FDSS7000EX FDSS/µCELL

SANOFI EXPLORATORY Unit

Use of Ca^{2+} oscillations in primary neuron culture: a new HTS model for neuronal diseases

Introduction

Even though they have made their proof in the discovery of many broad spectrum antiepileptic drugs (AEDs), phenotypic screening strategies are limited by the use of in vivo models thus rendering the screening of large chemical libraries virtually impossible. Ca^{2+} oscillations play a physiologic role in the central nervous system and are thus involved in the study of epilepsy, pathological pain states, Parkinson disease and neurological disorders. When neurons are cultured in high density in low magnesium concentration, they enter a stable firing mode mimicking epilepsy. This model, widely used in academic research, had not been characterized before in 384 wells format for MTS. This study demonstrates that AEDs are active in this assay, thus confirming its relevance in MTS. Moreover, tests with ion channels modulators, GPCR or transporters show that this model can be used to study basic anticonvulsant MOAs.

Materials and Methods

Cell culture

Hippocampal neuronal cultures were prepared from Sprague-Dawley rat embryos at 17-18 days of gestation. After dissociation, cells were plated in Neurobasal-A medium containing Glucose 0.45 %, Hepes 10 mM, Sodium bicarbonate 1 %, glutamax 1X, 2 % B27 and 1 % N2 at a density of 4.5 X 10^5 cells/ml (18 000 cell/wells) onto 384 PolyD-lysin coated microplates (Greiner). Neurons were grown in a humidified atmosphere of 5 % CO_2 at 37 °C, and cultures were fed at day 3 and 7 by exchanging medium (40 µL).

$[Ca^{2+}]i$ measurement

$[Ca^{2+}]i$ oscillations were monitored with the FDSS6000 microplate reader. Cells were preloaded for 1h at 37 °C with HBSS pH=7.4 containing 4 µM Fluo-4-AM, 0.001 % pluronic acid F-127, 3 mg/ml BSA, 2 mM $CaCl_2$, 0.45 % Glucose and 1 µM glycine. Cells were then washed twice with HBSS, 0.1 mM $MgCl_2$. 40 µL of 0.1 mM $MgCl_2$ was added to the well and basal activity was recorded during 4 min, then 10 µL of a solution containing the appropriate test compounds was injected and activity was recorded during 10 min. Control experiments where HBSS with no drug was added showed no injection effect. Data points were collected at 0.8 s intervals and analyzed using FDSS6000 software and an internal software OSCAR for frequency and amplitude quantification. All experiments were performed at room temperature.

Results

The first part (top panel) shows the characterization of this model with known agonists/antagonists compared to DMSO control (top left yellow). As expected from literature, GABAA agonist caused a rapid cessation of Ca^{2+} oscillations whereas antagonists induced amplitude increase combined to frequency decrease (top row green). Tests showed that Ca^{2+} oscillations blockade can also be observed in this model with NMDA antagonists (top row orange), diverse ion channels blockers (bottom row pink and blue) as well as adenosine receptor agonist (bottom row green).

The second part consists of a characterization of this model with known AEDs (bottom panel). All AEDs in yellow abolish as expected Ca^{2+} oscillations at concentrations used in literature. The effect of the most recently discovered AEDs (bottom row pink and blue) needs to be further investigated mainly because their cellular targets (receptors and pathways) are not established yet.

Summary

Use of oscillations in neuron primary cultures is a new and powerful tool for MTS and HTS. Like all new tools, it needs characterization and proofs of its reproducibility. The data presented in this study are part of a large work where more than 60 compounds were tested and validated with this model in MTS. This study demonstrates the robustness and reproducibility of oscillations in neuron primary cultures as a MTS model in the FDSS.

Moreover, compounds tested in this study like ion channels blockers or other receptors agonists/antagonists show that this model can also be used to study other pathways like GPCRs, reuptake or enzymatic assays in large variety of diseases models (Parkinson's, pathological pain states, etc...). This opens a new and unique way to monitor compounds actions on a physiological model. With this in mind, a 28 K chemical library will be screened and the most interesting active compounds will be directly tested in vivo.

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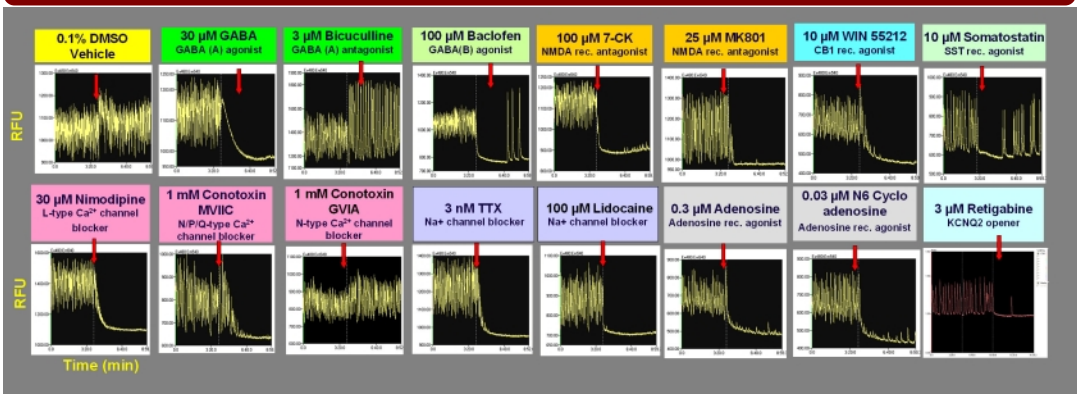
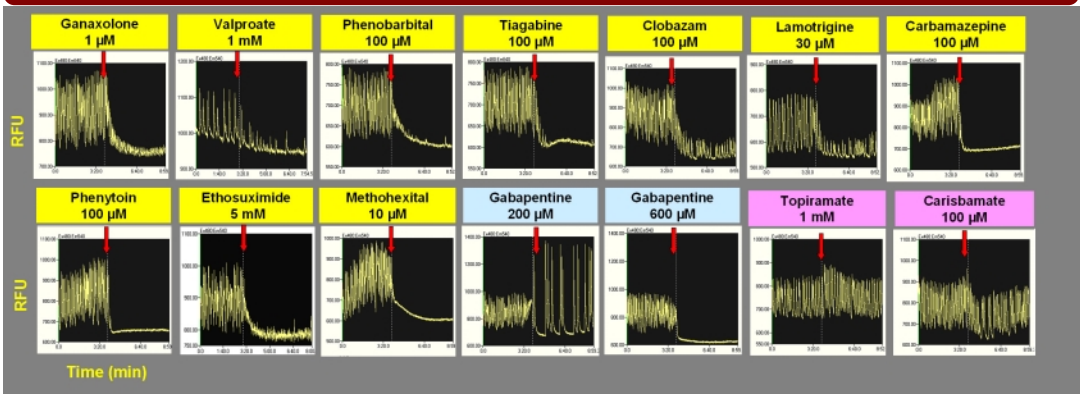
Involvement of various ion channels and receptors in Ca^{2+} oscillations



Effects of antiepileptic drugs



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