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Efficient high-throughput screening by ER Ca^{2+} measurement to identify inhibitors of ryanodine receptor Ca2+-release channels.

Murayama T[1], Kurebayashi N[2], Ikegami-Yuasa M[3], Mori S[3], Suzuki Y[2], Akima R[2], Ogawa H[4], Suzuki J[4], Kanemaru K[4], Oyamada H[5], Kiuchi Y[5], Iino M[4], Kagechika H[3], Sakurai T[2].

Author information

Abstract

Genetic mutations in **ryanodine** receptors (RyRs), **Ca2**+-**release channels** in the sarcoplasmic reticulum essential for muscle contractions, cause various skeletal muscle and cardiac diseases. Because the main underlying mechanism of the pathogenesis is overactive **Ca2**+ **release** by gain-of-function of the RyR channel, inhibition of RyRs is expected to be a promising treatment for these diseases. Here, to **identify inhibitors** specific to skeletal muscle type 1 RyR (RyR1), we developed a novel **high-throughput screening** (HTS) platform using time-lapse fluorescence **measurement** of **Ca2**+ concentrations in the endoplasmic reticulum (**ER**) ([**Ca2**+]**ER**). Because expression of RyR1 carrying disease-associated mutation reduces [**Ca2**+]**ER** in HEK293 cells through **Ca2**+ leakage from RyR1 **channels**, specific drugs that inhibit RyR1 will increase [**Ca2**+]**ER** by preventing such **Ca2**+ leakage. RyR1 carrying R2163C mutation and R-CEPIA1er, a genetically-encoded **ER Ca2**+ indicator, were stably expressed in HEK293 cells and time-lapse fluorescence was measured using a FlexStation II fluorometer. False positives were effectively excluded by using cells expressing wild-type (WT) RyR1. By **screening** 1,535 compounds in a library of well-characterized drugs, we successfully identified four compounds that significantly increased [**Ca2**+]**ER**. They include dantrolene, a known RyR1 inhibitor, and three structurally-different compounds; oxolinic acid, 9-aminoacridine and alexidine. All the hit compounds, except for oxolinic acid, inhibited [3H]**ryanodine** binding of WT and mutant RyR1. Interestingly, they showed different dose-dependencies and isoform specificities. The highly quantitative nature and good correlation

with the channel activity validated this HTS platform by [**Ca2**+]**ER measurement** to explore drugs for RyR-related diseases.

KEYWORDS: Calcium; Calcium imaging; Drug discovery; **High throughput screening**; Ion **channels**; **Ryanodine** receptors; Skeletal muscle

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Did you mean: *efficient high-throughput screening by er ca2+ measurement to identify inhibitors of ryanodine receptor **ca2+ release** channels* (1 items)