



A novel mechanism of hERG potassium ion channel blockade: implications for drug design?

Department of Pharmacology and Toxicology

Archis Bagati, Satpal Singh

ABSTRACT

Background:

The hERG (human Ether-à-go-go Related Gene) potassium ion channel is extremely critical for maintaining a normal heartbeat in humans. The inhibition of this channel by drugs may lead to cardiac arrhythmias resulting in sudden cardiac death. Therefore the FDA mandates a discontinuation of any drug in the development process that inhibits the hERG channel. As a consequence however, the mandate now accounts for over 30% of novel compound discontinuations.

Two key amino acid residues located intracellularly, a phenylalanine at position 656 (F656) and a tyrosine at position 652 (Y652) have been shown to be very important for binding of all drugs to the channel.

Significance:

This study indicates the presence of a potentially novel extracellular/alternative intracellular drug binding site, apart from Y652 and F656.

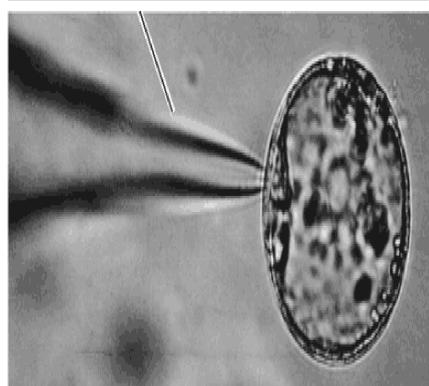
Preliminary results have demonstrated that the inhibition of the hERG channel by Celecoxib, an analgesic prescribed for arthritis and acute pain, is independent of both these binding sites, a first observation of its kind.

An increased understanding / identification of any novel molecular determinants of blockade of the hERG channel would allow for the refinement of the drug design process to bypass any interactions with the hERG channel. Thereby minimizing drug related cardiotoxicity (arrhythmia) and minimizing drug developmental costs.

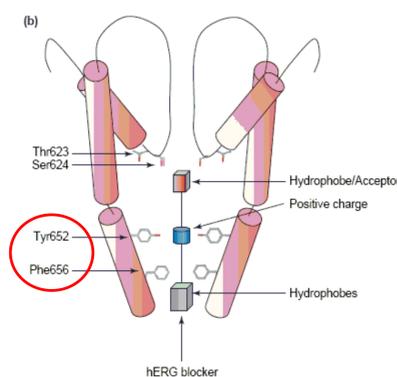
Study Goals: Determine-

1. the nature of inhibition of hERG by Celecoxib?
2. the key molecular determinants mediating the inhibition? (Drug binding site?)

Patch Clamping



hERG K⁺ channel



METHODS

1. Record currents from cells expressing hERG K⁺ channels: **Control conditions**
2. Add drug (Celecoxib)
3. Record currents in the presence of drug: **with Drug**

Goal #1: Nature of inhibition ?

Biophysical Properties:

4. Assess changes in current kinetics
5. Determine implications for drug block

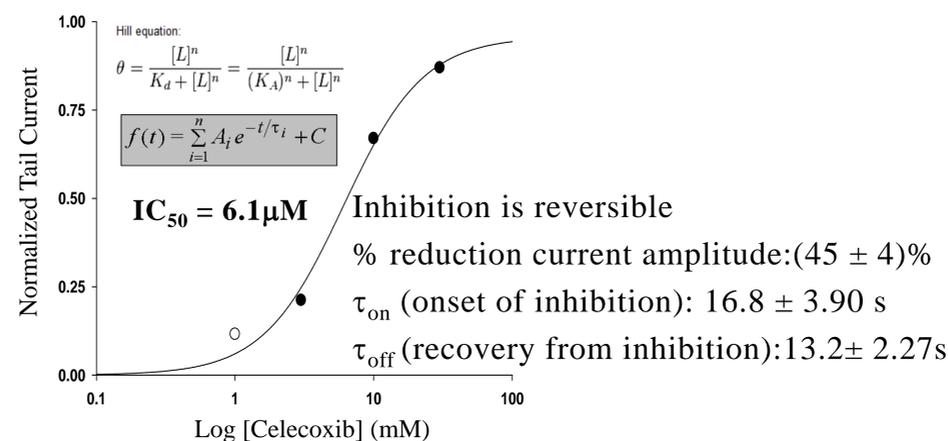
Goal #2: Drug binding site ?

Mutagenesis Approach:

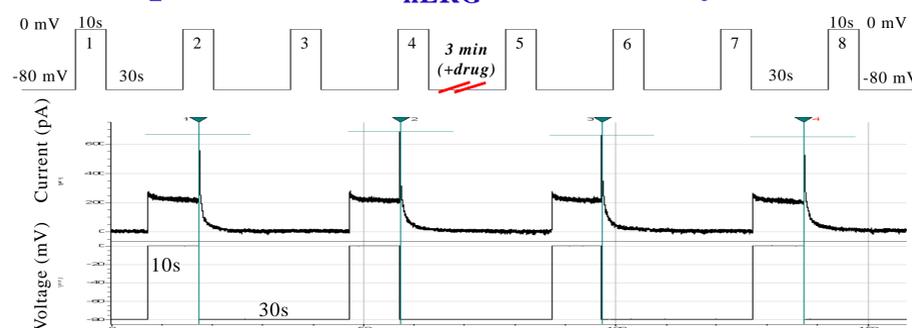
1. Mutate key amino acid residues (**Tyr652 and Phe656**) known to be important for the binding of several drugs to the hERG channel.
2. Determine if either mutation changes the inhibition profile of celecoxib.
3. Determine whether the binding site is intracellular or extracellular?

RESULTS

Concentration-Response Curve



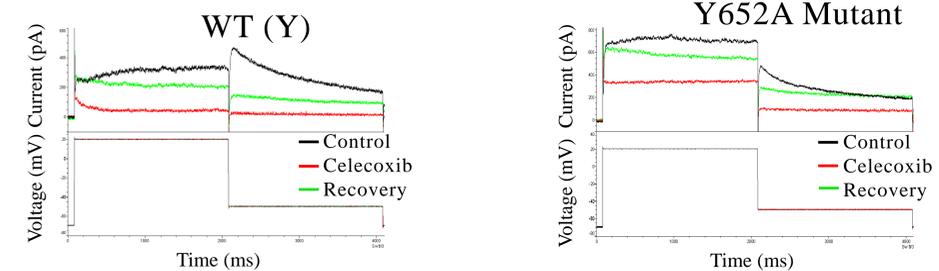
State Dependence of I_{hERG} blockade by Celecoxib



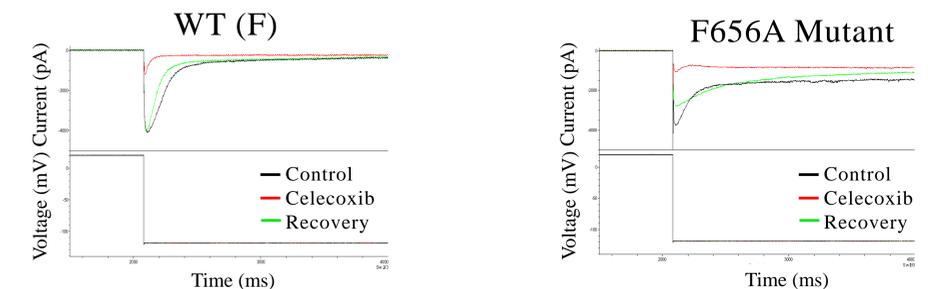
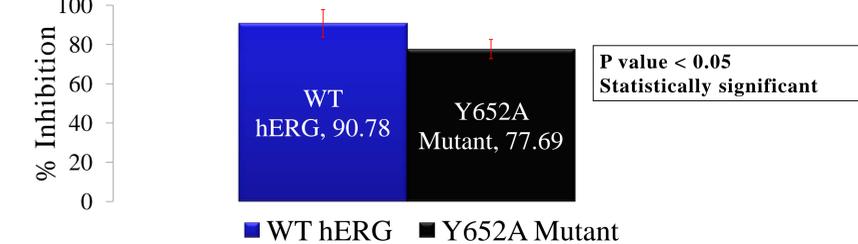
No increase in inhibition: No Open Channel Block

No relief of inhibition: No Closed Channel Block

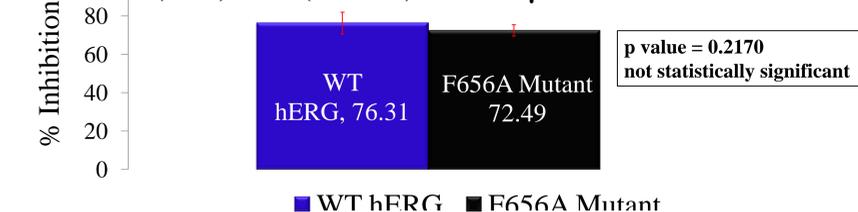
Mutant Studies



(WT) and (Y652A) with 10mM Celecoxib



(WT) and (F656A) with 30μM Celecoxib



CONCLUSIONS

Biophysical characterization

1. Celecoxib inhibits the hERG channel in a reversible concentration dependent manner with an IC₅₀ = 6.1 μM
2. No open channel or closed channel block; possible gating modifications

Mutant Studies

1. % Inhibition:
 - WT (Y652A) = $90.78 \pm 6.99\%$
 - Y652A = $77.69 \pm 4.87\%$
 - WT (F656A) = $76.31 \pm 5.66\%$
 - F656A = $72.49 \pm 2.93\%$
2. No significant attenuation in blockade in the absence of the (Y652) or (F656)
3. Celecoxib does not mediate its inhibition via Y652 or F656