Molecular Pharmacology

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

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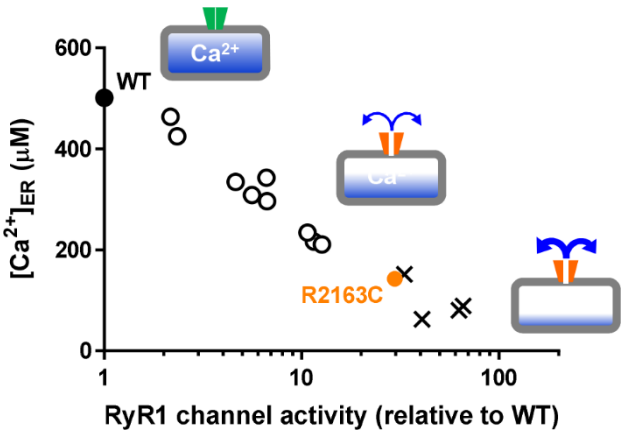
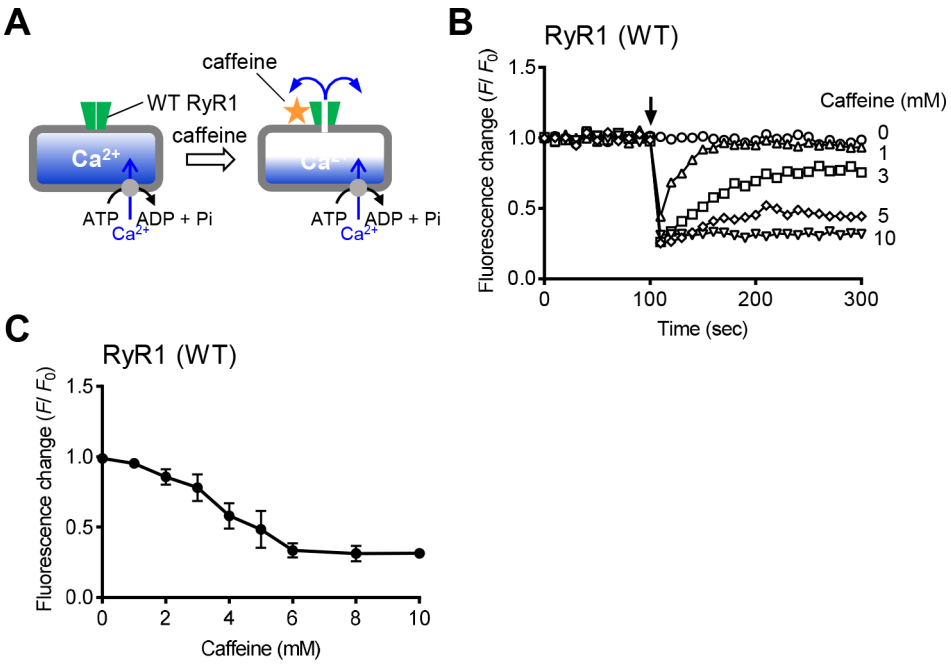


Supplementary Fig. 1. Relationship between RyR1 channel activity and $[Ca^{2+}]_{ER}$ in HEK293 cells expressing wild-type (WT, *filled circle*) or mutations associated with MH (*open circles*) and MH/CCD (*crosses*) in the central region. Data are modified from Murayama et al. (Murayama et al., 2016). Schematic illustrations represent $[Ca^{2+}]_{ER}$ and Ca^{2+} leakage through RyR1 in the indicated states. Note that $[Ca^{2+}]_{ER}$ are reduced to inversely correlate with the channel activity of the mutant RyR1, indicating Ca^{2+} leakage from the ER.



Supplementary Fig. 2. Effect of caffeine on $[Ca^{2+}]_{ER}$ in the WT RyR1 cells. (*A*) In the WT RyR1 cells, caffeine activates the RyR1 channels to cause Ca^{2+} leakage from ER. (*B*) Effect of caffeine on time-lapse R-CEPIA1er fluorescence measurement of the WT RyR1 cells. Application of caffeine at 1-10 mM (*arrows*) resulted in a rapid decrease in $[Ca^{2+}]_{ER}$ followed by gradual recovery toward their respective steady-state levels. (*C*) Caffeine dose-dependency of steady-state level of R-CEPIA1er fluorescence of the WT RyR1 cells. The steady-state level was reduced with caffeine in a dose-dependent manner. Data are the mean ± SD (n = 3).

Supplementary TABLE 1. Compounds that increased F/F_0 in WT cells by >3 SDs.

Compounds	Mean F/F_0 (RC)	Mean F/F_0 (WT)	ΔF (w/o cell)	ΔF (w cell)
Daunorubicin	3.88	2.36	18.4±3.0	17.3±0.2
Doxorubicin	4.97	3.79	28.2±2.0	26.9±1.8
Epirubicin	3.75	3.05	20.6±2.0	17.9±0.5
Erythrosine	10.15	6.11	53.0±2.4	48.7±1.5
Homidium Bromide	2.66	1.69	6.5±0.6	7.3±0.7
Oxidopamine	2.93	3.13	13.4±1.1	12.8±2.2
Pararosaniline	1.37	1.2	2.5±0.1	2.4±0.4
Pyrvinium	1.66	1.35	5.3±0.4	7.6±0.8

Mean F/F_0 represents ratio of the averaged R-CEPIA1er fluorescence for the last 100 seconds (F) to that for the first 100 seconds (F_0) in R2163C (RC) and WT RyR1 cells induced by the compounds. ΔF represents fluorescence changes by compounds in the presence (w cell) and absence (w/o cell) of the WT RyR1 cells. All the 8 compounds that increased F/F_0 in WT cells by >3 SDs exhibited autofluorescence.

Supplementary TABLE 2. Effects of hit and related compounds on $[Ca^{2+}]_{ER}$ of mutant and WT RyR1 cells.

Compounds	Mean F/F_0 (Mutant RyR1)	Mean F/F_0 (WT RyR1)
Oxolinic Acid	**1.66**	**1.01**
Cinoxacin	1.05	0.98
Ciprofloxacin	1.04	0.98
Enoxacin	0.96	0.97
Enrofloxacin	0.97	1.02
Flumequine	0.98	1.01
Gatifloxacin	1.00	0.99
Gemifloxacin	0.92	1.00
Levofloxacin	0.94	0.99
Lomefloxacin	0.89	0.95
Moxifloxacin	0.87	0.95
Norfloxacin	0.99	0.99
Ofloxacin	0.95	0.96
Orbifloxacin	0.95	0.94
Pefloxacine	0.96	0.93
Pipemidic Acid	0.94	0.90
Piromidic Acid	0.97	0.97
Sarafloxacin	1.01	0.94
9-Aminoacridine	**1.62**	**0.98**
Amsacrine	0.88	1.04
Ethacridine	1.06	1.04
Mepacrine	1.00	0.96
Alexidine	**1.71**	**0.84**
Chlorhexidine	0.93	0.97
Metformin	0.89	1.01
Phenformin	0.99	1.00
Phenylbiguanide	0.96	0.98
Proguanil	0.96	0.95
Compounds described in Rebbeck et al. (2017)		
Chloroxine	0.80	0.96
Disulfiram	0.60	0.78
Tacrolimus	0.70	1.00

Hit compounds are indicated in *bold* and the related compounds in the library used in this study are listed below. Values indicate F/F_0 of R-CEPIA1er fluorescence in mutant (R2163C) and WT RyR1 cells induced by the compounds (see **Fig. 3**).