hERG drug application comparison

Reliable drug delivery and increased throughput

Ion channel: hERG

Cell type: HEK293

Chip type: DF-8 Pro II

Data and report courtesy of Gedeon Richter Ltd., Budapest, Hungary

Methods

A comparative study of Dynaflow and conventional whole-cell patch clamp methods on hERG ion channels was performed. In the conventional patch clamp set up a pressure driven multi-barreled ejection pipette was used to apply compounds (WPI Inc.). In the Dynaflow experiments the DF-8 Pro II microfluidic chip was used for compound application.

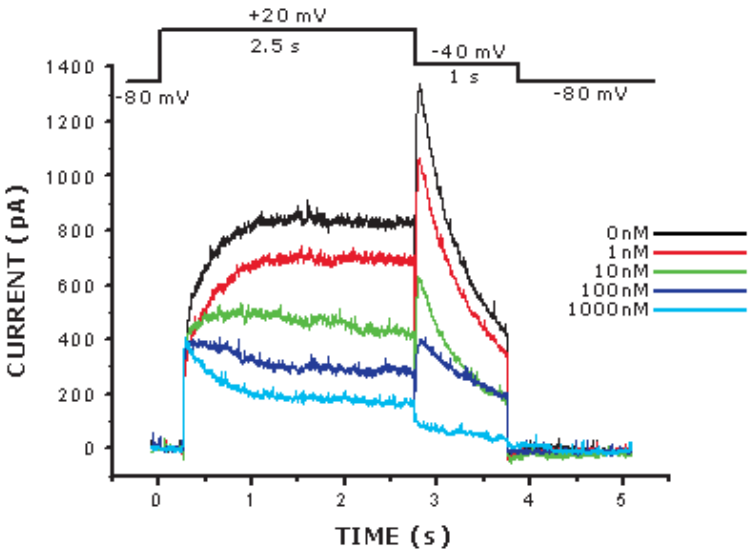
Results

Most of the cells showed outward potassium current in response to a +20 mV command step followed by a return potential of –40 mV **(Fig. 1)**. The average maximum peak amplitude of the tail current evoked by the return potential of –40 mV was 804±91 pA (*n*=42) in the conventional patch clamp studies, and 256±24 pA (*n*=40) in the Dynaflow experiments.

Dofetilide dose dependently inhibited the hERG current in the nanomolar range **(Fig. 1 and 2).** Mean inhibition values for various concentrations (pooled data from different experiments) were used for the concentration-response curve and calculated IC_{50} value. The IC_{50} values of 14.5 and 22.5 nM were obtained for dofetilide in conventional and Dynaflow patch clamp experiments respectively. The dose-response curves obtained with the two methods do not show a statistically significant difference.

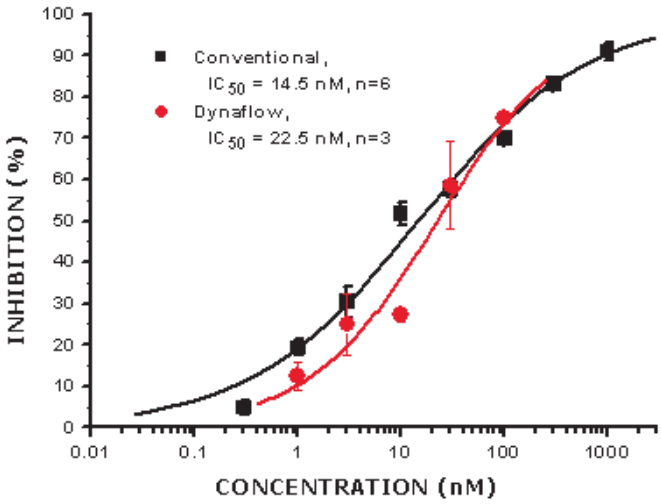
The effect on hERG channels of six Richter compounds was also tested in order to compare Dynaflow and conventional whole-cell patch clamp methods [1]. These compounds were tested at 3 µM, and the percentage inhibition of hERG tail current was calculated. No significant difference was seen with any of the tested compounds regarding the inhibition values determined by using the two methods **(Fig 3)**.

Figure 1



Representative current traces from an experiment. Traces from top to bottom were recorded in the presence of 0, 1, 10, 100, and 1000 nM dofetilide. The applied voltage command protocol is shown above the traces.

Figure 2



Concentration dependent inhibition of hERG potassium current by dofetilide determined by the two methods. Data are presented as mean ± sem.

Results (cont.)

The inhibition of current exerted by Richter compounds when studied with the Dynaflow system developed rapidly. Compound 'F' (3 µM) needed two seconds to evolve its maximum effect **(Fig. 4)**. The washout of the drug was complete and fast taking 10-20 seconds. To illustrate the time-course effect of this compound, the peaks of the tail current were plotted against time in **Fig. 5**. The drug effect was slower when the conventional system was used, and, in several cases, the washout was incomplete. The recording times for individual cells were longer with Dynaflow, on average. In general, the drug application in the Dynaflow system proved to be much more reliable then in the conventional system.

Discussion

The peak current amplitudes were significantly higher in the conventional patch clamp experiments than in the Dynaflow experiments. This finding may be explained by a selective loss of large-diameter cells during the cell suspension procedure used prior to running Dynaflow. However, the low electrical noise-level in the Dynaflow experiments still allowed reliable recordings suitable for studying drug effect. The IC_{50} values for dofetilide were in good agreement with data from other laboratories working under similar experimental conditions [2, 3]. There was no significant statistical difference (by two-way ANOVA) in concentration-response relationships of dofetilide when determined using conventional and Dynaflow patch clamp methods. The experiments with the six Richter compounds bolster that using the Dynaflow system yields practically the same pharmacological results as the conventional system. Drug application is more reliable and the fluid exchange is much faster when the Dynaflow system is used, which results in an increased throughput of drug testing experiments.

References

1. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers.Arch.* 1981; **391**:85-100.

2. Rampe D, Roy ML, Dennis A, Brown AM. A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett.* 1997; **417**:28-32.

3. Snyders DJ, Chaudhary A. High affinity open channel block by dofetilide of HERG expressed in a human cell line. *Mol.Pharmacol.* 1996; **49**:949-955.

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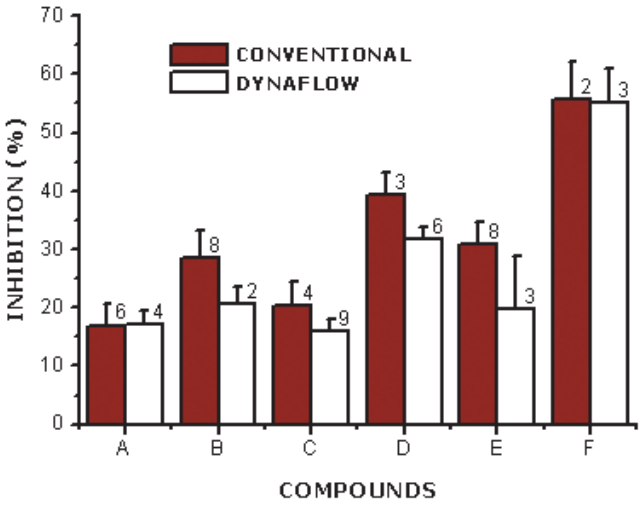
Figure 3



Percent inhibition values of different compounds yielded by the two methods.

Figure 4

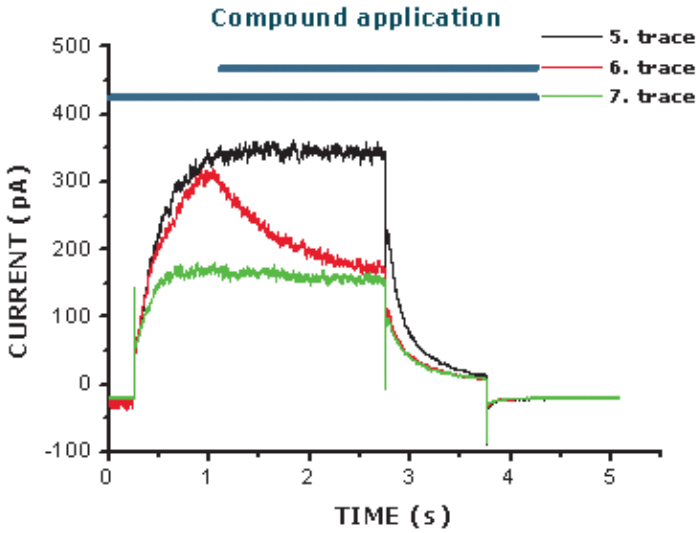
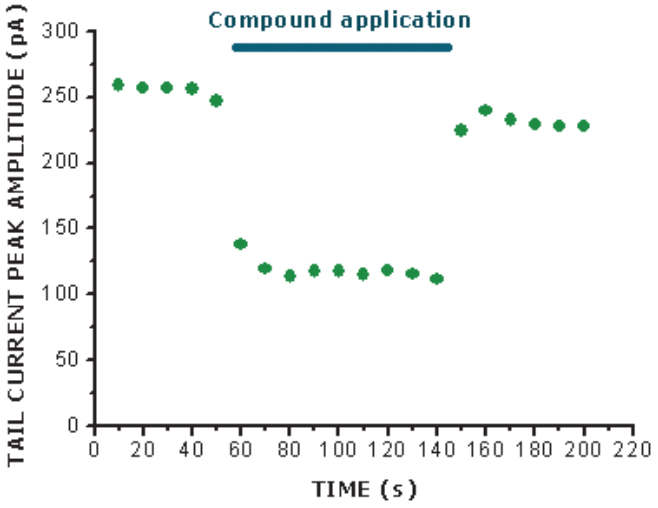


Illustration of rapid onset of drug effect, current traces from a representative experiment when the Dynaflow system was used. Traces from top to bottom were recorded right before (trace 5), at the start of application (trace 6) and in the continuous presence (trace 7) of a Richter compound as shown by the bars, representing drug application.

Figure 5



Time course of the effect of a Richter compound on hERG current when the Dynaflow system was used. Peaks of tail currents (traces 5, 6 and 7 are shown in Fig. 4) were plotted against time.

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