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Lucifer Yellow CH modifies voltage-gated channels and a ligand-gated channel in a light-dependent manner

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Abstract. Lucifer Yellow CH (LY), a fluorescent dye, has been used to visualize the cell investigated electrophysiologically. Recently, we showed that LY slowed the inactivation of voltage-gated Na^+ channels in a light-dependent manner by increasing their open time. Here we show that LY increases the current magnitude of outwardly rectifying and inwardly rectifying K^+ channels and AMPA receptor channels but has no effect on that of HVA-Ca^{2+}, Cl^-, NMDA, kainite and $5HT_3$ channels. The mechanism of how light-exposed LY modifies ion channels is discussed. © 2006 Published by Elsevier B.V.

Keywords: Voltage-clamp; Lucifer Yellow CH; Voltage-gated channel; Ligand-gated channel; Light

1. Introduction

Many fluorescent probes are shown to modify cellular responses when they are exposed to light. Fluorescein derivatives activated and inhibited ATP-sensitive K^+ channels [1] and Fluo-3 and Fura-red, calcium-sensitive indicator dyes enhanced persistent Na^+ current [2]. However, Lucifer Yellow CH (LY), a fluorescent membrane-impermeable cell-marker dye, has been added to the intracellular recording electrode to visualize the cell investigated electrophysiologically without considering its pharmacological effect. In a previous study, we showed that LY irreversibly slowed the inactivation of TTX-sensitive voltage-gated Na^+ channel current by increasing the mean open time of the channel when the LY-injected cell was exposed to the light at the intensity for a usual fluorescent microscopy (Fig. 1A),

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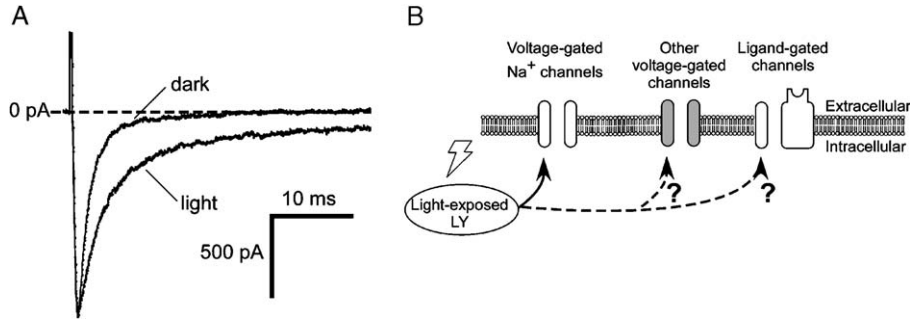

Fig. 1. Retarded inactivation of TTX-sensitive, voltage-gated Na^+ currents of a LY-injected hippocampal neuron by the exposure to light (A) and a schematic representation of this study (B). *A*, the membrane potential was depolarised from a holding potential of −70 to −20 mV in dark conditions, before (dark) and after exposure to 4900 lx light for 310 s (light).

and suggested that LY radicals generated by the exposure modified the channel protein, since dithiothreitol (DTT), a free-radical scavenger, antagonized the LY effect [3]. In this study, we investigated the effect of illuminated LY on other voltage-gated currents and ligand-gated currents of cultured mouse hippocampal neurons under voltage-clamp conditions (Fig. 1B).

2. Materials and methods

Experimental procedure was similar to the previous study [3]. In brief, we placed primary cultures of mouse hippocampal neurons under a fluorescent microscope with a 60× water-immersed objective, injected 4.2 mM LY into cultured neurons through recording patch electrodes in the dark, and exposed injected neurons to the light through the objective. The wavelength of the light was 427 nm optimized to excite LY, and its intensity was 4900 lx at source, a usual intensity for fluorescent microscopy. We continuously irrigated LY-injected neurons with a physiological saline, and added agonists or DTT into the bathing solutions.

3. Results

AMPA receptor channels elicited an inactivating current that reached a steady-state (Fig. 2). After 4-min exposure, the magnitude of the peak current was decreased and that of the steady state current was increased to 2.7 times of control (Fig. 2). Both effects were irreversible. The reversal potential of the steady-state inactivating current remained unchanged, indicating LY hardly changed the ionic selectivity of AMPA receptor channels. Although $5HT_3$ receptor channels also elicited inactivating current, LY had no effects on their currents. In addition, LY had no effects on NMDA, kainite, and $GABA_A$ receptor channel currents.

LY also irreversibly increased the magnitude of outwardly rectifying K^+ currents to 1.3 times of the control at +65 mV, and that of inwardly rectifying K^+ channel currents to 1.8 times at −135 mV, after 4-min exposure. The magnitude of other voltage-gated channel currents such as Cl^- and HVA-Ca^{2+} channel currents remained unchanged.