TECHNICAL NOTE

HEK-hERG Cell Line Electrophysiology

Methods: Patch pipettes were polished to obtain a tip resistance of 2-3 mΩ in the external bath solution. 200B patch clamp amplifiers (Axon Instruments) were used for voltage clamp measurements. Voltage clamp protocols were controlled via PC using pClamp9 acquisition and analysis software. To elicit hERG K^+ currents, depolarizing voltage pulses were applied to various levels, from a holding potential of –80 mV, for 3.5-sec followed by stepwise repolarization first to -40 mV and then to –120mV to measure outward and inward tail currents. Signals were analog-filtered at 2,000 Hz and sampled from 5-10,000 Hz. Whole cell capacitance (generally 10-30pF) was compensated electronically through the amplifier. Whole cell series resistance of 6-12MΩ was compensated by 75-90% through amplifier circuitry such that the voltage errors for currents of 2nA were always less than 6 mV. Only cells with seal resistances >10 mM are included for analysis. Pipette solution consisted (in mM) of KCl 126, MgSO4 2, CaCl2 0.5, EGTA 5, Mg-ATP 4, and HEPES 25 (pH 7.2, osmolality: 280±10 mM). External bath solution consisted (in mM) of NaCl 150, CaCl2 1.8, KCl 5, MgCl2 1, glucose 5, and HEPES 10 (pH7.4; osmolality: 320±10mM). Pipette offset potential in these solutions was corrected to zero just prior to gigaseal formation. Junction potential for our experimental solutions were calculated to be between 3-4mV (by pClamp 9 analysis software) and was not corrected for analysis. Experiments were conducted at room temperature, 20-22ºC.

Explanation of Figures:

Figure 1. Activation Protocols for HEK-hERG cells.

(A) Raw traces of hERG current in response to activation voltage protocol (shown below the currents).
(B) Isochronal current-voltage relationship taken 3-seconds after the start of each depolarizing pulse (as in panel A), divided by the cellular capacitance and then plotted against the depolarizing step voltage.
(C) Voltage-dependence of activation measured by plotting the current density during the -40mV repolarizing step (as in panel A) and plotted against the preceding depolarizing step voltage.

Figure 2. Deactivation and Tail current protocols for HEK-hERG cells.

(A) Raw traces of hERG current in response to activation voltage steps up to 50mV followed by repolarizing steps to various voltages (protocol is shown below the currents).
(B) Peak current density taken during the repolarizing steps plotted against the command voltage. Figures 2A & 2B show the open channel conductance at various voltages AFTER they have been activated by a strong depolarizing step (+50mV in this case). This does not mean that significant activation occurs at -70mV, rather it means that there is a conductance at -70mV after a strong depolarizing step but before the channel deactivates.

Figure 3. Effects of E-4031 (1µM) on HEK-hERG cells during Activation Protocol.

(A) Raw traces of hERG current in response to an activation voltage protocol. Small currents elicited during depolarizing step are seen but no tail currents during the repolarizing steps.
(B) Isochronal current-voltage relationship taken 3-seconds after the start of each depolarizing pulse (as in panel A), divided by the cellular capacitance and then plotted against the depolarizing step voltage.
(C) Peak current density taken during the repolarizing steps plotted against the command voltage as described in Figure 2.

Figure 4. Time course of E-4031 (100nM) use-dependent block in HEK-hERG cells.

(A) Graph shows the time course of block by 100nM E-4031. Y-axis represents the fraction of tail-current (at -40mV) remaining after application of E-4031. Data points indicate numbers of depolarizing pulses. Data is given for wild-type hERG
(B) Summary data of E-4031 from wild-type.

Notes:

1. The native outward HEK conductance is maximally ≈10pA/pF at +60mV, and often less. It is also sensitive to tetraethylammonium (TEA). The hERG outward currents within that voltage range are between 25-70pA/pF and the hERG tail current is between 100-400pA/pF outward at -40mV, and between 500-1000pA/pF inward at -120mV. Thus, the native current is not problematic provided the hERG tail currents are examined, but if one wanted the hERG to be pure, one could add a little TEA. However, under some culture conditions one can get a shift in the voltage-dependence of activation of hERG such that significant current is activated at prolonged depolarization at even -60mV. With a long pulse at -60mV there will be always be some small amount of current activated, but usually this is less than 5-10% of the maximal.

2. E4031 is well known to require a series of depolarizing pulses to achieve full block with the drug.

FIGURES

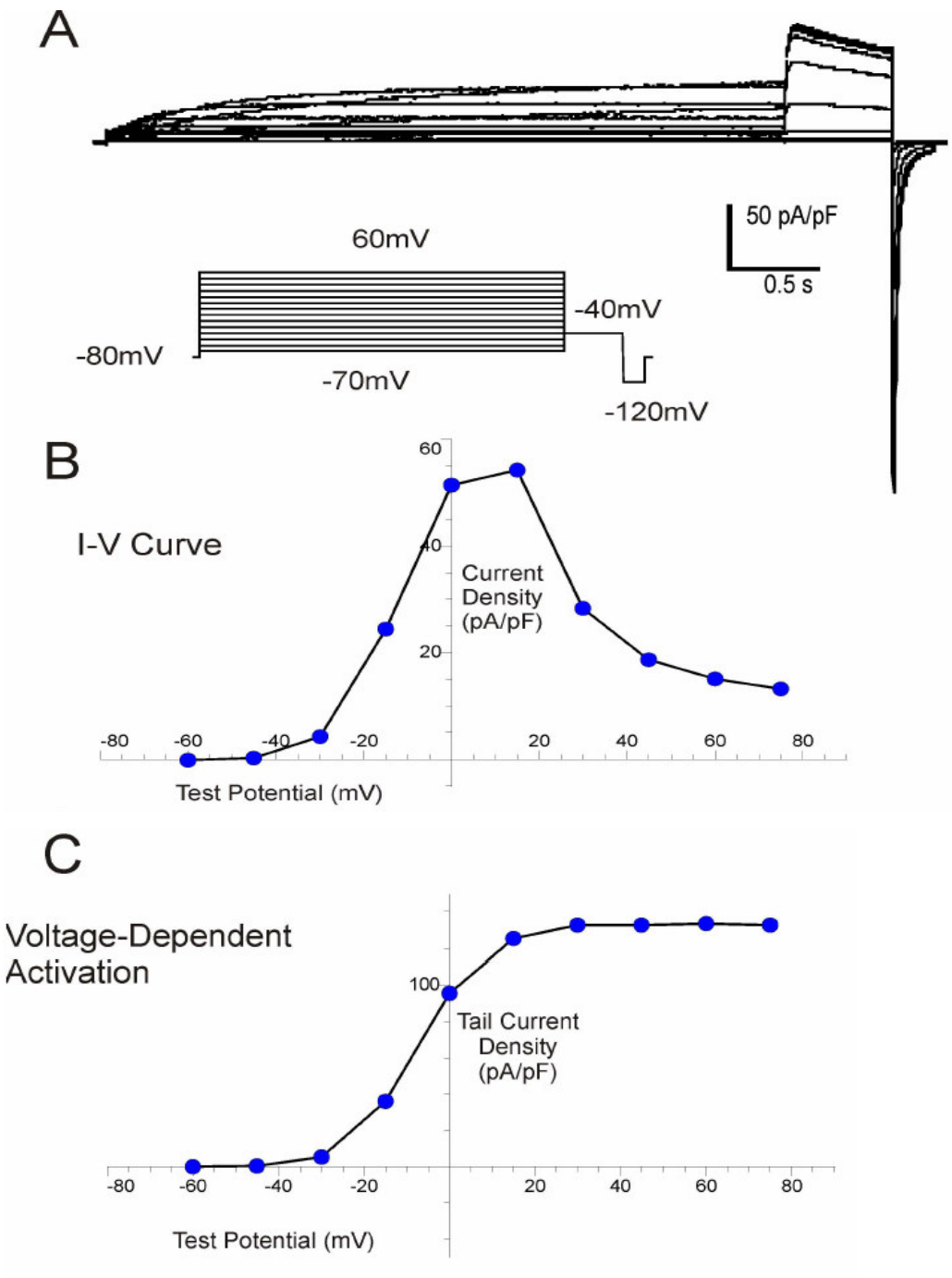

Figure 1

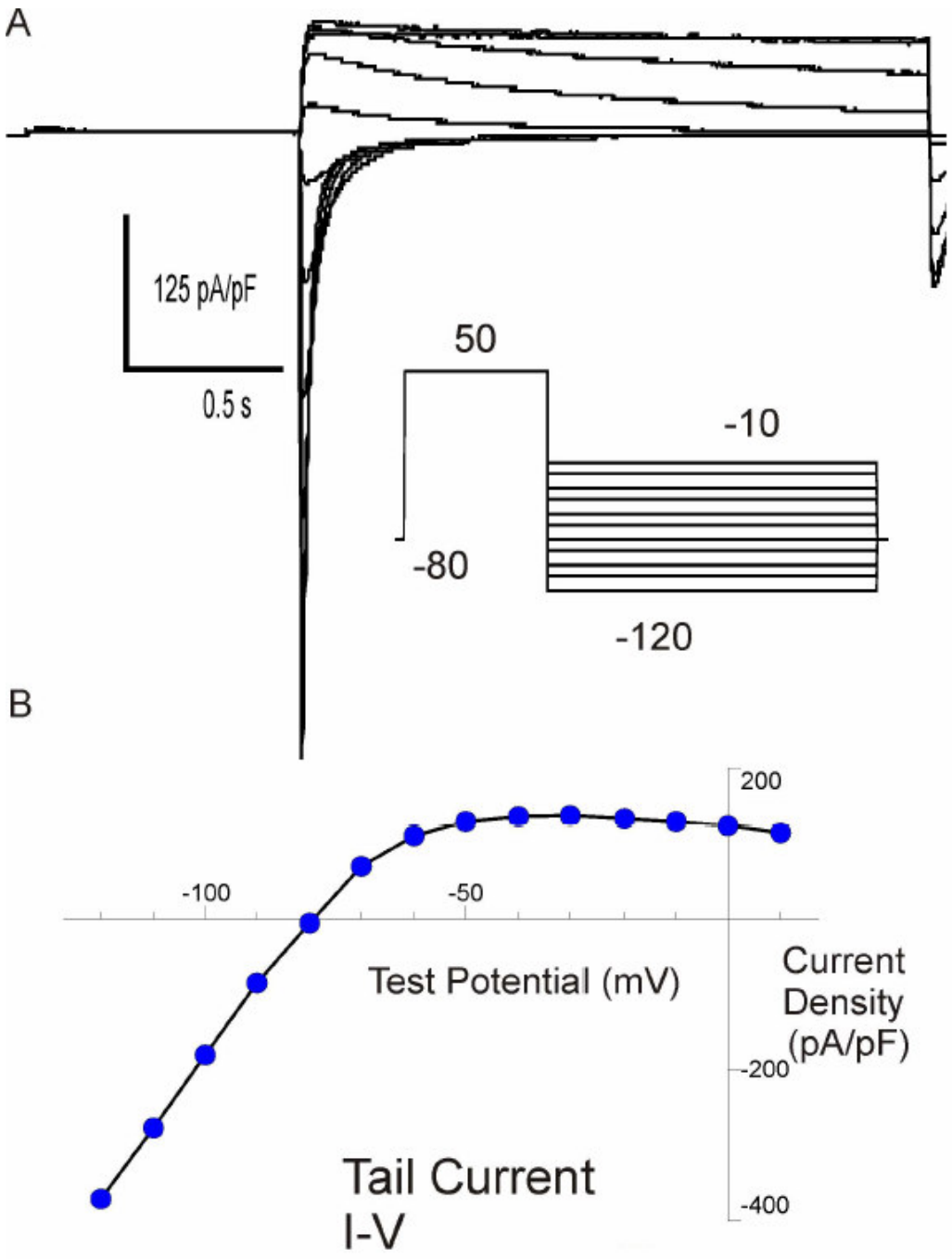
A
125 pA/pF
0.5 s
50
-10
-80
-120
B
200
-100
-50
Test Potential (mV)
Current Density (pA/pF)
-200
-400
Tail Current I-V

Figure 2

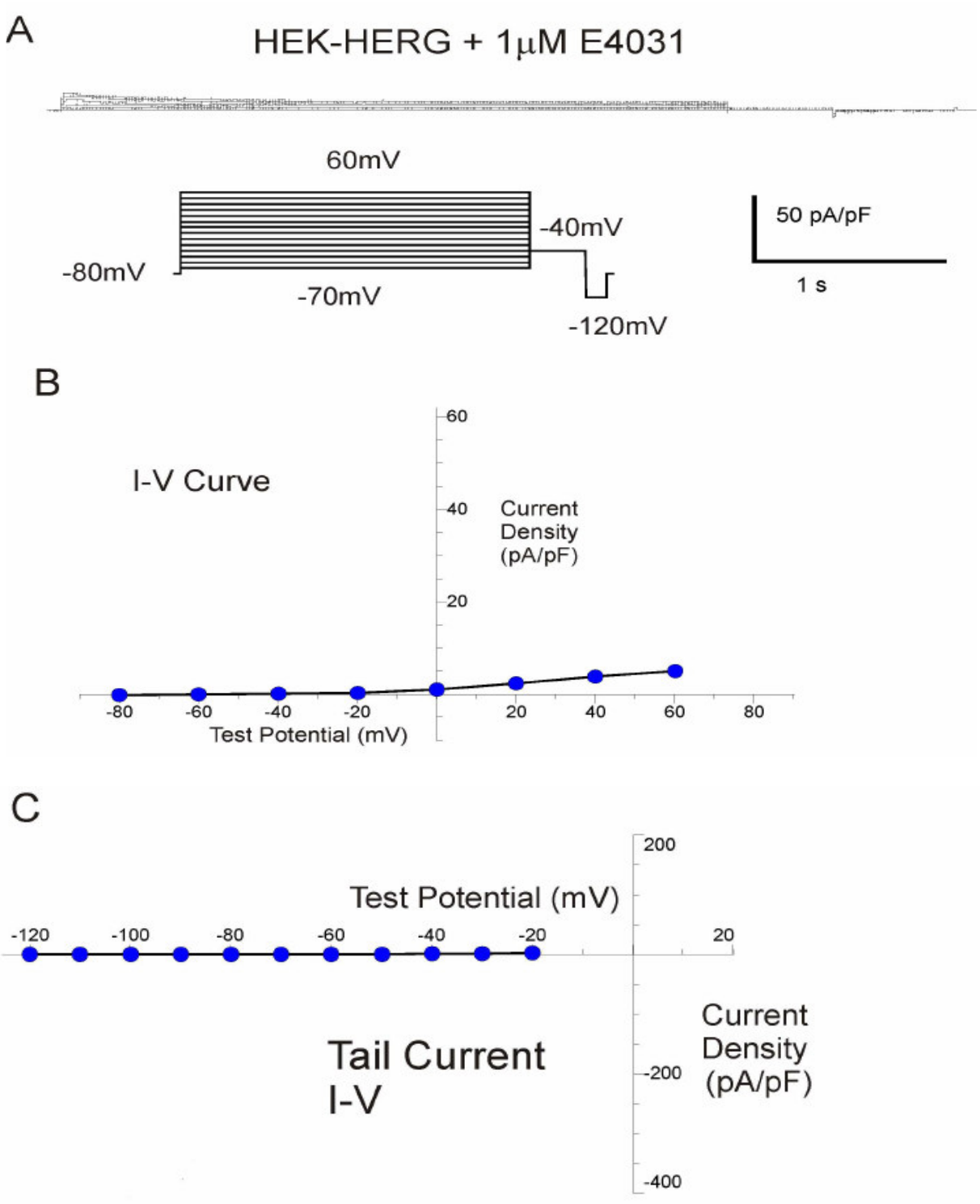
A
HEK-HERG + 1μM E4031
60mV
-40mV
-80mV
-70mV
-120mV
50 pA/pF
1 s
B
I-V Curve
60
40
20
Current
Density
(pA/pF)
-80
-60
-40
-20
20
40
60
80
Test Potential (mV)
C
200
Test Potential (mV)
-120
-100
-80
-60
-40
-20
20
Current
Density
(pA/pF)
Tail Current
I-V
-200
-400

Figure 3

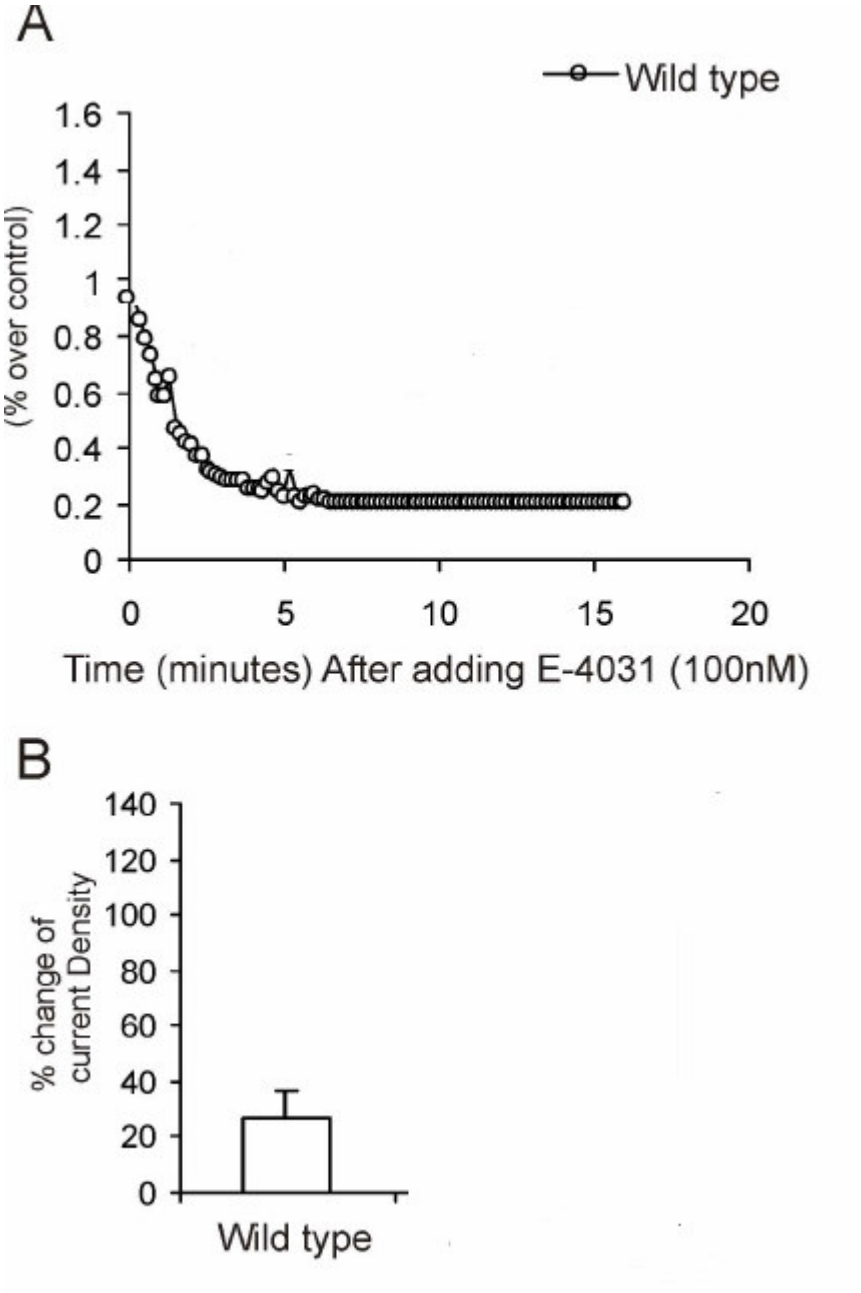
A
Wild type
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(% over control)
0
5
10
15
20
Time (minutes) After adding E-4031 (100nM)
B
140
120
100
80
60
40
20
0
% change of current Density
Wild type

Figure 4