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Generation of biochemical response patterns of different substances using a whole cell assay with multiple signaling pathways

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Abstract

Distinctive generation of biochemical response patterns of eight different substances, using an assay based on pigment containing cells, was demonstrated. *Xenopus laevis* melanophores, transfected with human β_2-adrenergic receptor, were seeded in a 96 well microplate and used to generate individual biochemical images through a two transient measuring protocol that contributes to highlight the response signatures of the agents. Adequate signal processing creates distinctive patterns in a time–concentration response space suitable for substance classification. The concept of biochemical images is introduced here.

The assays were evaluated both with a standard microplate reader and with a computer screen photo-assisted technique (CSPT) yielding similar results. Since CSPT platforms only demand standard computer sets and web cameras as measuring setup, applications for these kind of assays outside main-laboratories were discussed.

Keywords: Biochemical response patterns; Biochemical images; Natural nanosystems; Computer screen photo-assisted technique; Bioassays; Genetically modified organisms

1. Introduction

Pigment containing cells, such as those in *Xenopus laevis* African frogs, comprise an intact biochemical pathway, which consists of a signal amplification mechanism and a nanoscopic transduction machinery that provide conveniently readable physiological responses to target drugs (Potenza and Lerner, 1992; Lundström and Svensson, 2002). In *X. laevis* the color changes caused by these cells, termed melanophores, serve to camouflage or signaling purposes, and are triggered by specific substances such as melatonin or melanocyte stimulating hormones (MSH; Nery and Castrucci, 1997; DeOliveira et al., 1996), which activate a biochemical signaling cascade within the cell, leading to the relocation of pigment granules. The sensitivity to different stimuli depends on the presence of specific receptors in the cell membrane, and can be tailored by genetic engineering of guanine nucleotide binding protein (G-protein) coupled receptors (McClintock et al., 1993; Karlsson et al., 2002).

In the present study, the transfection of a human β_2 adrenergic receptor (β_2-AR) in a *X. laevis* cell line confers the cells sensitivity to adrenergic hormones, such as adrenaline and noradrenaline, as well as to adrenoceptor agonists (Potenza et al., 1992; McClintock and Lerner, 1997).

Assays of such cells can be used for screening pharmaceuticals with agonist or antagonist behavior to certain receptors, in which case, dose-response characteristics are typically recorded and compared. These steady state features, although simple to obtain, do not fully exploit the complexity of the physiological response to the different drugs, for example different capture efficiencies of the receptor towards target agents, or the varied degrees of cellular incorporation that determine the permanence of the stimulus.

We demonstrate the generation of distinctive response patterns suitable for classifying different β_2-adrenergic

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drugs, adrenergic hormones, serotonin and MSH, using a melanophore cell line with only one foreign receptor besides the endogenous ones.

The combined use of a particular measuring protocol with a proper signal composition revealed differences in the dynamic responses towards each individual substance, while keeping the experimental requirements comparable to those of standard procedures.

Assays as the present one can be arranged in microplates and evaluated with regular microplate readers. However, for applications outside specialized laboratories such as in primary care units or doctor's offices this kind of instrumentation might become unaffordable. As an alternative, the computer screen photo-assisted technique (CSPT; Filippini et al., 2003a,b) provides commonly available measuring platforms, just involving standard computer sets and web cameras, the ability of CSPT to evaluate the assay was also investigated.

2. Experimental

2.1. Materials and methods

X. laevis melanophores and fibroblasts were propagated as previously described (McClintock and Lerner, 1997). The melanophore cell line, expressing human β_2-AR was generously provided by Dr. Michael R. Lerner, Arena Pharmaceuticals Inc., San Diego, CA, USA.

Melanophores were seeded at a concentration of 40 000 cells per well in a flat bottom 96-well microplate and cultured at 27 °C for 1 day. Twelve hours before the experiment, the culture medium was changed to 50 μl serum-free media (70% L-15). Experiments were started by recording the absorbance of the plate for calibration purposes. Induction of pigment aggregation was initiated by adding 50 μl 70% L-15 containing 4 nM of melatonin. Steady aggregation was achieved after about 70 min. The target agents (inducing dispersion) were dissolved in 100 μl of medium, and then added.

After 25 min of drug exposure the wells were emptied and re-filled with 50 μl 70% L-15 containing 4 nM melatonin. This step induced a new aggregation for the remainder of the experiment.

(*R*;*R*)-formoterol ($C_{19}H_{24}N_2O_4$) (*S*;*S*)-formoterol (*R*;*R*/*S*;*S*)-formoterol, salbutamol ($C_{13}H_{21}NO_3$, all purchased from Sepracor, Marlborough, MA, USA), α-melanocyte stimulating hormone (MSH, Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2), adrenaline ($C_9H_{13}NO_3$), noradrenaline ($C_8H_{11}NO_3$) and serotonin ($C_{10}H_{12}N_2O$, Sigma, St. Louis, MO, USA), were pipetted in triplicates in adjacent wells, whereas different rows of the microplate (A–F) contained five drug dilutions from 0 to 10^{-4} M. Row G contained the maximum concentration of the substance but without cells, and was used to correct for the absorbance of the medium. Row H contained the blank references used by the microplate reader. Absorbance measurements were performed at intervals using a microplate reader (FuoStar Galaxy) operating at a wavelength of 620 nm.

CSPT measurements were performed using a Dell Latitude D800 computer with a WXGA screen (1280 pixels × 800 pixels) operating at a refresh frequency of 60 Hz. A part of the computer screen was used as a large area light source (130 mm × 80 mm) illuminating the assay with a particular light color, in this case pure red (*rgb* = 255 000 000). A web camera (Philips PCVC740K ToU Cam Pro, with a CCD detector operating at a resolution of 320 pixels × 240 pixels) captured the image of the assay under the screen illumination at one frame/s during 5 s at each acquisition time. A mirror to deflect the screen illumination, a removable holder keeping the microplate horizontally positioned, and a light shield to prevent external illumination completed the setup.

Matlab® software controlled the illumination, the camera acquisition and processing steps including the masking of the images, the image composition and the routines for principal component analysis (PCA, Joliffe, 1986).

2.2. Measuring protocol

Evaluation of pigment containing cell assays for drug–receptor interactions usually considers the dose-response curves in steady state upon exposure to different drugs (Fig. 1). The concentration corresponding to the inflection point and the amplitude of these curves is used to distinguish between different responses. Fig. 1b displays a steady state dose-response characteristic and illustrates how the first derivative, which is used later in our protocol for the composition of the biochemical images, characterizes both inflexion point and slope through the position of its maximum and its width respectively.

Evaluating steady state responses implies dismissing potentially important differences in the dynamic responses. In order to further emphasize these dynamic characteristics, a second transient was used in our protocol. In this case, after 25 min of target drug exposure the system was forced into a new melatonin induced aggregation. The rest of the procedure consisted of the generation of distinctive response patterns in a time–concentration response space.

Fig. 2a shows the raw data of one single well along the time dimension and particular points considered for calibrations and signal scaling.

Natural variability in the final number of cells per well induced spurious differences in the absorption readout that were compensated by recording the initial light absorption of each well *j*, $A_0^{\langle j \rangle}$ (with $j \in [1,96]$) as the corresponding averages in the interval [to, ta]. The relative discrepancy of a particular well was then computed as:

$$\Delta^{\langle j \rangle} = \frac{A_0^{\langle j \rangle}}{\max(A_0)} \quad (1)$$

where $\max(A_0)$ is the maximum absorbance of all the wells.