The challenge of interpreting drug potency under non-equilibrium conditions using Ca²⁺ assays

1. Introduction

The development of FDSI (FDSS/µCell) has made it possible to assess the most relevant cellular response to the concentration of agonist (log[agonist] in KH buffer) of interest. This is only true for the rapid and complete response of cell signal, the option for the physiological condition of interest. Thus, an essential issue to solve is the interpretation of drug potency at non-equilibrium conditions. For this, PdCa (PdCa) receptor assays are used. The first step is to determine the antagonist potency by titrating the resulting displacement, typically from 10 to 14 log cycles. The pA² (pA2) is a useful estimate of the antagonist potency, since the pA² tends towards the pKᵦ for the high antagonist concentration. Additional experimental parameters should be taken into account when performing studies. The different properties of agonists are important to be considered during evaluation of experimental parameters. Most of the defined agonist properties have to be closely observed for any given FCPI when performing the cell line response. When these factors are identified, the pA² becomes the useful parameter. The antagonists are often used to determine the potency and potency of agonists. The antagonist potency can be determined by subtracting the antagonist concentration from the maximal fluorescence counts (cmax – 0). This is a major step in determining the concentration response curve and as a result, the pA² as well as the agonist activity.

2. Experimental procedures

Agonist (4 mM for cAMP) and compound wells (25 mM for THF) are washed with KH buffer (pH 7.4) for 15 min. Cells are seeded into 15 µl of 100 mM NaCl in a 384-well plate (150 µl per well) in XF culture plates (384-well plates) and incubated at 37 °C for 1 h. The plates are washed with 0.5 % PBS in 300 µl. Antagonist solutions are added and added immediately is performed in duplicate wells at each concentration. The plates are added to the XF culture plates (150 µl per well). For agonist concentration (related volume), the plates are transferred to the XF culture plates (150 µl per well). For antagonist concentration (related volume), the plates are transferred to the XF culture plates (150 µl per well).

3. Principle of Schild plot

To interpret the relationship between the log(pA²) for various concentrations of antagonists and the pA², a Schild plot is used. The Schild plot is a graphical representation of the relation between the log(pA²) for various concentrations of antagonists and the pA². The relationship is shown in the following graph:

4. Results

Results were performed on a CEPI with expressing a recombinant antagonists at the Hamamatsu FDSS/µCell. The agonist injection was performed at different depths (4 µm for the readings.

5. Summarized results

In summary, the effective agonist concentration is obtained using Ca²⁺ assay. The results show that the pA² is a useful estimate of the antagonist potency. The antagonist potency can be determined by subtracting the antagonist concentration from the maximal fluorescence counts (cmax – 0). This is a major step in determining the concentration response curve and as a result, the pA² as well as the agonist activity.

6. Conclusions

(a) EC₅₀ = 835 nM
(b) pA² = 6.23
(c) pA² = 7.05

Bibliography

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- N. M. J. Angeli, jangeli@hamamatsu.fr
- Dr. T. Calmels

Fig. 1: principle of shild regression analyses

Fig. 2: Calcium assays performed on CHO cells expressing a recombinant aminergic GPCR at the Hamamatsu FDSS/µCell.

Fig. 3: Relationship between the pA² for various mechanisms of antagonism and the pKᵦ.