

Effects of Various Vehicles on hERG Current and Countermeasures

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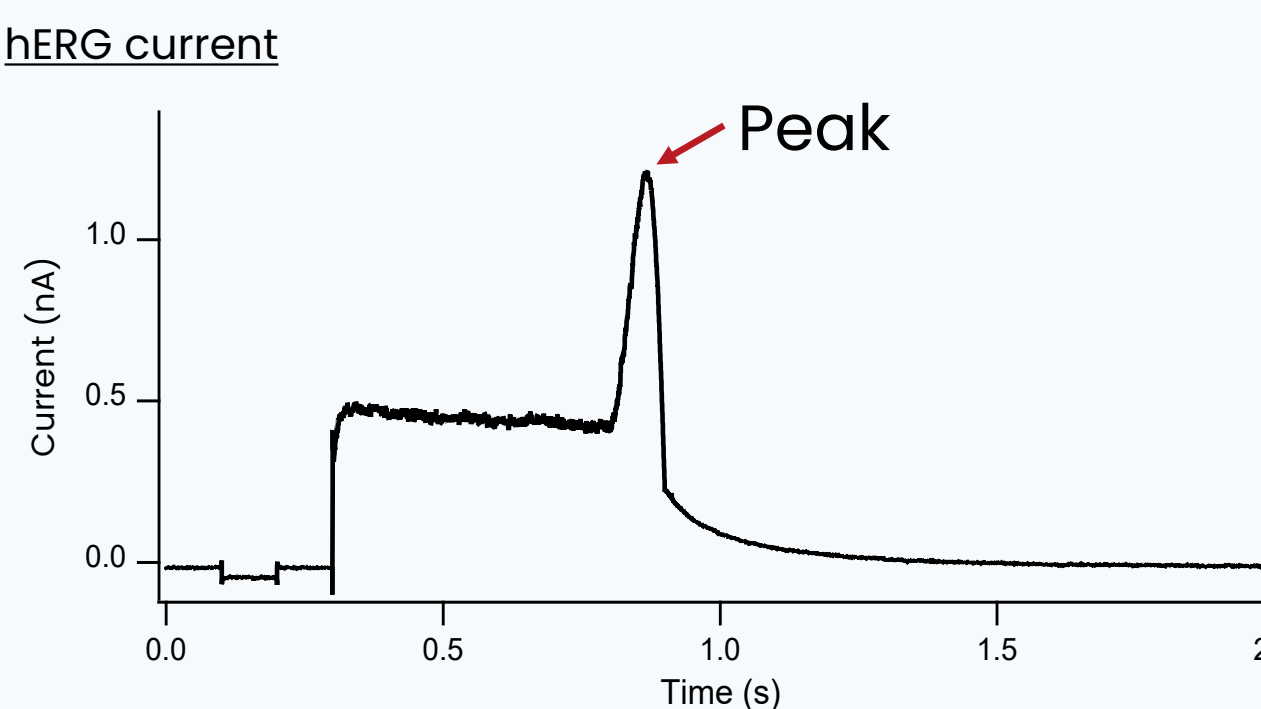
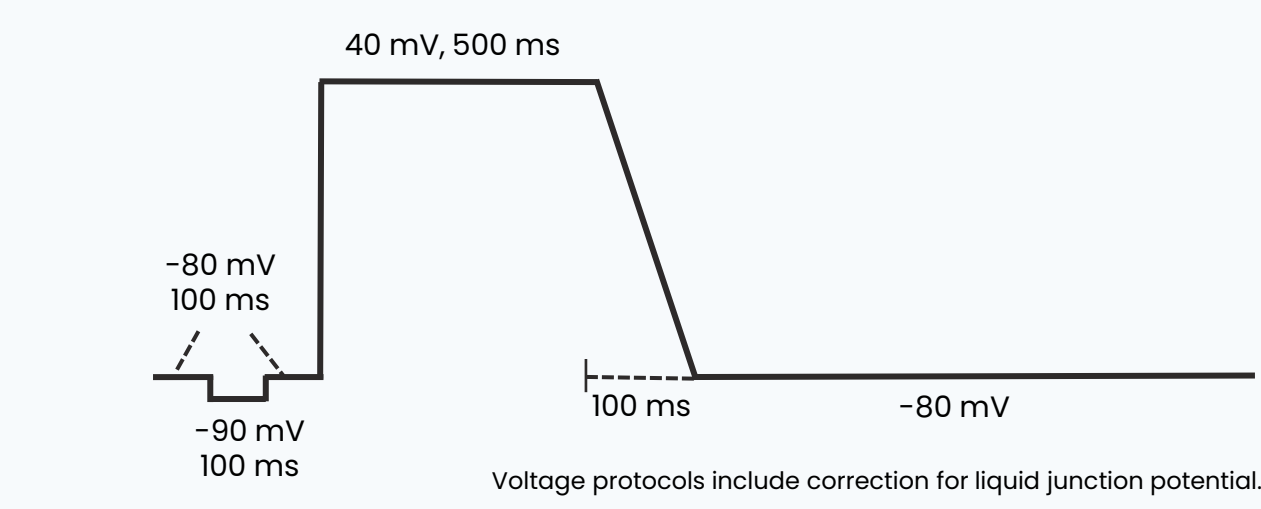
Background and Objective

The hERG assay is a required preclinical test used to evaluate the cardiovascular safety of test articles before Phase I clinical trials. In the hERG assay, test articles are typically dissolved in a vehicle and added to aqueous physiological salt solutions before application to cells. Dimethyl sulfoxide (DMSO) is commonly used as the standard vehicle due to its high solubility and relatively low cytotoxicity. However, DMSO is known to inhibit hERG currents at high concentrations. Furthermore, some test articles exhibit poor solubility in DMSO or may precipitate upon addition to aqueous physiological salt solutions, potentially affecting assay reliability. Given these limitations, identifying alternative vehicles is essential to enhance the accuracy and robustness of the hERG assay. In this study, we evaluated the effects of seven vehicles on hERG currents. For vehicles that demonstrated any effect on hERG currents, we further investigated their impact on assay outcomes and explored strategies to mitigate these effects.

Materials and Method

● Patch clamp assay

Voltage protocol



Whole cell patch-clamp (manual)

Cell lines

hERG-HEK293

Recording temperature

Physiological temperature (36.0 ± 1.0 °C)

Stimulation frequency

0.2 Hz (5s)

External solution

130 mmol/L NaCl, 5 mmol/L KCl, 10 mmol/L HEPES, 1 mmol/L CaCl₂, 1 mmol/L MgCl₂, 12.5 mmol/L Glucose adjusted to pH 7.4 ± 0.1 with 5 mol/L NaOH

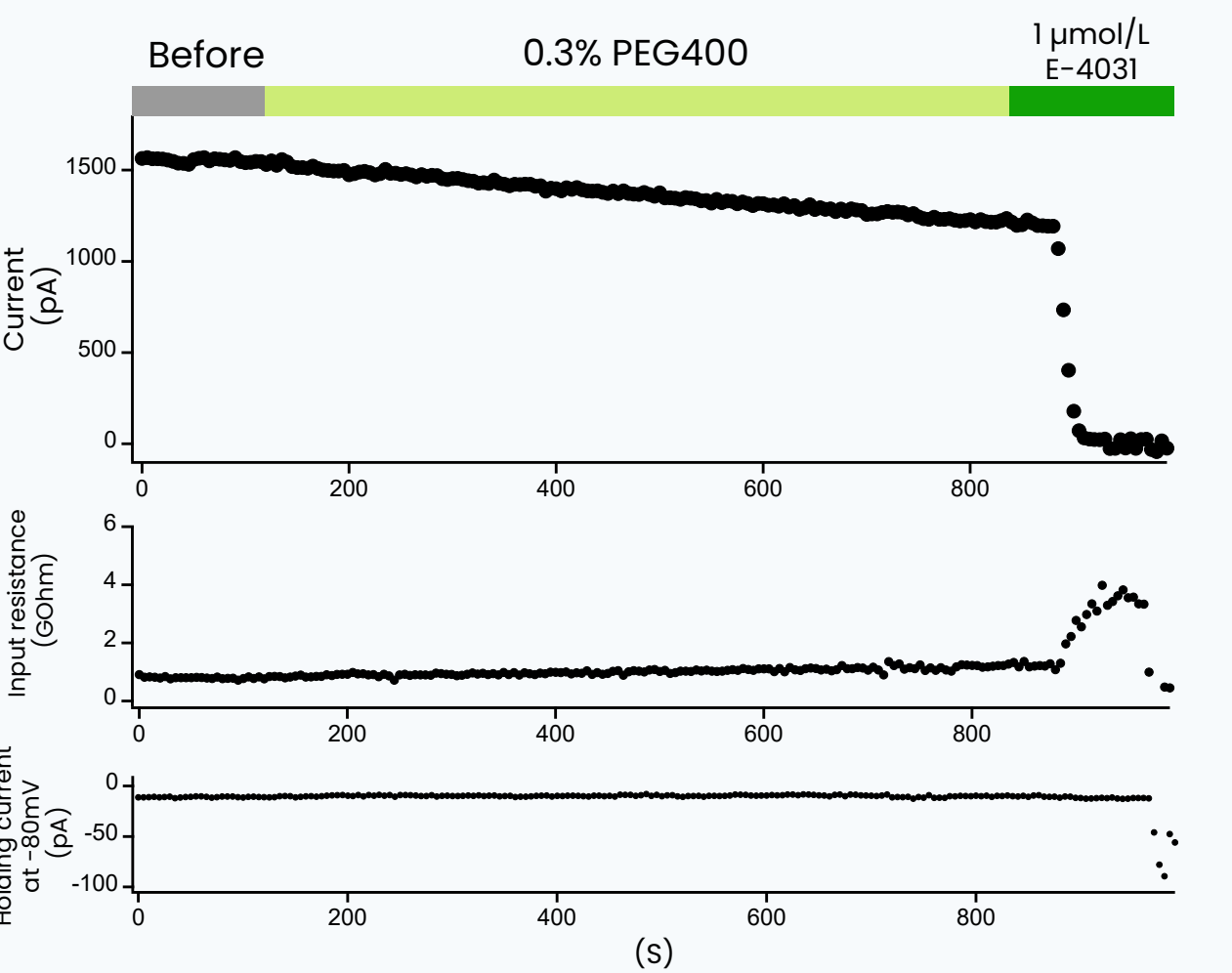
Internal solution

120 mmol/L K-gluconate, 20 mmol/L KCl, 5 mmol/L EGTA, 10 mmol/L HEPES, 1.5 mmol/L MgATP adjusted to pH 7.3 ± 0.1 with 1 mol/L KOH

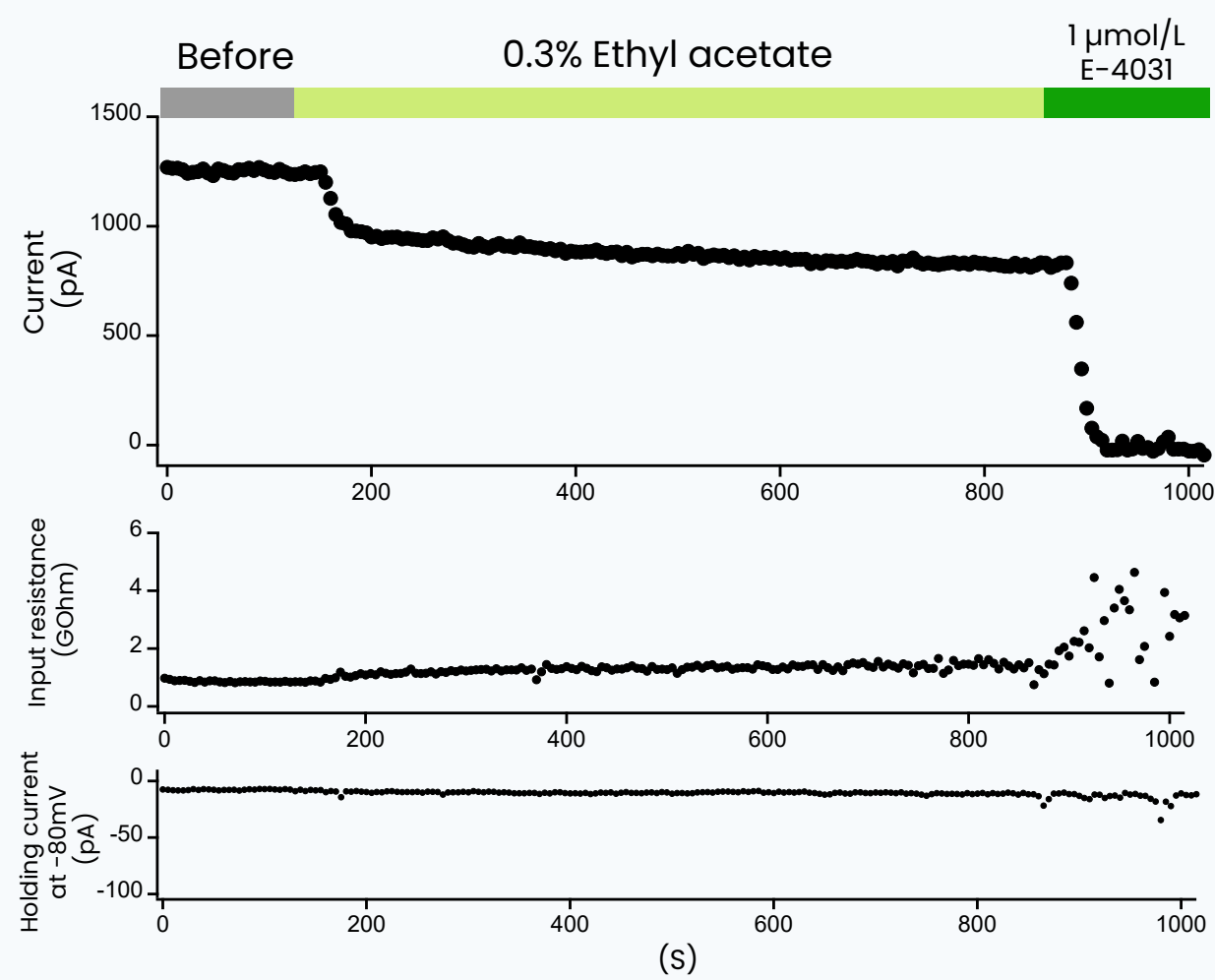
- This experiment was conducted in accordance with **best practices and the CIPA* protocol**.
- Recommended voltage protocols to study drug-cardiac ion channel interactions using recombinant cell lines; 2021 Jul 30 [cited 2022 Jun 1].

Result 1. Effect of Vehicles on hERG current

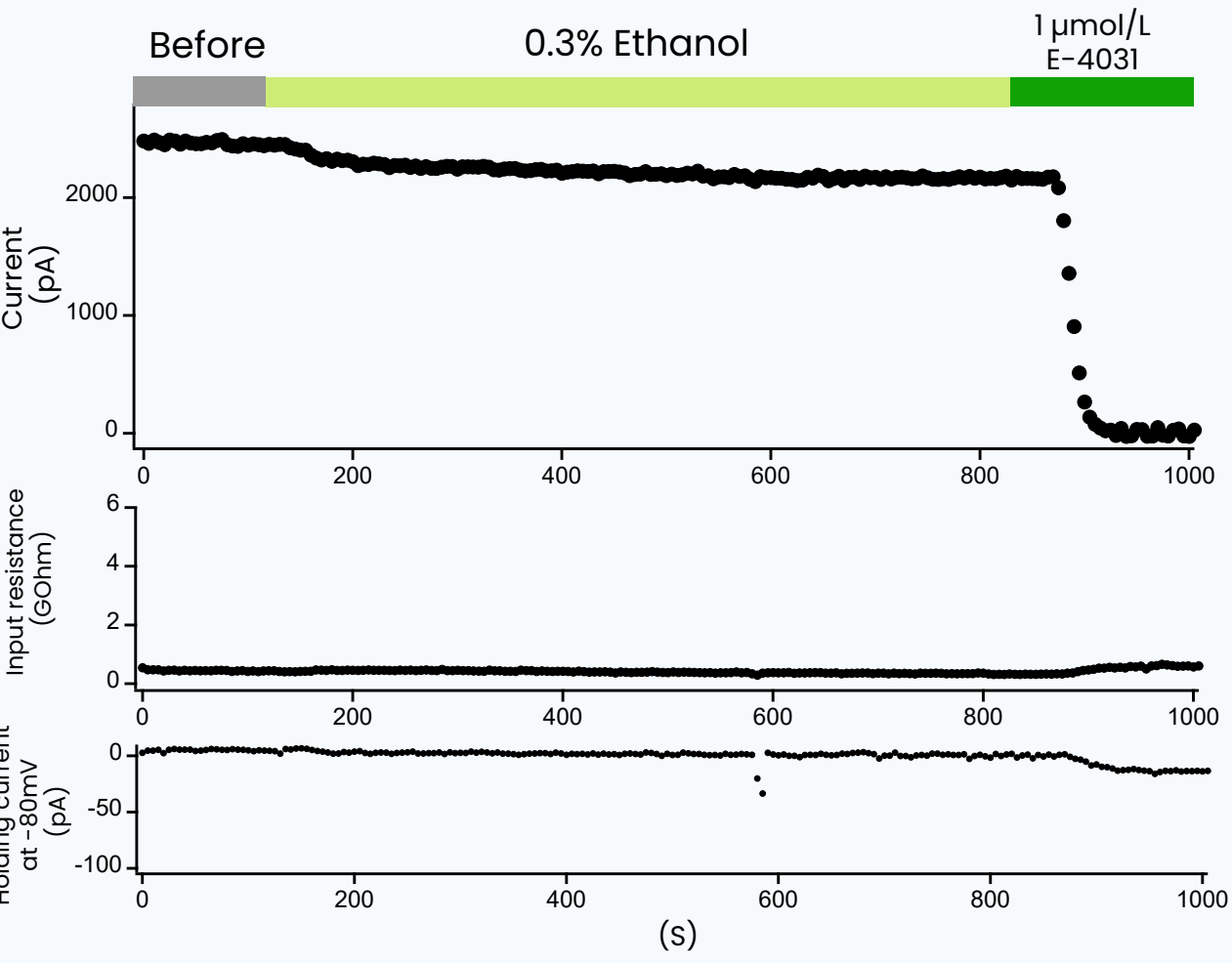
● Polyethylene glycol 400 (PEG400)



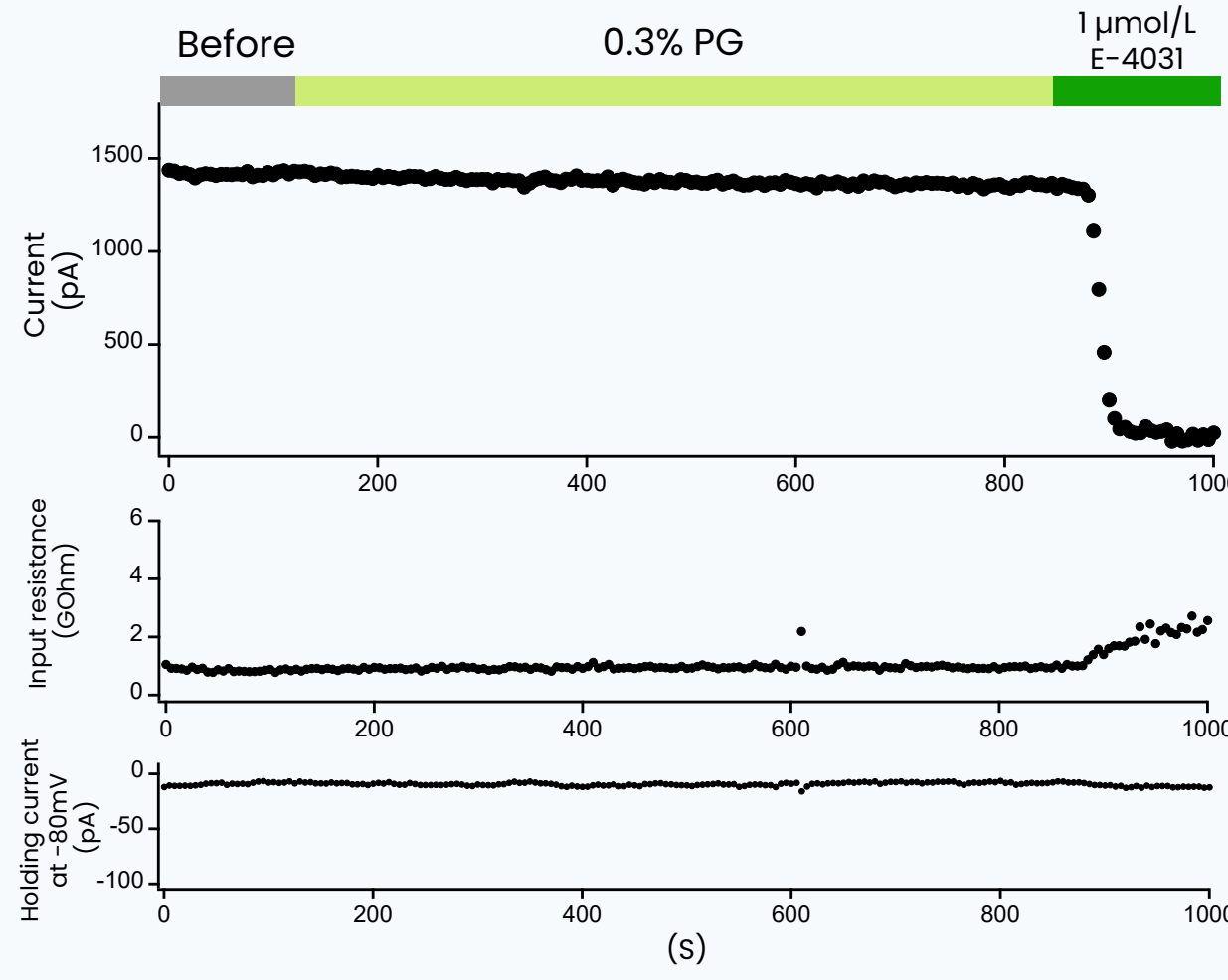
● Ethyl acetate



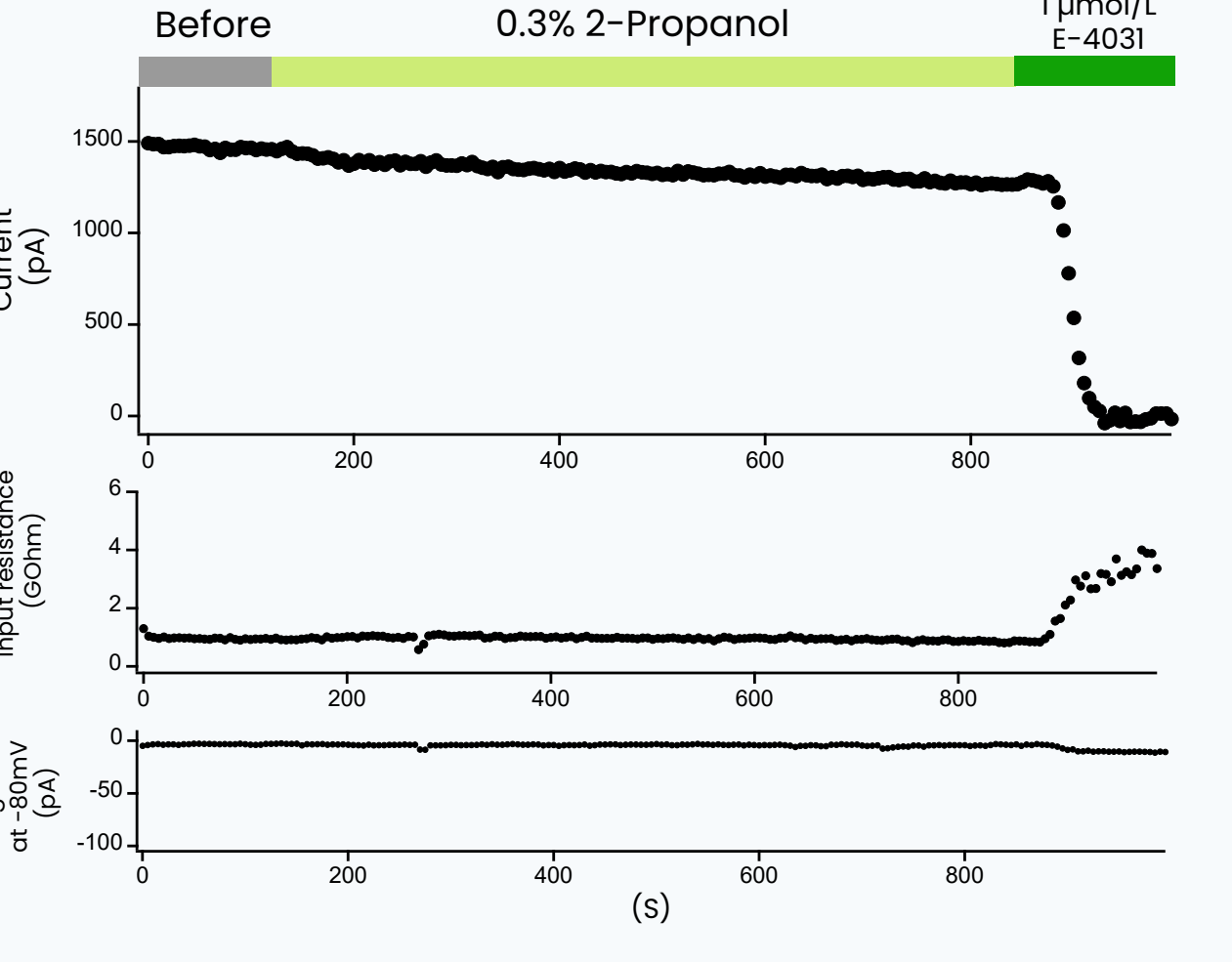
● Ethanol



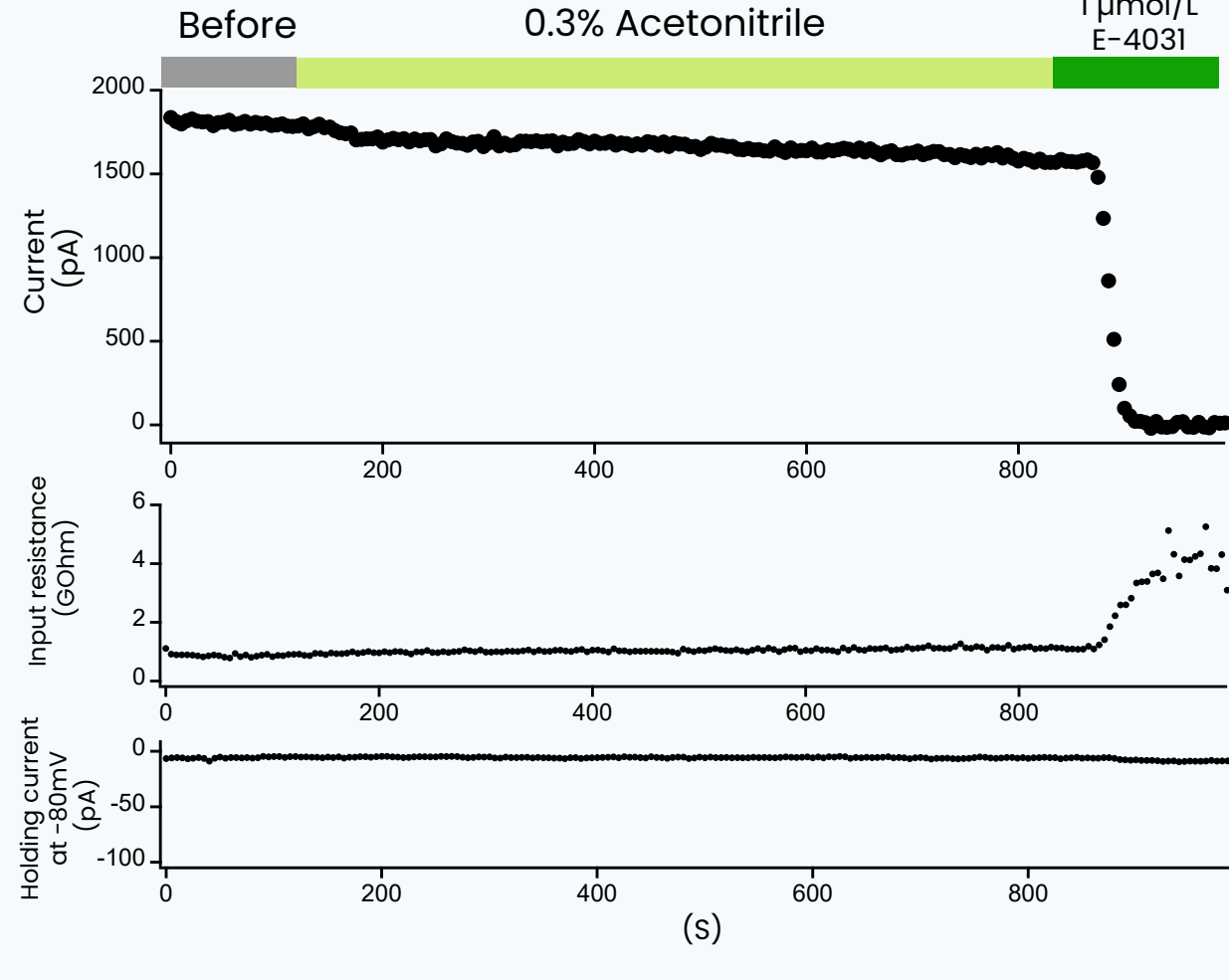
● Propylene glycol (PG)



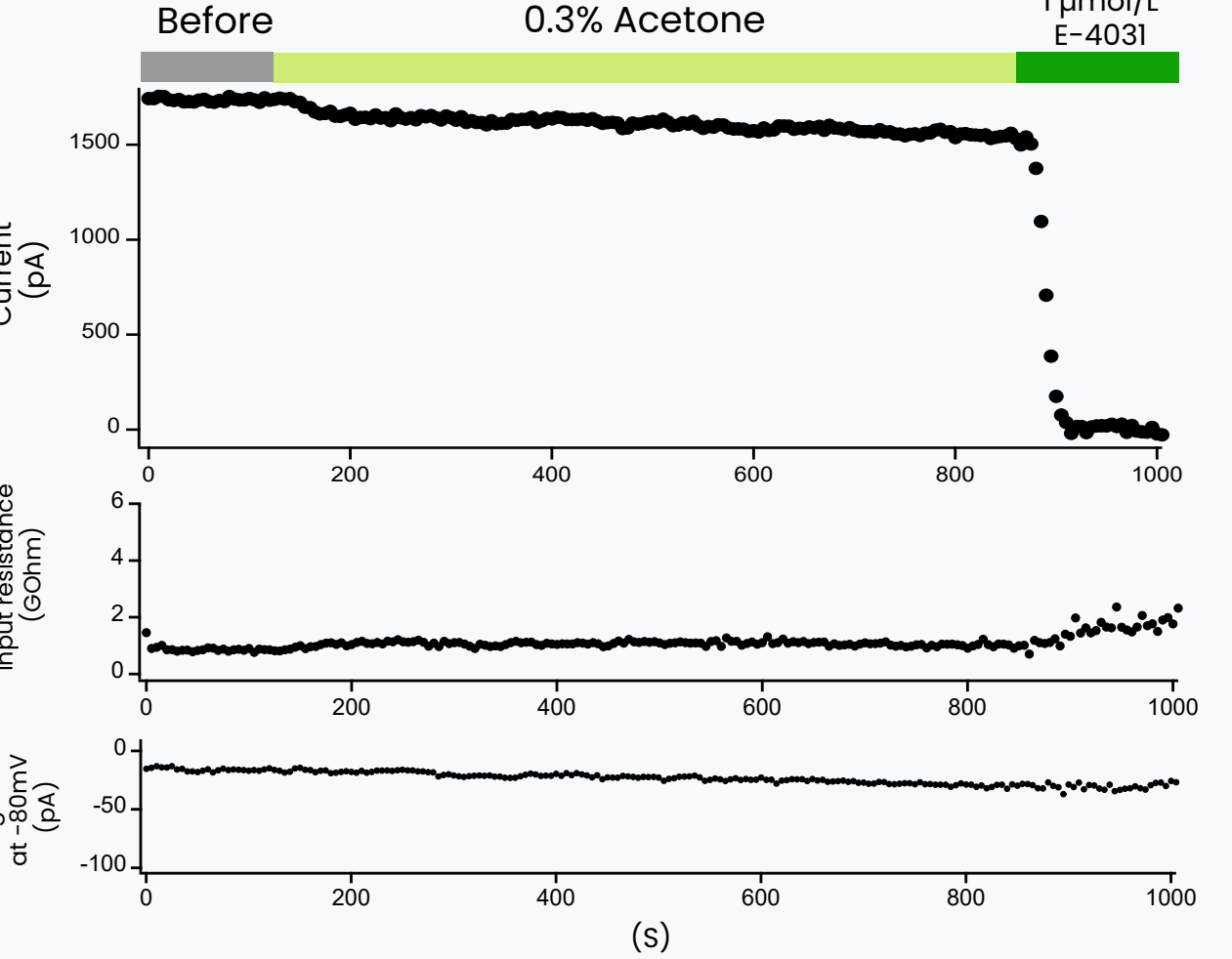
● 2-Propanol



● Acetonitrile



● Acetone



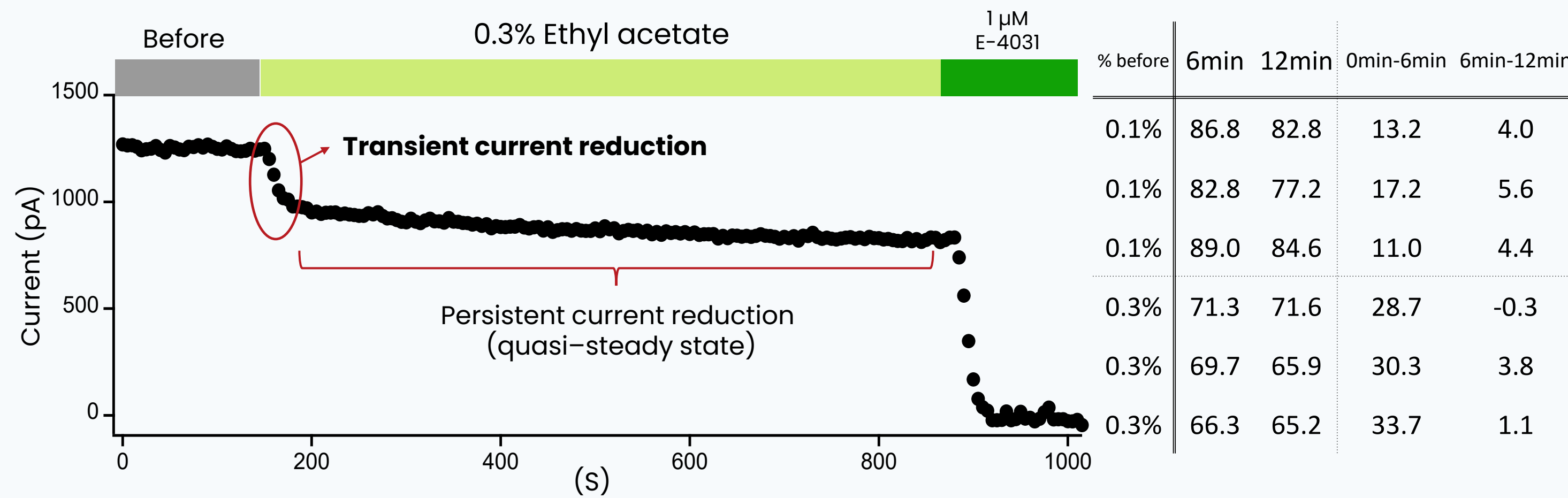
Representative plots of results with 0.3% vehicles

After 12 minutes of application, 1 μM E-4031 was applied. After recording, the current waveform was subtracted and **% before** was calculated.

% before =
(Mean Peak Value After Application /
Mean Peak Value Before Application)*100

Result 2. Assessment of vehicle correction (Comparison of IC₅₀ using moxifloxacin)

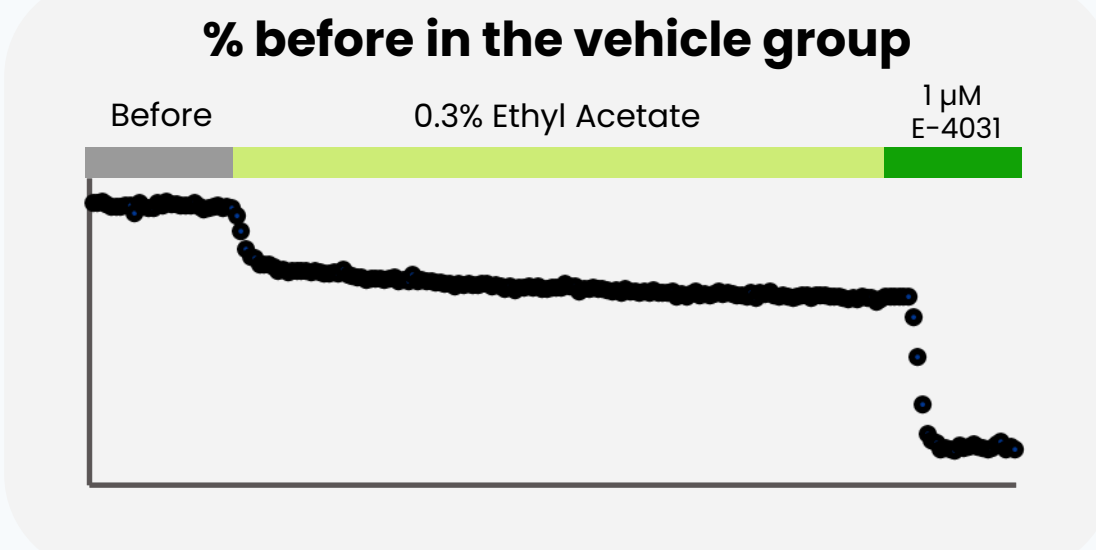
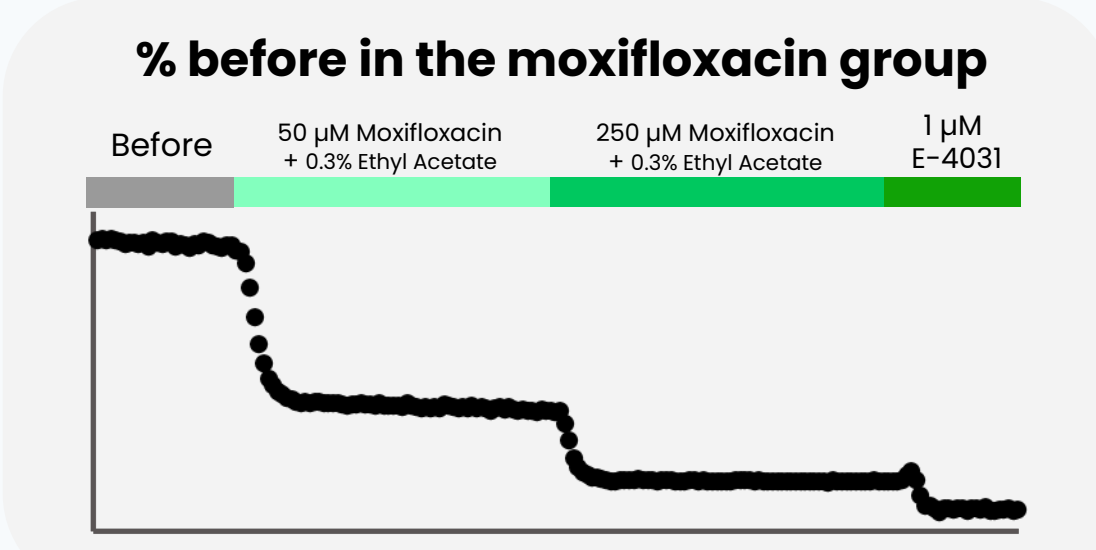
Ethyl acetate causes a strong transient reduction in hERG current



Correction 1

Inhibition (%) = 100 - (% before in the moxifloxacin group*1 / % before in the vehicle group) × 100

*1: The moxifloxacin group includes vehicle at the same concentration as in the vehicle group.

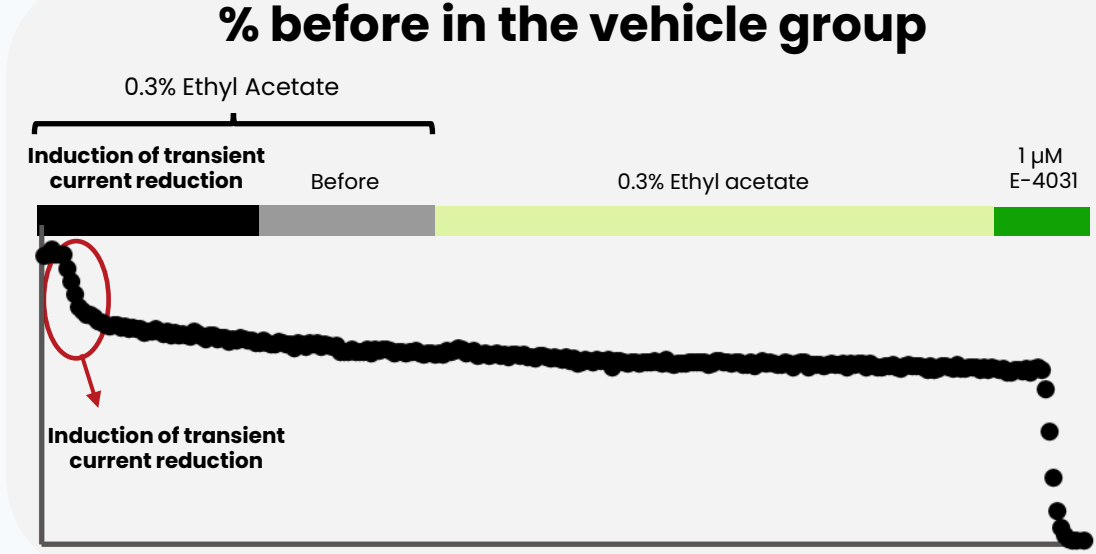
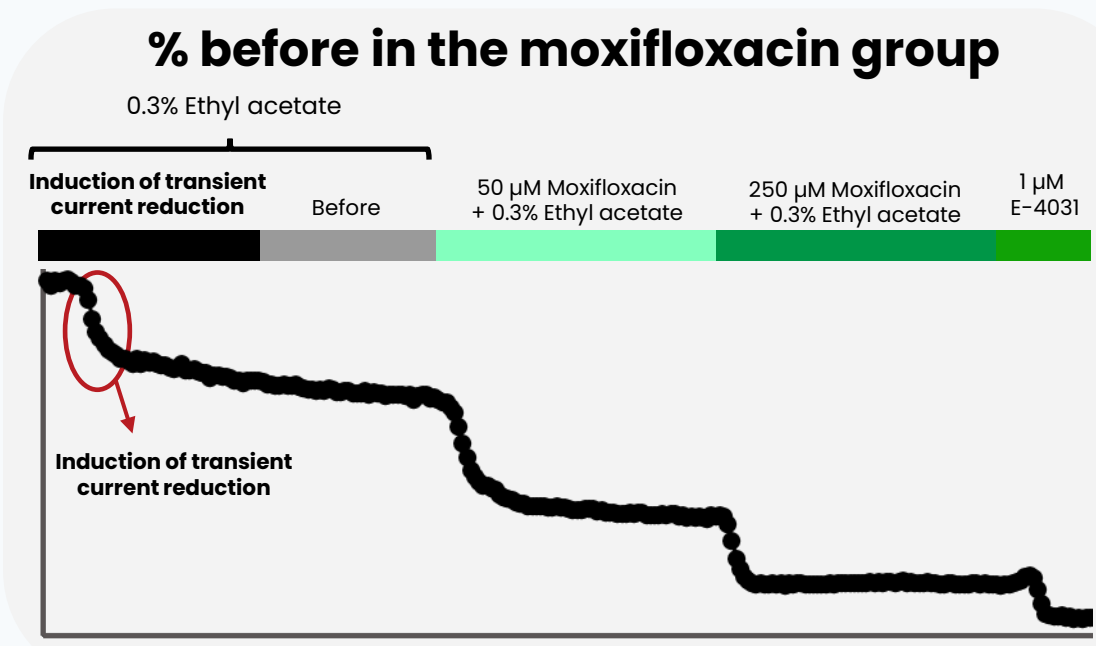


	before (pA)	6min (pA)	% before	12min (pA)	% before	w/o correction 1 inhibition(%)	Correction 1 inhibition(%)
0.3% Ethyl acetate	1633	1164	71.3	1169	71.6		
0.3% Ethyl acetate	1251	872	69.7	824	65.9		
0.3% Ethyl acetate	837	555	66.3	546	65.2		
Average			69.1		67.6		
Moxifloxacin 20μM	1596	959	60.1			39.9	13.0
Moxifloxacin 20μM	2133	1250	58.6			41.4	15.2
Moxifloxacin 20μM	1666	968	58.1			41.9	15.9
Moxifloxacin 50μM	1891	791	41.8			58.2	39.5
Moxifloxacin 50μM	1383	667	48.2			51.8	30.2
Moxifloxacin 50μM	863	359	41.6			58.4	39.8
Moxifloxacin 125μM	1596			442	27.7	72.3	59.0
Moxifloxacin 125μM	2133			533	25.0	75.0	63.0
Moxifloxacin 125μM	1666			339	20.3	79.7	69.9
Moxifloxacin 250μM	1891			331	17.5	82.5	74.1
Moxifloxacin 250μM	1383			260	18.8	81.2	72.2
Moxifloxacin 250μM	863			141	16.3	83.7	75.8

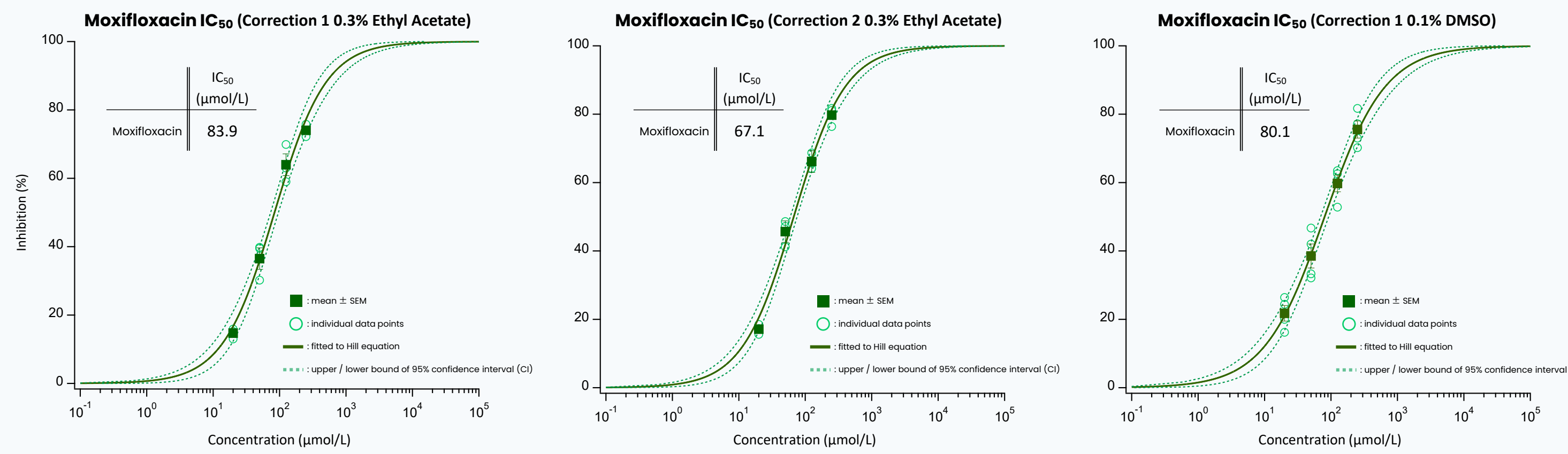
Correction 2

Inhibition(%)=100-% before*1 in the moxifloxacin group after transient current reduction/
% before for the vehicle group after transient current reduction)×100

*1: The moxifloxacin group includes vehicle at the same concentration as in the vehicle group.



	before (pA)	6min (pA)	% before	12min (pA)	% before	w/o correction 2 inhibition(%)	Correction 2 inhibition(%)
0.3% Ethyl acetate	809	745	92.1	729	90.1		
0.3% Ethyl acetate	1496	1405	93.9	1338	89.4		
0.3% Ethyl acetate	954	866	90.8	816	85.5		
Average			92.3		88.4		
Moxifloxacin 20μM	1868	1425	76.3			23.7	17.3
Moxifloxacin 20μM	1300	978	75.2			24.8	18.5
Moxifloxacin 20μM	1467	1142	77.8			22.2	15.6
Moxifloxacin 50μM	4765	2586	54.3			45.7	41.2
Moxifloxacin 50μM	1984	941	47.4			52.6	48.6
Moxifloxacin 50μM	2047	998	48.8			51.2	47.2
Moxifloxacin 125μM	1868			566	30.3	69.7	65.7
Moxifloxacin 125μM	1300			360	27.7	72.3	68.7
Moxifloxacin 125μM	1467			465	31.7	68.3	64.1
Moxifloxacin 250μM	4765			772	16.2	83.8	81.7
Moxifloxacin 250μM	1984			331	16.7	83.3	81.1
Moxifloxacin 250μM	2047			426	20.8	79.2	76.4



Moxifloxacin	0.3% Ethyl Acetate				0.1% DMSO	
	Correction 1	Correction 2	w/o correction 1	w/o correction 2	Correction 1	w/o correction 1
IC ₅₀ (μM)	83.9	67.1	33.1	55.1	80.1	76.3
95% CI	(73.7 – 95.5)	(60.0 – 75.0)	(29.1 – 37.7)	(50.4 – 60.2)	(70.0 – 91.8)	(66.7 – 87.2)
nH	1.1	1.1	0.8	1.1	1.0	0.9
95% CI	(0.9 – 1.3)	(1.0 – 1.3)	(0.7 – 0.9)	(0.9 – 1.2)	(0.8 – 1.1)	(0.8 – 1.1)

Discussion & Conclusion

Results

- In 0.1% vehicles, % before was ≥90%, except for ethyl acetate and acetone.
- In most 0.3% vehicles, % before was ≤90%.
- Vehicle-related current reduction was either transient or persistent.
- Moxifloxacin IC₅₀ differed between ethyl acetate and DMSO
- Moxifloxacin IC₅₀ for Corrections 1 and 2 with ethyl acetate (0.3%) did not differ markedly.
- Moxifloxacin IC₅₀ for Corrections 1 and 2 with ethyl acetate (0.3%) was similar to that for Correction 1 with DMSO.
- These results indicate that:
 - PG up to 0.3% and ethanol, PEG400, 2-propanol, and acetonitrile up to 0.1% did not affect hERG current.
 - Ethyl acetate and acetone were suggested to strongly affect hERG current.
 - Corrections 1 and 2 were suggested to reduce the effect of strong, vehicle-related current reduction.

The hERG assay should be conducted using vehicles with minimal effects on hERG current; however, appropriate correction was suggested to mitigate vehicle-related effects. At Mediford, in addition to DMSO, the seven vehicles reported here can be used as options for the hERG assay.