

Evaluation of membrane potential assay kit using the FDSS/ μ CELL imaging plate reader: Detection ion channel in nicotinic acetylcholine receptors (nAChRs)

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels. nAChRs are normally triggered by the neurotransmitter acetylcholine (ACh), but they may also be triggered by nicotine. Nicotinic receptors do not operate with a second messenger, but open themselves forming an ion channel. Opening of the channel allows positively charged ions to enter or leave the cell. The nAChR are permeable to Ca^{2+} , but also to Na^+ and K^+ which will flow according to their concentration gradients. The neuronal nAChR subunit family includes nine α subunits, designated $(\alpha)2$ - $(\alpha)10$ and three β subunits designated $\beta 2$ - $\beta 4$. The $\alpha 4\beta 2$ nAChR is the most abundant nAChR subtype expressed in the brain, and studies have demonstrated that this receptor subtype is located presynaptically on GABAergic and dopaminergic terminals. This nAChR subtype is elevated in brain tissue from smokers (Benwell et al. 1988; Breese et al. 1997)

While nicotinic receptors are frequently studied with a calcium-sensitive dye, the physiology of these receptors suggest that dyes that are sensitive to membrane potential may be more sensitive as they detect membrane potential changes that may be triggered by the

different cations that may flow through these channels. The aim of this study was to compare the responses of these receptors with 2 different type of dyes, including the calcium-specific Fluo-4 dye (Invitrogen) and membrane potential-sensitive assay fluorophores (Molecular Devices) after agonist stimulation of $\alpha 4\beta 2$ nicotinic receptors with either Nicotine, or with the partial agonist Varenicline.

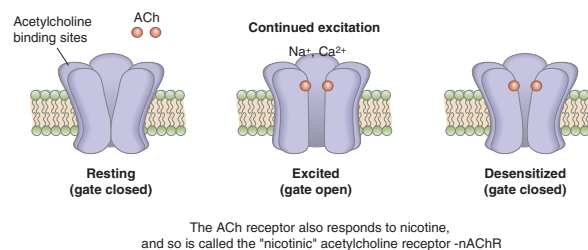


Fig 1: nACh receptors response to Acetylcholine or Nicotine

Principle

Membrane Potential Assay Kits (i.e. FMP dyes) detect ion channel modulation by increasing or decreasing the fluorescent signal as cellular membrane potential changes. The fluorescent signal increases in intensity during membrane depolarization as dye follows the positively charged ions inside the cell. During membrane hyperpolarization, fluorescent signal decreases in intensity as dye follows the positively charged ions out of the cell. Membrane Potential Assay Kits are uniquely suited for use with the simultaneous pipet and read capability of FDSS/ μ CELL to capture fast kinetics associated with ion channel activation. The kits employ a quenching dye to reduce background fluorescence and improve the signal-to-noise ratio.

Experimental procedure

HEK-293 cells expressing $\alpha 4\beta 2$ nicotinic receptors were plated (50 000 cells/well) in 96 well clear bottom polylysine-coated plates 48 hours before the experiment. On the day of the experiment, cells were washed once and incubated with the dye (2 hours for Fluo-4 and 30 minutes for FMP dyes). Fluorescence signals were collected by the FDSS/ μ CELL (Hamamatsu Photonics), a new imaging plate reader for kinetic cell-based assays. The latter is equipped with one injection head (96, 384) and enables simultaneous injection and detection of all the wells. (Fluo-4: 200 ms impulses each 700 ms; excitation wavelength = 480 nm / emission wavelength = 540 nm; FMP: 200 ms impulses each 700 ms; excitation wavelength = 531 nm / emission wavelength = 593 nm). Fluorescence signal was measured during 10 s for the base line before the treatment was applied and measured for another 3 minutes. Peak fluorescence following treatment was quantified with the FDSS/ μ CELL software, and pharmacological data were derived from the concentration-response curve using GraphPad PRISM software "Sigmoidal Dose-Response (variable slope)" built-in equation.

FDSS7000EX

FDSS/μCELL

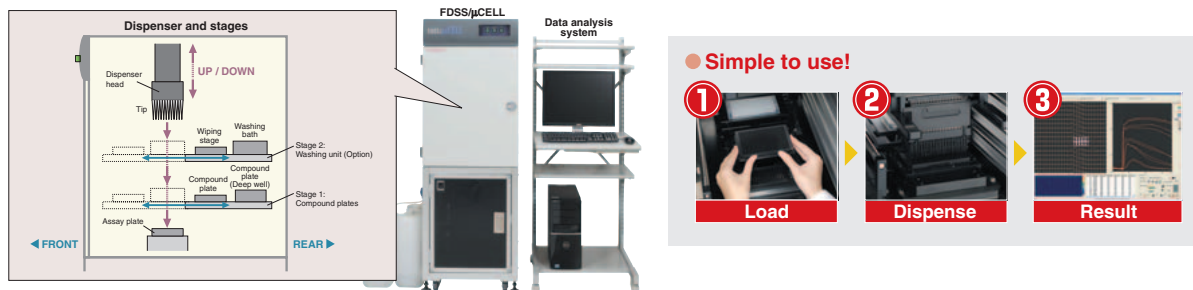
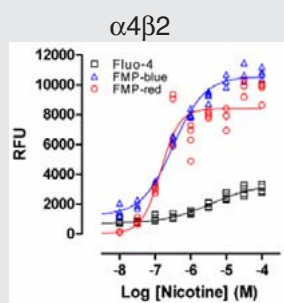


Fig 3: Overview of the FDSS/μCELL

Simple to use!

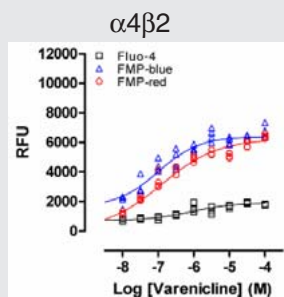


Results



Full agonist

| Nicotine | Fluo-4 | FMP-blue | FMP-red |
|---------------------|------------|------------|------------|
| Best-fit values | | | |
| BOTTOM | 696.6 | 1234 | 33.19 |
| TOP | 3335 | 10550 | 8402 |
| LOGEC ₅₀ | -5.430 | -6.492 | -6.841 |
| HILLSLOPE | 0.6719 | 0.9847 | 1.658 |
| EC ₅₀ | 3.717e-006 | 3.221e-007 | 1.442e-007 |



Partial agonist

| Varenicline | Fluo-4 | FMP-blue | FMP-red |
|---------------------|------------|------------|------------|
| Best-fit values | | | |
| BOTTOM | 716.1 | 1640 | 88.74 |
| TOP | 1896 | 6407 | 6242 |
| LOGEC ₅₀ | -6.093 | -6.996 | -6.900 |
| HILLSLOPE | 0.7396 | 0.7490 | 0.5525 |
| EC ₅₀ | 8.070e-007 | 1.009e-007 | 1.259e-007 |

Fig 4: EC₅₀ Dose-Response curve. The stimulation by Nicotine (a) and Varenicline (b) is dose-dependent with HEK-293 cells expressing α4β2 nicotinic receptors

Conclusions

While all 3 dyes gave exploitable agonist concentration-response curves, the two FMP dyes gave greater amplitude of fluorescence upon agonist stimulation. These latter dyes also allowed a better detection of the activity of the partial agonist varenicline. Moreover, the potencies of the 2 agonists were greater (concentration-response curves shifted to the left) with the FMP dyes, thus suggesting a greater sensitivity of these dyes toward agonist stimulation.

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HAMAMATSU PHOTONICS K.K. www.hamamatsu.com

HAMAMATSU PHOTONICS K.K., Systems Division

812 Joko-cho, Higashi-ku, Hamamatsu City, 431-3196, Japan, Telephone: (81)53-431-0124, Fax: (81)53-435-1574, E-mail: export@sys.hpk.co.jp

U.S.A.: Hamamatsu Corporation: 360 Foothill Road, Bridgewater, N.J 08807, U.S.A., Telephone: (1)908-231-0960, Fax: (1)908-231-1218 E-mail: usa@hamamatsu.com

Germany: Hamamatsu Photonics Deutschland GmbH.: Arzbergerstr. 10, D-82211 Herrsching am Ammersee, Germany, Telephone: (49)8152-375-0, Fax: (49)8152-265-8 E-mail: info@hamamatsu.de

France: Hamamatsu Photonics France S.A.R.L.: 19, Rue du Saule Trapu, Parc du Moulin de Massy, 91882 Massy Cedex, France, Telephone: (33)1 69 53 71 00, Fax: (33)1 69 53 71 10 E-mail: infos@hamamatsu.fr

United Kingdom: Hamamatsu Photonics UK Limited: 2 Howard Court, 10 Tewin Road, Welwyn Garden City, Hertfordshire AL7 1BW, UK, Telephone: (44)1707-294888, Fax: (44)1707-325777 E-mail: info@hamamatsu.co.uk

North Europe: Hamamatsu Photonics Norden AB: Torshamnsgatan 35 16440 Kista, Sweden, Telephone: (46)8-509-031-00, Fax: (46)8-509-031-01 E-mail: info@hamamatsu.se

Italy: Hamamatsu Photonics Italia S.r.l.: Strada della Moia, 1 int. 6 20020 Arese (Milano), Italy, Telephone: (39)02-93581733, Fax: (39)02-93581741 E-mail: info@hamamatsu.it

China: Hamamatsu Photonics (China) Co., Ltd.: B1201 Jiaming Center, No.27 Dongsanhuan Beilu, Chaoyang District, Beijing 100020, China, Telephone: (86)10-6586-6006, Fax: (86)10-6586-2866 E-mail: hpc@hamamatsu.com.cn