Development of a Novel Photochromic Ion Channel Blocker through Azologization of FomoCaine

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ABSTRACT:

Photochromic blockers targeting voltage-gated ion channels serve as powerful tools for manipulating neuronal systems with exceptional spatial and temporal precision. We are excited to introduce fotocaine, an innovative photochromic channel blocker derived from the long-lasting anesthetic fomocaine. Fotocaine is efficiently absorbed by neurons in brain slices and allows for optical modulation of action potential firing by alternating between light wavelengths of 350 and 450 nm. Additionally, it exemplifies the concept of "azologization," which refers to the systematic transformation of an established drug into a photoswitchable variant.

KEYWORDS: Photopharmacology, local anesthetics, fomocaine, action potential firing control, azologization, azobenzenephotoswitch.

INTRODUCTION:

Optical techniques for manipulating neuronal functions have significantly advanced neuroscience in recent years. Photoswitchable local anesthetics have emerged as effective tools for targeting native voltage-gated ion channels and have been applied in areas such as pain research and vision restoration. Notable examples include the bis-quaternary ammonium ion QAQ and the quaternary ammonium ion DENAQ, both of which are photoswitchable azobenzene derivatives of QX-314— a permanently charged derivative of lidocaine. The positive charge characteristic of these photoswitchable ion channel blockers hinders their ability to penetrate biological barriers. Consequently, QAQ must be introduced through a patch pipette or via auxiliary ion channels like P2X7 or TRPV1. While this method can be beneficial for studying single cells, it complicates the application of QAQ in tissues lacking these pathways. DENAQ, with only a single permanent charge, does not require auxiliary ion channels to mediate its effects, such as on HCN channels, but its tissue diffusion capability remains limited.

In our pursuit to develop new and enhanced photoswitchable ion channel blockers, we aimed to explore alternative pharmacophores that might improve biodistribution and pharmacokinetic attributes. Local anesthetics possess a long history of medical use and their mechanisms are fairly well understood. They primarily act as use-dependent open channel blockers, particularly for voltage-gated sodium channels (NaV), but can also influence other targets. For instance, cocaine has served as a topical anesthetic in ophthalmology since the early 1900s, despite its notable central nervous system effects. Novocaine, a simpler analog of cocaine, represents the ester class of local anesthetics. In the late 1960s, the morpholine derivative fomocaine was introduced, sharing minimal structural resemblance to cocaine aside from its

tertiary amine group. Like lidocaine and novocaine, fomocaine is not permanently charged, yet it is more lipophilic and exhibits less systemic toxicity, making it a popular long-lasting topical anesthetic.

We now present fotocaine, a photoswitchable version of fomocaine that functions as a photochromic ion channel blocker enabling light-mediated control of neuronal activity. Fomocaine's unique molecular structure, which includes a benzyl-phenyl ether moiety, aligns with our concept of "azologization"—the strategic incorporation of azobenzenes into existing drugs. Logical targets for this process include compounds with stilbenes, 1,2-diphenyl ethanes, 1,2-diphenyl hydrazines, N-benzyl anilines, as well as benzyl-phenyl ethers, thioethers, diaryl esters, amides, and their heterocyclic derivatives. An examination of existing databases reveals that numerous established drugs contain these moieties. By substituting them with azobenzenes, we can create photoswitchable analogs, or "azosters," that maintain similar size and shape to their parent compounds but ideally alter their efficacy in response to light. Applying this rationale to fomocaine leads to the creation of fotocaine, where a CH2−O moiety is replaced with a diazene unit (N=N). We hypothesized that this modification would yield a photoswitchable ion channel blocker retaining similar pharmacokinetic and pharmacodynamic properties. The synthesis process involves a three-step procedure.

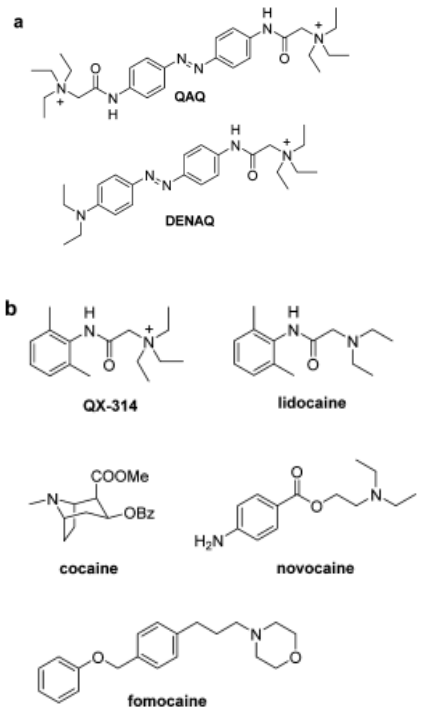

Figure 1. (a) Photoswitchableazobenzene derivatives QAQ and DENAQ used as photoswitchable blockers of voltage gated ion channels. (b) Structures of local anesthetics. QX-314 is a permanently charged derivative of the local anestheticlidocaine. Cocaine is a tropane alkaloid, and novocaine is an ester local anesthetic. All of these compounds feature a tertiary amine pharmacophore. Fomocaine is an ether local anesthetic with a morpholino group

of fotocaine from commercially available starting materials is described in the Supporting Information. To test fotocaine'sphotoswitching properties, we first utilized UV/vis spectroscopy (Figure 3). A 50 μM solution of fotocaine in DMSO was placed in a quartz cuvette with 1 cm diameter and illuminated from above using a monochromator. The lightinduced isomerization of fotocaine and the corresponding absorption spectra of cis- and trans-isomers are depicted in Figure 3a. As a classical azobenzene, isomerization could be followed by monitoring the π to π* transition at 330 nm

over time. Toggling between 350 and 450 nm light switched the molecule into its cis- and trans-state, respectively (Figure 3c, i). As it is known for regular azobenzenes, photostationarycis/trans ratios of up to 90:10 can be achieved by irradiation with ultraviolet light.3 Wavelengths between 400 and 350 nm could be used to install mixtures with different cis/trans ratios (Figure 3c, ii). As expected from a "classical" azobenzene, the thermodynamically less stable cis-state remained stable in the dark (Figure 3c, iii).16,17 Thus, fotocaine provides the desired reversible light-mediated cis/trans-isomerization. As an added advantage, it shows bistability and stays in its cis-state for several minutes even without continuous UV-illumination.

Next, we investigated the ability of fotocaine to optically control neuronal function. To this end, we resorted to patch clamp electrophysiology using dissociated mouse hippocampal neurons (Figure 4). Fotocaine was applied at 50 μM concentration in the external bath solution. At a starting potential of −80 mV, action potential (AP) firing of neurons was induced by injecting a 50 pA current. When the illumination wavelength was set to 450 nm, AP firing was inhibited. However, when switching to 350 nm, AP firing triggered by the same current took place reliably. This process could be repeated with a variety of different illumination protocols (Figure S1). The same effects were observed at higher concentration (100 μMfotocaine, Figure S1b). The single action potential at the beginning of each current injection under 450 nm indicates that trans-fotocaine acts as an open channel blocker as is the case for its permanently charged relatives.9,18 To test the action of fotocaine in a functional neuronal circuit and assess its distribution in tissues, we performed further patch clamp experiments using acute hippocampal mouse brain slices.

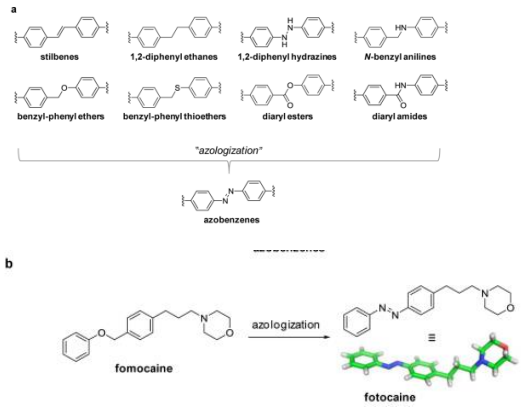

Figure 2. Logic of azologization. (a) Prime isosters of azobenzenes, that is, azosters, are stilbenes, 1,2-diphenyl ethers, 1,2.diphenyl hydrazines, N-benzyl anilines, benzyl-phenyl ethers, benzyl-phenyl thioethers, diaryl esters, and diaryl amides. (b) Application of the concept of azologization to fomocaine. Replacement of the benzyl-phenyl ether bridge by a diazene yields the azobenzene derivative fotocaine. The X-ray structure of fotocaine is deposited at the Cambridge crystallographic data center, ID: 991565.

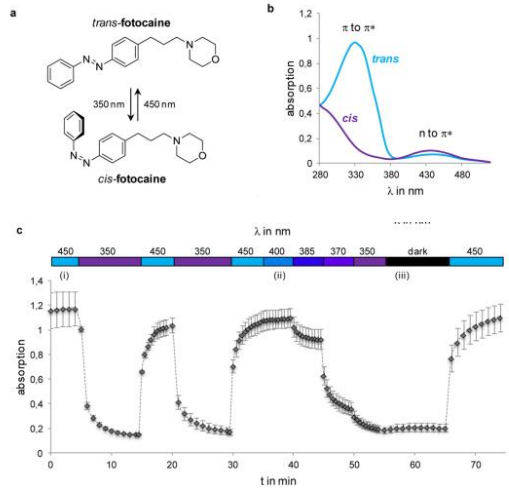

Figure 3. Photoswitching of fotocaine followed by UV/vis spectroscopy. (a) UV-light (e.g., 350 nm) isomerizes the azobenzene functional group in fotocaine to itscis-isomer, which is the thermodynamically less stable state. Blue light (e.g., 450 nm) triggers isomerization

to trans. (b) Absorption spectra of trans-fotocaine (blue line) and cis-fotocaine (purple line) are distinct. The π to π* band decreases starkly upon isomerization to cis, while the n to π* band slightly increases. (c) In-time photoswitching by following the fotocaine absorption at 330 nm. Fotocaine can be reversibly isomerized by switching between, for example, 450 and 350 nm (i). Wavelengths between 400 and 350 nm lead to graded effects (ii). Once switched to cis, fotocaine stays in its excited state without further illumination (iii). No decay was detected for the investigated time of 10 min (n = 3, error bar indicates standard deviation).

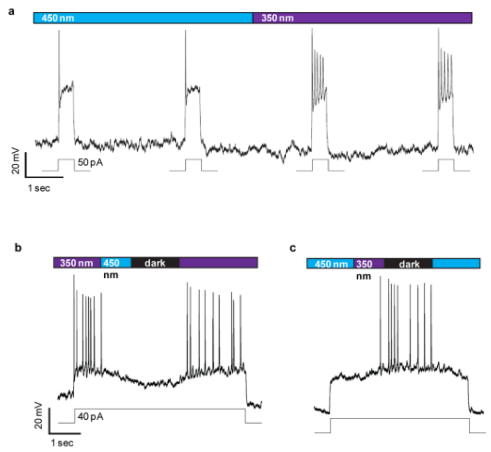

Figure 4. Photocontrol of action potential (AP) firing mediated by fotocaine investigated in whole cell patch clamp experiments (representative traces). (a) Dissociated mouse hippocampal neurons, current clamp mode, 50 μMfotocaine. AP-firing was triggered by injecting 50 pA for 300 ms (cells were held at −80 mV). Under 450 nm (trans-fotocaine), AP-firing was suppressed, while 350 nm illumination (cis-fotocaine) allowed AP-firing. The initial AP under 450 nm is indicative of the action of an open channel blocker. (b and c) Acute mouse brain slice, hippocampal CA1 neurons, current clamp mode, after 10 min wash-out of fotocaine. Currents of 40 pA were injected, and illumination wavelengths were changed simultaneously. Effects of cis- and trans-fotocaine were identical to those in (a). In addition, when 350 or 450 nm was turned off after short application, the thereby installed effect maintained.

Initially, the tissue preparation was incubated with 100 μM fotocaine for 5 minutes to enable the cells to absorb the photochromic drug. Following this, a buffered Ringer solution was perfused for 10 minutes to wash out the photoswitchable blocker from the extracellular environment. As anticipated for a long-lasting open channel blocker, we observed that action potential (AP) firing could be controlled optically without the need for a continuous supply of extracellular fotocaine. AP firing was stimulated by injecting a 40 pA current for several seconds, during which the illumination wavelengths were alternated.

In accordance with our findings from dissociated neurons, we found that AP firing was inhibited under 450 nm illumination and facilitated by 350 nm light. Additionally, the bistability of fotocaine, confirmed through UV/vis spectroscopy, was also evident in this physiological experiment. Neuronal silencing induced by 450 nm light persisted in darkness but could be reversed with 350 nm light. Conversely, AP firing initiated by 350 nm light continued even after the light was turned off but could be halted by shifting to 450 nm.

In conclusion, we have successfully applied the principle of azologization to the local anesthetic fomocaine, thereby creating a novel photochromic ion channel blocker, fotocaine. Our results demonstrate that fotocaine is readily absorbed by neurons in brain slices, allowing for light-

mediated control of action potential firing with enduring effects. Its relatively straightforward structure may support the design and synthesis of enhanced versions with desirable traits, such as red-shifted action spectra. Future investigations will explore the applications of fotocaine in neurophysiology, particularly its potential use as an analgesic for photopharmacological pain management.

METHODS

UV/Vis Spectroscopy. UV/Vis spectroscopy was conducted using a VARIAN Cary 50 Scan UV/Vis spectrometer. A PCL solution was placed in a standard quartz cuvette (1 cm path length), which was illuminated from above using a fiber optic light source.

Cell and Tissue Preparation. Dissociated mouse hippocampal neurons were prepared and cultured on an astrocyte feeder layer as previously described. For the preparation of acute hippocampal brain slices, BL6 wild-type mice (postnatal days 9–13) of either sex were rapidly decapitated, and the brain was removed. Horizontal slices (250 μm) were created using a vibrating microtome (7000smz-2, Campden Instruments). These slices were incubated for 30 minutes at 34 °C in a carbogenated sucrose medium (composition in mM: 87 NaCl, 2.5 KCl, 7 MgCl2, 0.5 CaCl2, 25 Glucose, 1.25 NaH2PO4, 25 NaHCO3, 75 sucrose, total osmolarity 319 mOsm). Following this, the slices were perfused with a solution containing 100 μM fotocaine for 5 minutes, and then subjected to a 10-minute wash with bath solution. Whole-cell patch clamp recordings were performed on CA1 hippocampal neurons.

Electrophysiology. Whole-cell patch clamp recordings were carried out using a standard electrophysiological setup, which included a HEKA Patch Clamp EPC10 USB amplifier and PatchMaster software. Micropipettes were fabricated from "Science Products GB200-F-8P with filament" pipettes using a vertical puller (PC-10, Narishige), achieving resistance values between 5 and 7 MΩ. The bath solution for dissociated hippocampal neurons comprised the following (in mM): 140 NaCl, 3 KCl, 2 CaCl2, 1 MgCl2, 10 D-Glucose, and 20 HEPES (adjusted to pH 7.4 with NaOH). The pipette solution for these neurons contained (in mM): 107 KCl, 1.2 MgCl2, 1 CaCl2, 10 EGTA, 5 HEPES, 2 MgATP, and 0.3 Na2GTP (adjusted to pH 7.2 with KOH). For acute brain slices, the bath solution consisted of (in mM): 125 NaCl, 2.5 KCl, 1 MgCl2, 2 CaCl2, 10 Glutamate, 1.25 NaH2PO4, and 26 NaHCO3 (osmolarity 290–295 mOsm). The pipette solution for acute brain slices contained (in mM): 140 K-gluconate, 4 NaCl, 12 KCl, 10 HEPES, 4 MgATP, and 0.4 Na2ATP (adjusted to pH 7.3 with KOH). Action potentials (APs) were evoked using a 50 pA current injection. Fotocaine was prepared in the bath solution from a 1000× DMSO stock solution for both tissue preparations.

Illumination. For irradiation during electrophysiology and UV/Vis experiments, a TILL Photonics Polychrome 5000 monochromator was used, controlled by the PolyCon software and the patch clamp amplifier, respectively.

ASSOCIATED CONTENT

*S Supporting Information

Representative traces of action potential firing with varying concentrations of fotocaine and illumination timing (Figure S1). Details on the synthesis and characterization of organic compounds.

This material is freely available online at http://pubs.acs.org.

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