Evaluation of Various Ionic Currents Generating Cardiac Action Potential by Patch-Clamp Method
心筋活動電位を形成する各種イオンチャネル電流のパッチクランプ法による評価

DSTC
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Introduction

The hERG assay is now a familiar measure of predicting drug-induced QT prolongation in the preclinical stage. Recently effects on cardiac ion channels other than hERG is coming under a spot light, and many are suggesting assessing effects on those ion channels in an early stage. We investigated methods of assessing the currents through those ion channels by patch-clamp technique with stably expressing cell lines.

Materials and Methods

■ Cell Line

Cell Line	Supplier	Current
CHO-h$Ca_V1.2/\beta_2/\alpha_2\delta_1$	ChanTest	I_{Ca}
HEK-h$Na_V1.5$	SB Drug Discovery	I_{Na}
HEK-hK_VLQT1/minK	Cytocentrics	I_{Ks}
CHO-h$K_{ir}2.1$	B'SYS	I_{K1}
CHO-h$K_V1.5$	Cytocentrics	I_{Kur}
CHO-h$K_V4.3$	B'SYS	I_{to}

■ Patch Clamp

- Whole-cell patch-clamp method
- Application: single or cumulative
- Flow rate: 3-5 mL/min
- Pipette resistance: 1-5 MΩ

■ Solution

・*Extracellular solution*

	I_{Na}	I_{Ca}	I_{Ks}	I_{K1}, I_{Kur}, I_{to}
NaCl	140	145	145	137
KCl	4	4	4	4
$CaCl_2$	2	10	2	1.8
$MgCl_2$	1		1	1
glucose	10	10	10	10
HEPES	10	10	10	10
pH	7.35	7.4	7.4	7.4

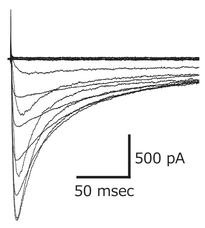
・*Intracellular solution*

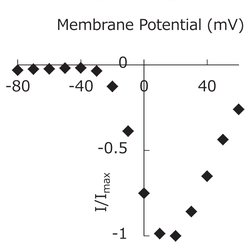
	I_{Na}	I_{Ca}	I_{Ks}, I_{K1}, I_{Kur}, I_{to}
KCl			130
CsCl	50	112	
NaCl	10	2	
CsF	60	27	
$MgCl_2$	2		1
EGTA	20	8.2	5
MgATP		4	5
HEPES	10	10	10
pH	7.25	7.2	7.2

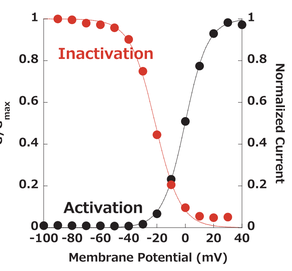
Results

■ $Ca_V1.2$

・***Electrophysiological Property***

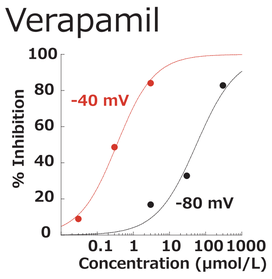




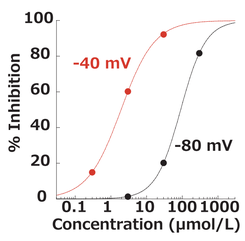


・***Holding Potential Dependence***

Verapamil



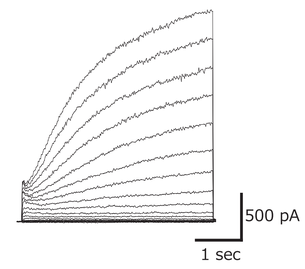
Diltiazem

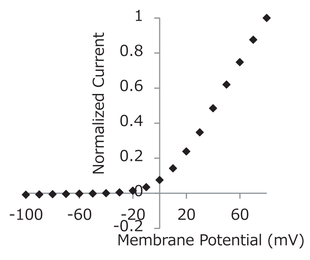


- V_{hold}: -80 mV or -40 mV
- V_{depo}: 0 mV
- Inhibitory activities of compounds depend on holding potentials.

■ $K_VLQT1/minK$

・***Electrophysiological Property***

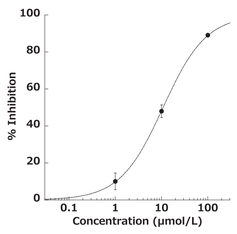


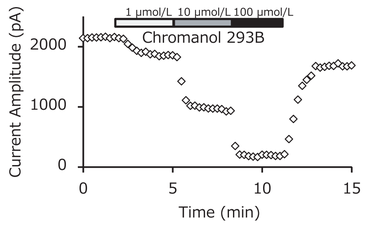


- V_{hold}: -80 mV
- V_{depo}: +60 mV
- Stable measurements of large outward currents were enabled.

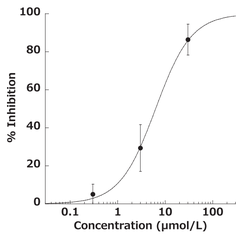
・***Responce to Compounds***

Chromanol 293B



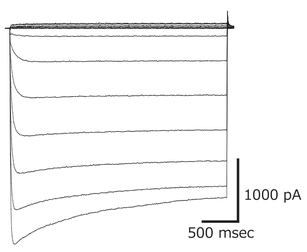


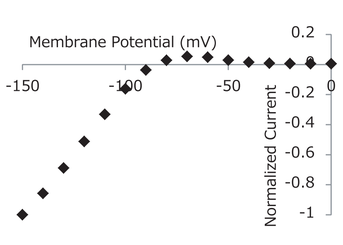
Bepridil



■ $K_{ir}2.1$

・***Electrophysiological Property***





・***Responce to Compounds***

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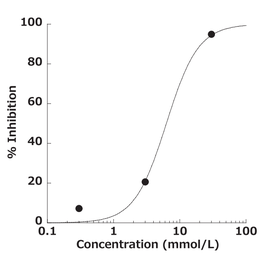
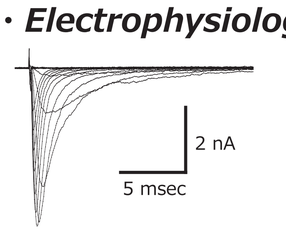
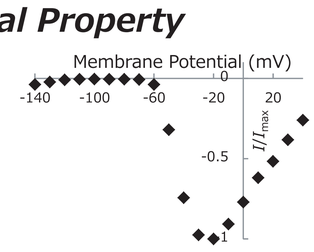


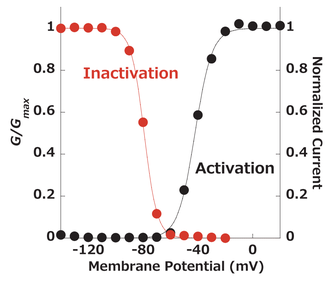
- V_{hold}: -20 mV
- V_{hypo}: -120 mV
- Notable inward-rectifying current was identified.

■ $Na_V1.5$

・***Electrophysiological Property***

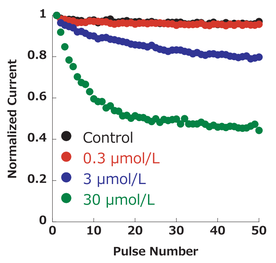




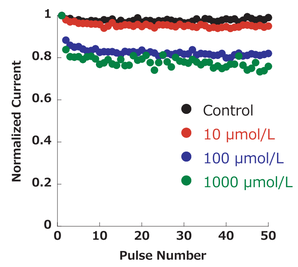


・***Use Dependence (1 Hz)***

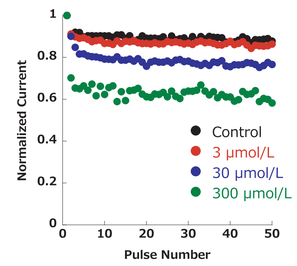
Flecainide



Lidocaine

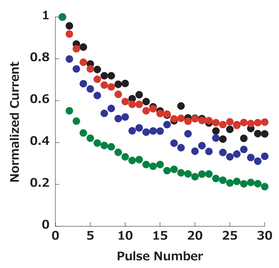


Mexiletine

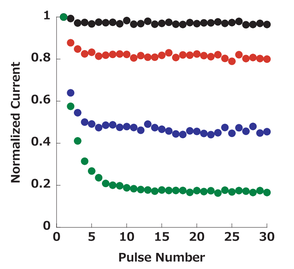


・***Frequency Dependence***

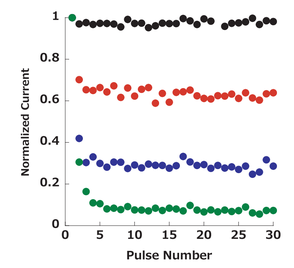
Flecainide (30 μmol/L)



Lidocaine (100 μmol/L)



Mexiletine (300 μmol/L)



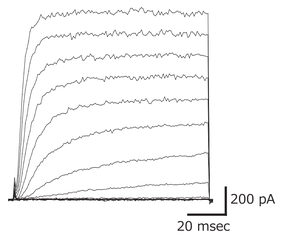
・***Results from various protocols (comparison of IC_{50}s; μmol/L)***

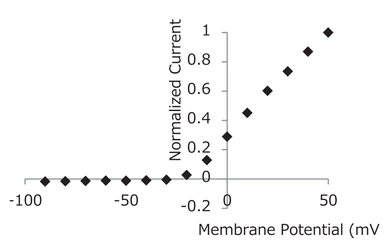
Protocol		Flecainide	Lidocaine	Mexiletine
Tonic Block		> 30	274.4	87.0
UD 0.5 Hz	50th/1st	19.6	> 1000	> 300
	$50th_{cpd}/50th_{ctrl}$	5.6	466.8	291.2
UD 1 Hz	50th/1st	23.6	> 1000	> 300
	$50th_{cpd}/50th_{ctrl}$	6.2	141.6	45.3
UD 2.5 Hz	50th/1st	11.7	> 1000	85.7
	$50th_{cpd}/50th_{ctrl}$	2.7	31.3	39.6
UD 10 Hz	50th/1st	8.7	25.6	17.4
	$50th_{cpd}/50th_{ctrl}$	4.3	21.5	10.1
Inactivation		> 30	18.2	15.0

- V_{hold}: -100 mV
- V_{depo}: -20 mV
- Inhibitory activities of compounds depend on setting protocols.

■ $K_V1.5$

・***Electrophysiological Property***





・***Responce to Compounds***

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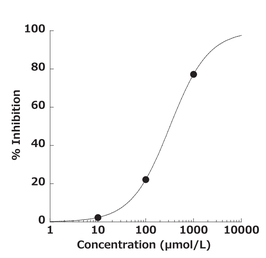
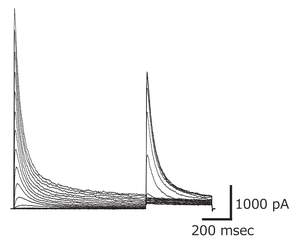
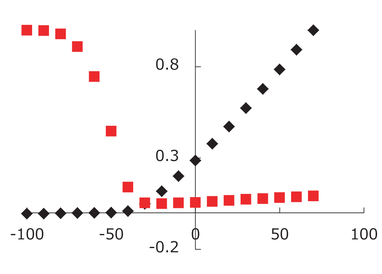


- V_{hold}: -80 mV
- V_{depo}: +20 mV
- Stable measurements of large outward currents were enabled.

■ $K_V4.3$

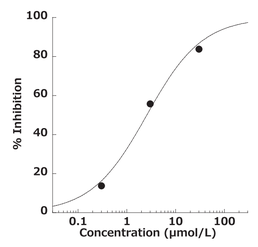
・***Electrophysiological Property***





・***Responce to Compounds***

Dapoxetine



- V_{hold}: -80 mV
- V_{depo}: +20 mV
- Stable measurements of transient outward currents were enabled.

Conclusion

Now we are able to perform stable measurements of cardiac ion-channel currents. Protocols and experimental conditions must be optimized depending on purposes, since the degree of inhibitory activity depends on experimental setting. For evaluating effects of compounds on these cardiac ion channels, standardization of experimental conditions appropriate to the evaluation purpose (safety pharmacology, target assay, etc), based on knowledge of both electrophysiological and pharmacological properties of each ion channel, would be necessary.