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CASE REPORT

Method for optical measuring variations of cell membrane conductance

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Method for optical measuring variations of cell membrane conductance

Summary

This document describes an optical method for the measurement of changes in membrane permeability. A specific field of application is the ***screening of candidate compounds putatively acting on ion channel activity***. Indeed, the proposed method allows to derive experimentally ***a dose-response curve concerning specific agonist-receptor interactions using a procedure that fits the requirements of high throughput screening better than traditional electrophysiological techniques and does not require specific expertise of the operator.***

The method can also advantageously used for ***evaluating a cell status, namely a differentiative or a pathologic status.***

The competitive advantage of this invention is related to:

Time resolution: the instant invention makes possible monitoring changes in membrane conductance (typically caused by drug acting on ion channels) in the subsecond time scale, i.e. better than in conventional optical drug screening;

Spatial resolution: the instant invention works at the single cell level, thereby allowing the study of conductance changes in identified cells within heterogeneous populations as well as in subcompartments of cells with complex morphology;

Sensitivity: the instant invention does not require overexpression of channels/transporters to have a proper readout of their activity;

Specificity: the instant invention provides a highly specific readout, with no restrictions on the nature of the molecular mechanism responsible for the change in conductance;

Scalability: the instant invention makes cell-based high-content-screening compatible with the requirements of high throughput screening.

Technology

This new approach requires the use of fast response Voltage Sensitive Dyes (fast-VSD), which are typically characterized by a submillisecond temporal resolution, thanks to their extremely rapid intramolecular charge distribution shift. However, their main limitation is low sensitivity (e.g. a change in signal value <10%/100mV for di-4-ANEPPS and di-8-ANEPPS), a characteristic that prevents the possibility to analyze small changes when VSD are used conventionally to monitor the effect of agonist activity on membrane potential.

The method can be proficiently exploited to:

- combine the requirements of high-throughput screening (HTS) with those of high-content screening (HCS) for drug discovery;

- study the cellular and sub-cellular localization of permeability events in identified domains (even in complex and heterogeneous cellular models);

Development stage

1. Pharmacology: No limitations, i.e. in principle any type of channels, transporters and membrane porators (e.g. antibiotics and antimicotics) can be studied
2. Cell substrate: multiwells (typically 96, up to 384 wells)
3. Field of measurement: limited only by the field of view and the magnification of the objective employed (typically 25 mm and 40x, respectively, i.e. a square of ~400 μm).
4. Spatial resolution: subcellular, limited only by the wavelength and the NA of the objective employed.
5. Acquisition time of a single measurement: <200 ms.
6. Throughput at the present time: measurement of a dose of drug ~ 5 min; dose-response curve ~50 min.
7. Expected throughput in one year: measurement of a dose of drug < 12 s; dose-response curve <2 min.

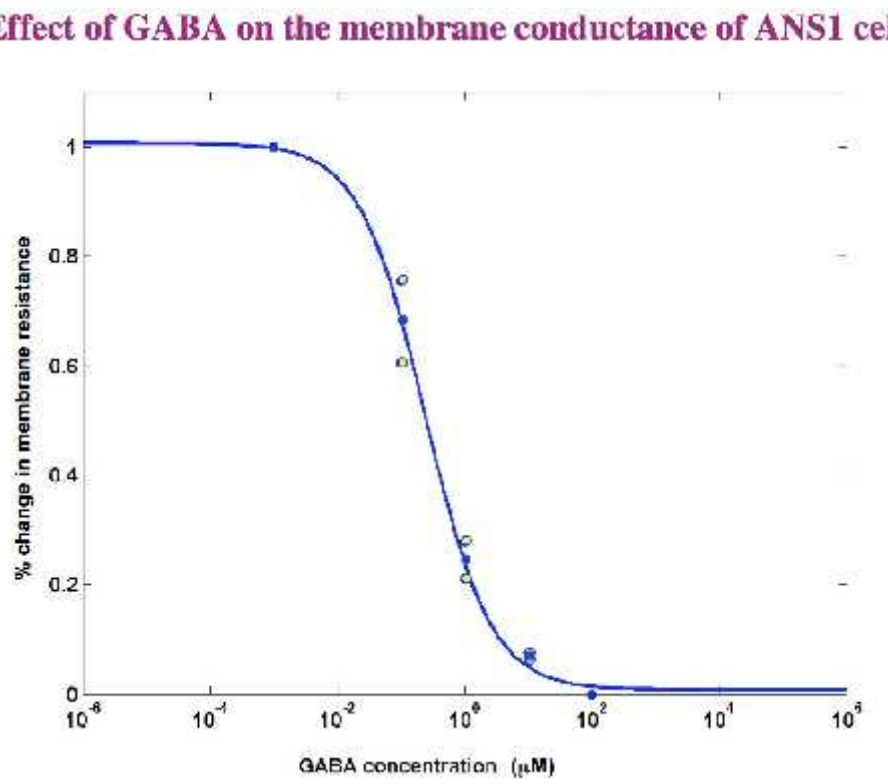
We have obtained the following preliminary results:

-Time course of changes in membrane conductance after triton X-100 administration;

-Concentration/response relationship in HeLa cells exposed to streptolysin-O;

-Capsaicin concentration/response relationship for CHO cells expressing VR1/TRPV1;

-GABA concentration/response relationship in gabaergic differentiated Adult Neuronal Stem cells.



Market/Opportunity

Low fluorescence emission and sensitivity of commercially available fast-VSD are critical issues and were addressed by two strategies: optimization of the instrumentation (by adopting most recent CCD and CMOS technologies) and proper analysis focused on responsive areas.

Therefore, under optimized conditions, the team propose the use of fast-VSD, as fast sensors of membrane permeability/conductance by imposing local field stimulation.

The method is applied to:

- evaluate direct or indirect activation, modulation or blockade of ion channels or transporters expressed on the plasma membrane, with the possibility to derive experimentally a concentration-response relationship;

- evaluate the direct permeabilizing effect of molecules with channel or transport characteristics (i.e. antibiotics);

- evaluate changes in the basal conductance of the plasma membrane as a marker of the different physiological states of the cell.

IP

European patent application No. 09 165 872.4 of 20/07/2009.

Not yet finalized the national applications

There are no constraints to the use of the patent.

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