Mediford Corporation

Rundown correction in $Ca_V1.2$ channels: a proposal for a novel testing approach

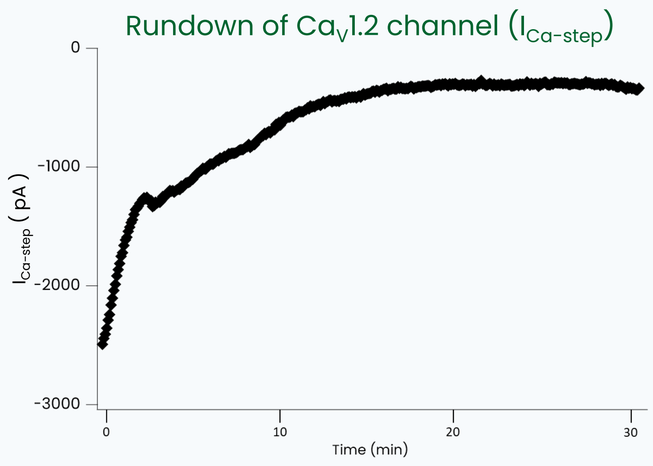
○Hiromu Amano[1)], Minami Arayama[1)], Mayumi Obo[1)], Hiromi Negishi[1)], Yoshio Matsumoto[1)], Atsushi Sugiyama[2) 3) 4)]

1) Mediford Corporation, 2) Faculty of Medicine, Toho University, 3) Yamanashi Research Center of Clinical Pharmacology, 4) Internal Medicine, Koshu Rehabilitation Hospital

Background and Objective

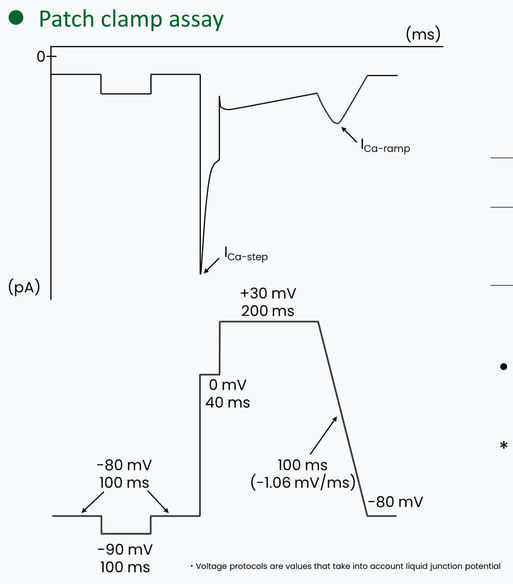
The effects of drugs on L-type calcium channels ($Ca_V1.2$) are a critical indicator in cardiovascular evaluations. Patch clamp recordings of ionic currents are employed for these evaluations; however, it is imperative to mitigate the influence of time-dependent current decline (rundown).
Notably, rundown phenomena are consistently observed in I_{Ca} recordings. At other institutions, $Ca_V1.2$ current measurements are performed either by correcting for rundown using values from the vehicle control group or by carrying out measurements after the rundown has nearly disappeared. However, since rundown in $Ca_V1.2$ varies from cell to cell, a uniform correction based on the vehicle group may lead to inaccurate evaluations. Therefore, to achieve greater accuracy in assessments, a correction method that reflects the rundown characteristics of each cell type is necessary. In this study, we propose a novel correction method that accounts for cell-specific rundown and validate it using verapamil.

Rundown of $Ca_V1.2$ channel ($I_{Ca\text{-}step}$)



Materials and Methods

- Patch clamp assay



Cell line	HEK–$Ca_V1.2$ Stable Cell Line
Temperature	36.0 ± 1.0 °C
Stimulation frequency	0.2 Hz
Drug	Verapamil

- This experiment was conducted in accordance with best practices and the CiPA* protocol.

* Recommended voltage protocols to study drug-cardiac ion channel interactions using recombinant cell lines; 2021 Jul 30 [cited 2022 Jun 1].

- In this study, the $I_{Ca\text{-}step}$ was used as the evaluation parameter.
- For each group, measurements were performed using vehicle (0.1% DMSO) and four concentrations of verapamil, with four replicates obtained under each condition. In total, data from 80 cells were used to determine the IC_{50} for four groups (designated as Group 1, Group 2, Group 3, and Group 4).

Results

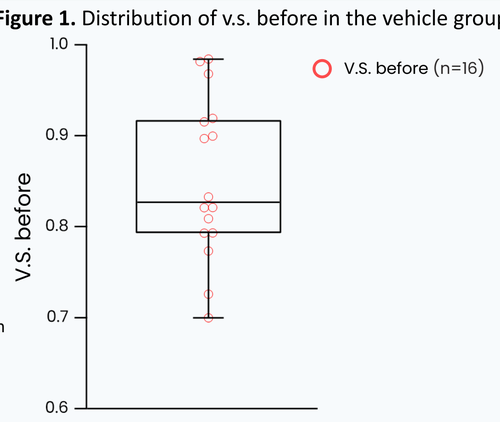
1. Rundown characteristics are cell-specific.

Table 1. Comparison of v.s. before in the vehicle group

0.1 % DMSO (n=4)	Group 1	Group 2	Group 3	Group 4
V.S. before	0.90	0.79	0.80	0.92
S.D.	± 0.07	± 0.09	± 0.03	± 0.09
S.E.	± 0.03	± 0.05	± 0.01	± 0.04

V.S. before = Average Peak Value After Application / Average Peak Value Before Application

Figure 1. Distribution of v.s. before in the vehicle group



Among the cells evaluated, the maximum intercellular difference in v.s. before was approximately 0.3, indicating that each cell has unique rundown characteristics.

2. Cell-specific rundown correction.

During verapamil application, the observed reduction in $Ca_V1.2$ current is influenced by both the drug's effect and rundown phenomena. Therefore, to perform rundown correction on a cell-by-cell basis, it is necessary to estimate the post-application peak value based solely on the rundown effect. In this study, the post-application peak value was estimated using the following three approaches.

- **Estimated value**

1. Estimation from changes in pre-application current (I_{Ca}) : E1
(For the estimation, the pre-application peak value and the pre-application rundown are used.
It is assumed that, in the absence of any drug effect, the rundown during application follows the pre-application rundown, thereby enabling the calculation of the post-application peak value.)

2 . Estimation based on current changes before and after application : E2
(This approach uses the pre-application peak value, pre-application rundown, and post-application rundown to estimate the post-application peak value. In the absence of drug effects, it is assumed that the rundown observed during application changes at a constant rate from the pre-application level to the post-application level.)

3 . Estimation based on current changes before, during, and after application : E3
(This approach uses the pre-application peak value, pre-application rundown, post-application rundown, and the change in peak value during application to estimate the post-application peak value. It is assumed that the drug-induced current reduction affects the rundown characteristics. The overall peak attenuation observed during application is defined as 100%. The post-application peak value is estimated by assuming that the rundown transitions proportionally from the pre-application level to the post-application level based on this percentage.)

Rundown correction was performed using the following methods:

① : Vehicle-based rundown correction

Vehicle correction = 100 - (Drug's v.s. before / Average v.s. before of the vehicle group) * 100

- **Cell-specific rundown correction**

② : Correction using estimation formula E1
Correction A

③ : Correction using estimation formula E2
Correction B

④ : Correction using estimation formula E3
Correction C

Cell-specific rundown correction (Correction A, Correction B, and Correction C) = 100 - ((Drug's post-application peak value / E1 , E2 or E3) * (Accuracy correction using vehicle group)) * 100

3. High-accuracy estimation of post-application peak value in the vehicle group.

Table 2. Comparison of post-application measured values and estimated values using the estimation formula in the vehicle group (n = 16).

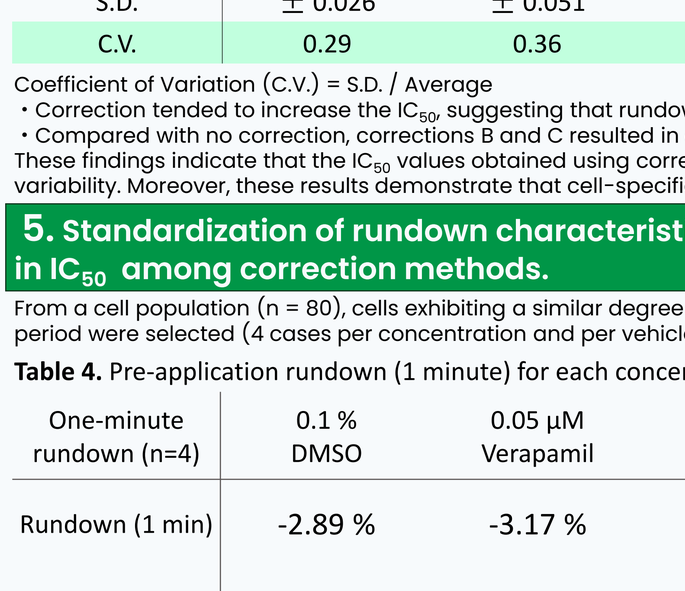
	E1	E2	E3
Actual value/ Estimated value	1.020	1.002	0.984
S.D.	± 0.148	± 0.050	± 0.034

The ratio of the actual value to the estimated value approaches 1 with increasing estimation accuracy.
All three estimation formulas produced highly accurate predictions of the post-application peak value.
Moreover, the variability observed with E2 and E3 was smaller compared to that with E1.
These results suggest that, within the vehicle group, the estimation formulas appropriately reflect the cell-specific rundown characteristics.

4. Verapamil's IC_{50} varies with correction methods.

Figure 2. Variation of verapamil's IC_{50} for each correction method

A. Variation in IC_{50} among groups for each correction method



B. Average IC_{50} values of all groups for each correction

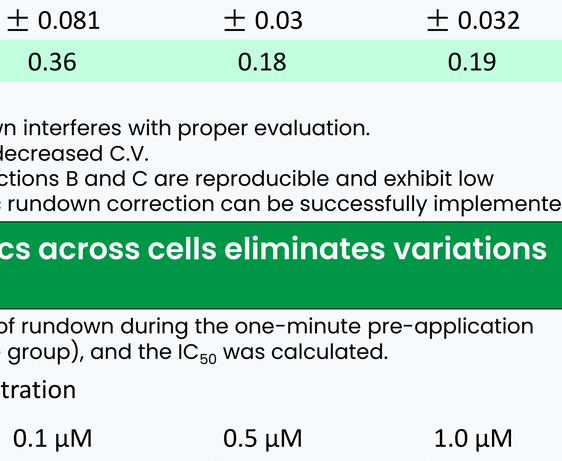


Table 3. Variation in IC_{50} among groups for each correction method

IC_{50} (µmol/L)	No correction	Vehicle correction	Correction A	Correction B	Correction C
Group 1	0.064	0.087	0.312	0.168	0.154
Group 2	0.063	0.118	0.088	0.119	0.123
Group 3	0.123	0.226	0.231	0.202	0.197
Group 4	0.109	0.141	0.186	0.173	0.203
Average	0.090	0.143	0.204	0.166	0.169
S.D.	± 0.026	± 0.051	± 0.081	± 0.03	± 0.032
C.V.	0.29	0.36	0.36	0.18	0.19

Coefficient of Variation (C.V.) = S.D. / Average
・Correction tended to increase the IC_{50}, suggesting that rundown interferes with proper evaluation.
・Compared with no correction, corrections B and C resulted in decreased C.V.
These findings indicate that the IC_{50} values obtained using corrections B and C are reproducible and exhibit low variability. Moreover, these results demonstrate that cell-specific rundown correction can be successfully implemented.

5. Standardization of rundown characteristics across cells eliminates variations in IC_{50} among correction methods.

From a cell population (n = 80), cells exhibiting a similar degree of rundown during the one-minute pre-application period were selected (4 cases per concentration and per vehicle group), and the IC_{50} was calculated.

Table 4. Pre-application rundown (1 minute) for each concentration

One-minute rundown (n=4)	0.1 % DMSO	0.05 µM Verapamil	0.1 µM Verapamil	0.5 µM Verapamil	1.0 µM Verapamil
Rundown (1 min)	-2.89 %	-3.17 %	-3.09 %	-2.90 %	-2.57 %
S.D.	± 1.51	± 0.30	± 0.54	± 0.72	± 0.63

Table 5. IC_{50} derived from a cell population with uniform rundown characteristics

	Vehicle correction	Correction A	Correction B	Correction C
IC_{50} (µmol/L)	0.157	0.146	0.154	0.157

The IC_{50} values obtained using vehicle correction were nearly identical to those obtained using corrections B and C. These results indicate that corrections B and C specifically address rundown effects.

6. Investigation of cell-specific correction using bepridil.

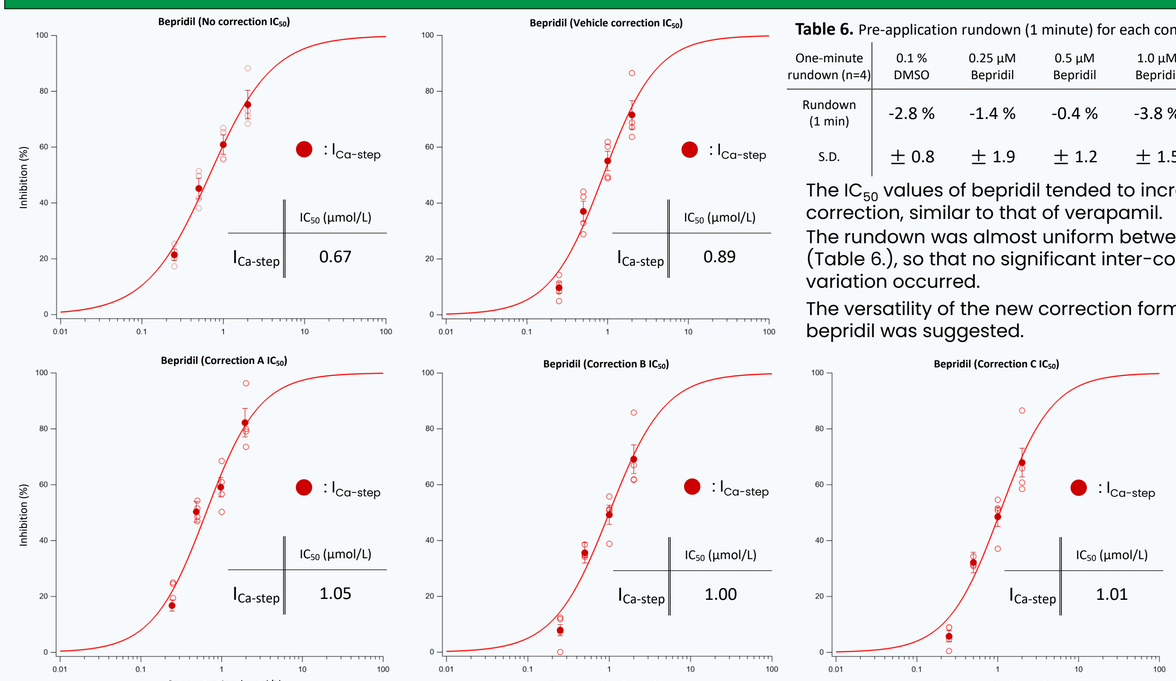


Table 6. Pre-application rundown (1 minute) for each concentration.

One-minute rundown (n=4)	0.1 % DMSO	0.25 µM Bepridil	0.5 µM Bepridil	1.0 µM Bepridil	2.0 µM Bepridil
Rundown (1 min)	-2.8 %	-1.4 %	-0.4 %	-3.8 %	-2.9 %
S.D.	± 0.8	± 1.9	± 1.2	± 1.5	± 1.5

The IC_{50} values of bepridil tended to increase with correction, similar to that of verapamil.
The rundown was almost uniform between cells (Table 6.), so that no significant inter-correction variation occurred.
The versatility of the new correction formula for bepridil was suggested.

Conclusion

Results

- The use of the correction method tended to increase the IC_{50}.
- In the vehicle group, the post-application peak value was estimated with high accuracy.
- Compared with no correction, corrections B and C reduced the variability of the IC_{50}.
- When rundown characteristics were standardized among cells, no differences in the IC_{50} were observed among the correction methods.

These results indicate that:

- Rundown occurring during $Ca_V1.2$ measurements clearly interferes with proper evaluation.
- The new correction method appears to appropriately address cell-specific rundown characteristics.
- When using the proposed correction method, there is no need to wait for a prolonged decrease in rundown; once a consistent rundown change is observed, drug evaluation can be performed. This approach is expected to significantly improve time efficiency.

Challenges

- We did not obtain direct evidence that the proposed correction method exclusively compensates for cell-specific rundown characteristics.
- In future studies, we plan to collaborate with other institutions and increase the sample size to validate its effectiveness.

mediford
A Member of PHC Group

aadlac INTERNATIONAL