme 1



Mechanism of photolysis of DECM-caged glutamate

product was purified over a silica gel column by FC (10% acetone: CH_2Cl_2) to yield 1.282 g (2.41 mmol, 85%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (d, $J = 9.0$ Hz, 1H), 6.57 (dd, $J = 9.0$, 2.6 Hz, 1H), 6.48 (d, $J = 2.6$ Hz, 1H), 6.1 (s, 1H), 5.2 (s, 2H), 5.08 (d, $J = 8$ Hz, 1H), 4.25 (m, 1H), 3.39 (q, $J = 7.1$ Hz, 4H), 2.4−2.6 (m, 2H), 2.24 (m, 1H), 1.96 (m, 1H), 1.45 (s, 9H) 1.41 (s, 9H), 1.19 (t, $J = 7.1$ Hz, 6H).

(7-N,N-Diethylaminocoumarin-4-yl)-methyl-glutamate-trifluoroacetate (4). (7-*N*,*N*-Diethylamino coumarin-4-yl)-methyl-*N*-*t*-BOC-glutamate (0.250 g, 0.47 mmol) was dissolved in 100 mL of dichloromethane, and the reaction mixture was cooled to 0 °C. Trifluoroacetic acid (5 mL) was added slowly, and the resulting solution was stirred in the dark for about 24 h, bringing it to room temperature. The solvent was removed under reduced pressure. The residue was purified over a Sephadex LH-20 column with water as the eluent.

^{1}H NMR (400 MHz, D_2O): δ 7.7 (d, $J = 9.0$ Hz, 1H), 7.35 (d, $J = 2.1$ Hz, 1H), 7.27 (dd, $J = 9.0$, 2.6 Hz, 1H), 6.42 (s, 1H), 5.27 (s, 2H), 3.83 (t, $J = 7.1$ Hz, 1H), 3.5 (q, $J = 7.1$ Hz, 4H), 2.62 (dt, $J = 7.1$, 2.1 Hz, 2H), 2.1 (dq, $J = 7.1$, 2.1 Hz, 2H), 0.97 (t, $J = 7.1$ Hz, 6H). Anal. Calcd for $C_{21}H_{25}N_2O_8F_3$: C, 51.42; H, 5.13; N, 5.71. Found: C, 51.21; H, 5.39; N, 5.49.

Hydrolysis in the Dark. The caged compound (1 mM) was rapidly dissolved in the buffer solution (150 mM NaCl, 1 mM $CaCl_2$, 10 mM HEPES; the pH was adjusted to 7.4 using 5 N NaOH), and the solution was immediately transferred to a cuvette (4 mL volume, 10 mm path length) and placed in an absorption spectrometer (OLIS-14C) at 22 °C. Whole spectra were recorded between 1 and 240 min after the compound was dissolved (Figure 1).

Laser-Flash Photolysis (Transient Spectral Measurements). Laser-flash photolysis experiments were done with 250 μM DECM-glutamate **4** (Scheme 1) in HEK extracellular buffer (see below for the composition), using a $XeCl_2$ excimer laser (Compex 101 Lambda Physik), with a single 10 ns pulse of 4.8 mJ. The energy of the beam was measured

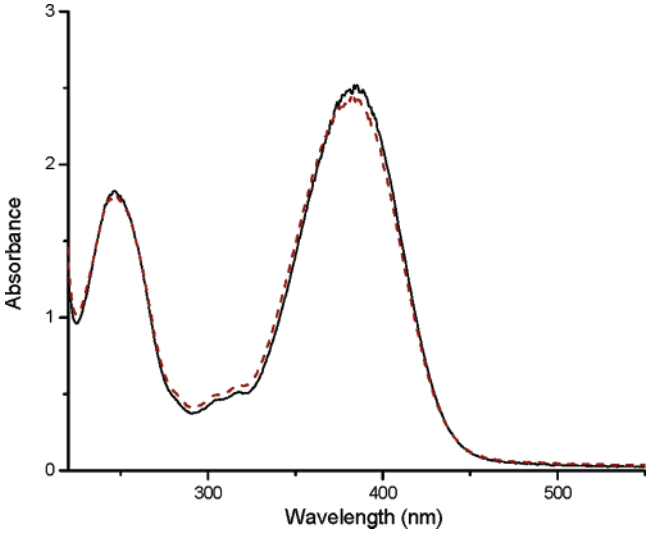


FIGURE 1: Thermal hydrolysis of DECM-caged glutamate: UV−vis spectrum of DECM-caged glutamate (250 μM in HEK extracellular buffer, pH 7.4, 22 °C) at times $t = 0$ min (solid line) and $t = 240$ min (dotted line) after the compound was dissolved. The data at $t = 5$, 10, 20, 30, 60, and 120 min are not shown. The path length of the cuvette was 10 mm.

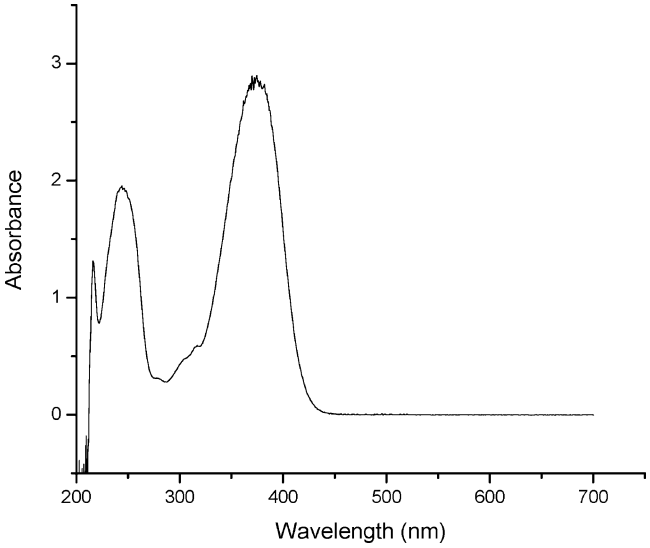


FIGURE 2: UV−visible absorption spectrum of compound **2** (Scheme 1), 7-*N*,*N*-diethylamino-4-hydroxymethylcoumarin, which is the DECM photolysis byproduct.

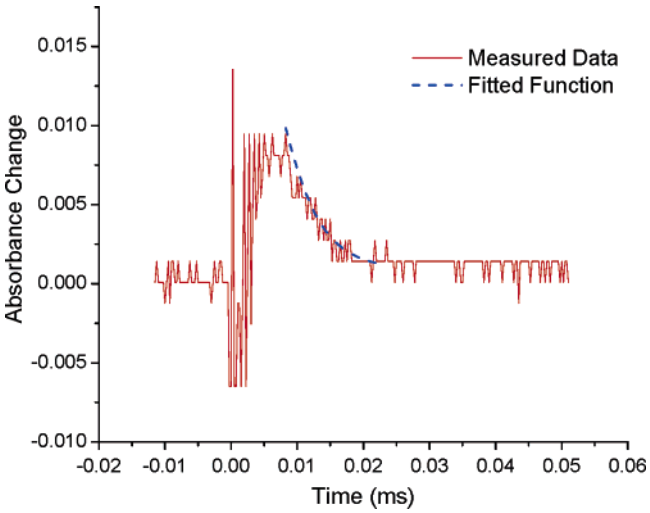


FIGURE 3: Absorption transient at 460 nm observed in the photolysis of DECM-caged glutamic acid (compound **4** in Scheme 1), produced by a light pulse at ≥400 nm in a 0.8 mM solution of compound **4** in 100 mM extracellular HEK buffer, pH 7.4 and 22 °C. The single-exponential absorbance decay has a time constant of 3 μs. The solid line represents the measured data and the dotted line the fitted curve. The absorbance change observed at zero time is produced by discharge of the laser power supply and is also observed in the absence of caged glutamate.

using a Molectron joule meter. Light of ≥400 nm produced by fluorescence from 4.9×10^{-4} M Exalite 404 dye (Exciton Inc.) was used to initiate photolysis. The decay of the transient intermediate absorbance was recorded by digitizing the photomultiplier output at rates up to 2 MHz. The transient absorption changes were monitored at a wavelength where there was significant change in the absorption on irradiation of the caged compound (ca. 460 nm). All photolysis rates and quantum yields were measured at room temperature. Nonlinear least-squares fitting of single exponential decays was used to determine the photolysis rate constants.

Quantum Yield. The quantum yield for the caged compound was determined by actinometric methods (*42*). The Oriel lamp used for photolysis was equipped with a filter (Schott glass filter GG400) to eliminate light at wavelengths below 400 nm.

The quantum yield of DECM-caged glutamate photolysis was also determined using the method published by Milburn et al. (*11*). A 3 mL sample of **4** (Scheme 1) was photolyzed with repetitive light pulses at ≥400 nm, and the concentration of **2** (Scheme 1) was determined spectrophotometrically.

Whole-Cell Current Recording. HEK 293 cells stably transfected with cDNA encoding the GluR6 were used. The cells were used for electrophysiological experiments between 24 and 72 h after each passage. Whole-cell currents evoked by glutamate (100 μM) were recorded using the whole-cell configuration (*43*) at room temperature, −60 mV, and pH 7.4. The experiments were carried out in the absence or presence of 2 mM DECM-caged glutamate (Figure 5A) or 1 mM 7-*N*,*N*-diethylamino-4-hydroxymethylcoumarin (**2**) (Figure 5B). The solution in the recording pipet contained 120 mM CsCl, 10 mM EGTA, and 10 mM HEPES, adjusted to pH 7.4. The HEK cell bath solution contained 150 mM NaCl, 1 mM $CaCl_2$, 10 mM HEPES, pH 7.4. The resistance of the recording electrode filled with buffer solution was typically 3−5 MΩ and the series resistance was 5−6 MΩ. The cells were held at a constant transmembrane voltage of −60 mV and room temperature (22 °C). Whole-cell currents were amplified by using an Axopatch 200B (Axon Instruments) amplifier and filtered at 1−5 kHz by using a 40-pole, low-pass, Bessel filter incorporated in the amplifier. The filtered signal was digitized by a Digidata 1322A controlled by pCLAMP9 software (Axon Instruments).

Cell-Flow Method. The use of a U-tube flow device for rapid solution exchange at the surface of a cell has been described in detail (*6*, *44*, *45*). The device, consisting of a U-tube and a preincubation tube, allows one to preincubate a solution of a compound with a cell before a solution containing this and/or other compounds, such as the receptor activating ligand, are allowed to flow over the cell (*45*). The maximum amplitude of the current is a measure of the concentration of open receptor-channels. In cell-flow experi-

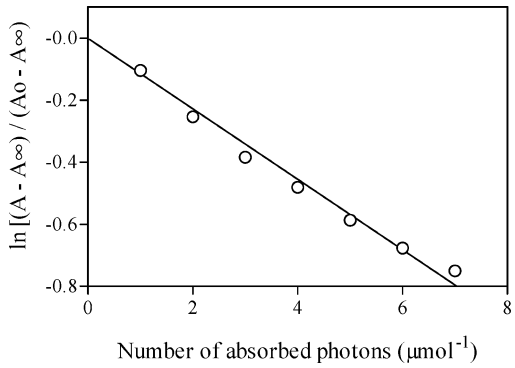


FIGURE 4: Absorbance (*A*) of a 250 μM DECM-caged glutamate solution in extracellular buffer at pH 7.4 and 22 °C measured as a function of the number of laser flashes at the excitation wavelength of 400 nm. The solution (3 mL) was irradiated in a 10 × 10 mm cuvette. The solution was stirred after every 20th laser flash. The line corresponds to the results of a linear regression representing the slope of 0.11 ± 0.08, which gives the quantum yield of the photolysis of the caged glutamate. The data were fitted to eq I.

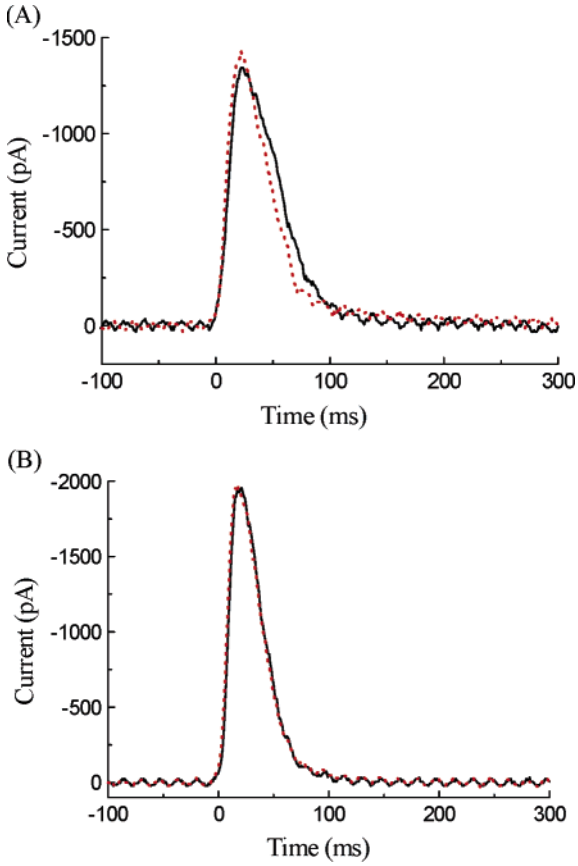


FIGURE 5: The DECM-caged glutamate and 7-*N*,*N*-diethylamino-4-hydroxymethylcoumarin, DECM (compound **2** in Scheme 1), do not inhibit the current evoked by glutamic acid. 100 μM glutamate, using the cell-flow technique (*6*, *44*), was allowed to flow over the surface of an HEK 293 cell transfected with cDNA encoding the GluR6 glutamate receptor ($V = -60$ mV, HEK buffer, pH 7.4, 22 °C) (A) in the absence (solid line) or presence (dotted line) of 2 mM DECM-caged glutamate, and (B) in the absence (solid line) or presence (dotted line) of 1 mM DECM. The induced current was recorded in the whole-cell configuration. Using the cell-flow technique for application of the ligand (*6*), experiments were carried out at least three times on at least two different cells.

ments, we correct the observed maximum current amplitude for the desensitization that occurs while the receptors equilibrate with the channel-activating ligand in the solution

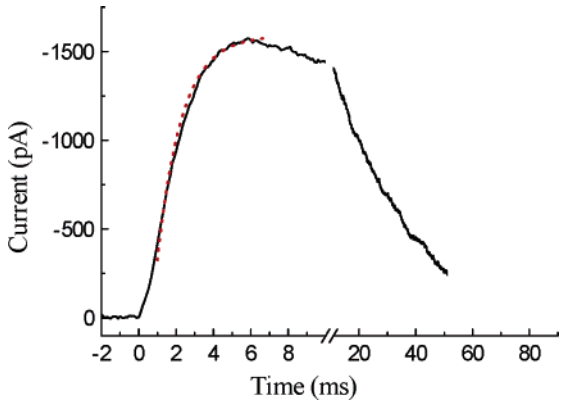


FIGURE 6: Whole-cell current recorded from an HEK 293 cell transfected with cDNA encoding the GluR6 glutamate receptor, at $V = -60$ mV, HEK buffer, pH 7.4, and 22 °C. The current was induced by the photolytic release of glutamate from 2 mM DECM-caged glutamate, which was equilibrated with the receptors for 400 ms before exposing it to a laser pulse of visible light, delivering 240 μJ of energy. The concentration of liberated glutamate was estimated to be ~300 μM (see text). The dotted line represents the best fit according to the equation $I_{(t)} = I_\infty[1 - \exp(-k_{obs}t)]$ (*47*) with $k_{obs} = 765 \pm 14$ s^{-1} and $I_\infty = -1590$ pA. $I_{(t)}$ is the current at time t, I_∞ is the current at $t = \infty$ (in the absence of desensitization), and k_{obs} is the apparent pseudo-first-order rate constant of the current rise.

flowing over the cell surface (*6*, *46*). This provides the maximum current amplitude corrected for the desensitization.

Laser-Pulse Photolysis. The laser light produced by the Exalite 404 dye (≥400 nm) was coupled into an optical fiber (300 μm internal diameter), which delivered the light to the cell. The concentration of DECM-caged glutamate used was 2 mM for the results in Figure 6. Typical laser energies were 150−250 μJ per pulse. The amount of glutamate liberated was calibrated by cell-flow experiments before and after the laser pulse with a standard glutamate solution (100 μM) and the known dose−response curve for glutamate (*47*). The pulse/flow system was computer-controlled with pClamp9 software (Axon instruments). Data were sampled at 5−100 kHz and low-pass filtered at 2−10 kHz. Data were analyzed with Microcal Origin3.5 software (Microcal, Northampton, MA).

RESULTS

Synthesis of 7-*N*,*N*-diethylamino 4-hydroxymethyl coumarin caged glutamic acid (compound **4**) is shown in Scheme 1, as described in detail in the Materials and Methods section.

The absorption spectrum and the thermal stability of the caged compound **4** (Scheme 1) were measured in HEK buffer solution at pH 7.4. The caged compound protected from light showed very little hydrolysis at room temperature during 2 h of measurements (Figure 1). No measurable hydrolysis was observed during 24 h when the caged glutamate dissolved in buffer, pH 7.4, was stored at −20 °C in the dark.

The stability of DECM-caged glutamate in aqueous solution was also tested using HEK 293 cells transfected with cDNA encoding the GluR6 glutamate receptor as a highly sensitive glutamate detector (*16*). The concentration of free glutamate in a 2 mM solution of DECM-caged glutamate was measured as a function of time after solubilization at pH 7.4 at room temperature, by whole-cell recording. After

24 h in the dark at −20 °C and pH 7.4, no free glutamate was detected in this experiment (data not shown).

The rate constant of glutamate release was estimated by measuring the rate constant for formation of 7-*N*,*N*-diethylamino-4-hydroxymethylcoumarin **2** (Scheme 1) (Figure 2). Formation of **2** is indirect evidence of release of free neurotransmitter. After a solution of caged glutamate was excited with a pulse of 10 ns, of ≥400 nm light, the absorbance of **2**, one of the products, was measured as a function of time, at 460 nm (Figure 3). The transient absorbance decays with a single-exponential component and a $t_{1/2}$ of 3 μs. Therefore, it appears that the caged compound is suitable for kinetic measurements of fast cellular processes in the microsecond time domain.

For a photolabile caging group to be useful for biological applications, the quantum yield of the photochemical step should be high (criterion 3, vide supra) so that the neurotransmitter is released in sufficient quantity at the desired site at a light intensity that is not harmful to the cell. The quantum yield of DECM-caged glutamate was determined at ≥400 nm as described previously (*11*). Figure 4 shows the change in the absorbance of the caged compound as a function of the number of photons absorbed from the light pulses at ≥400 nm (*11*). The absorbance A measured as a function of the number of consecutive light pulses was plotted in a semilogarithmic fashion (Figure 4) according to eq I (*11*):

$$An = \epsilon_m l C_0 \phi K_E \exp^{[-\phi K_E F(n-1)]} \quad \text{(I)}$$

An represents the absorbance after the *n*th pulse, ϵ_m the extinction coefficient of the product, *l* the path length, C_0 the initial concentration of the caged compound, ϕ the quantum yield, K_E the ratio of the absorbed photons to the number of target molecules (constant), and *F* the fraction of solution containing the caged compound through which the laser beam passes. The quantum yield was determined from the slope of the plot in Figure 4 and was calculated by linear regression analysis to be 0.11 ± 0.008 (Figure 4).

To be useful in investigations of neurotransmitter receptors, the caged compound and the photolysis byproducts must not inhibit or activate the receptors to be investigated. The HEK 293 cells transfected with cDNA encoding the GluR6 glutamate receptor were exposed to 100 μM glutamate in the presence and absence of 2 mM DECM-caged glutamate (compound **4** in Scheme 1) (Figure 5A) or in the presence or absence of 1 mM of the photolysis byproduct, 7-*N*,*N*-diethylamino-4-hydroxymethylcoumarin (compound **2** in Scheme 1) (Figure 5B) at pH 7.4 at 22 °C. The whole-cell currents recorded in the presence and absence of DECM-caged glutamate (Figure 5A) or the photolysis byproduct (Figure 5B) at concentrations as high as 2 mM were the same. These control experiments indicate that in this system the DECM-caged glutamate and its photolysis byproducts are biologically inert.

The laser-pulse photolysis at ≥400 nm of 2 mM caged compound was carried out with HEK 293 cells transfected with cDNA encoding the GluR6 glutamate receptor. The whole-cell current induced by the released glutamate was recorded as a function of time (Figure 6). A standard solution (100 μM) of glutamate flowed over the cell and the whole-cell current was recorded before and after the laser-pulse photolysis measurement to determine that neither the cell membrane nor the receptors were damaged. These experiments were also used to estimate the concentration of glutamate released from the caged compound (Figure 6). Laser-pulse photolysis of 2 mM DECM-caged glutamate at 400 nm induced currents, indicated the release of 300 μM glutamate as estimated from control cell-flow experiments (*6*) with a standard concentration of glutamate.

DISCUSSION

Photolabile DECM-caged glutamate was synthesized from readily available starting materials. Care had to be taken to protect the DECM-caged glutamate from light, particularly when aqueous solutions were handled. The caged compound was sufficiently soluble in water or the buffers used and was stable in the dark. Upon irradiation with a pulse of light, glutamate was released from the caged precursor in the visible wavelength region rapidly ($t_{1/2} \sim 3$ μs) and with sufficient quantum yield to be used in transient chemical kinetic investigations (*10*, *19*). One can assume that all the other carboxyl-group-containing neurotransmitters can also be caged with DECM and will all have similar favorable properties. This indicates that in transient kinetic investigations of these receptors in which the caged neurotransmitters were photolyzed in the UV region in previous experiments (reviewed *10*, *20*), inexpensive, hazard-free and simple-to-use light sources can now be used.

Many neurotransmitter receptors and their isoforms of unknown chemical mechanism (*48*) exist. Dysfunctions of neurotransmitter receptor-mediated reactions are implicated in many diseases of the nervous system (e.g. Huntington's disease, Parkinson's disease, epilepsy). Many clinically important compounds (e.g. tranquilizers, antidepressants) and abused drugs (e.g. cocaine) affect receptor function (*49*). In the case of the inhibition of the nicotinic acetylcholine receptor by cocaine the laser-pulse photolysis technique (*19*) using a caged receptor-activating ligand led to a new mechanism (*50*) and consequently to the first known compounds that prevent inhibition of this receptor by cocaine (*50*,*51*). More recently the laser-pulse photolysis technique using α-CNB-caged γ-aminobutyric (*14*) acid was used to elucidate the mechanism of dysfunction of a γ-aminobutyric acid receptor (*9*) linked to one form of epilepsy (*52*). On the basis of the mechanism of the mutated receptor (*9*), compounds are being developed to alleviate the dysfunction of the receptor (*53*).

The results obtained in investigations of only very few of the problems related to neurotransmitter receptor mechanisms, employing transient kinetic techniques using caged compounds that require ultraviolet light for photolysis and the use of expensive lasers, appear promising (reviewed, *10*). Caged compounds that can be photolyzed in the visible wavelength region are expected to greatly facilitate these investigations.

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