Effects of novel urea analogues of NS1643 on potassium and calcium fluxes in cardiac muscle cells

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NS1643 is a commercially available urea compound that enhances IK_r current in hERG transfected *Xenopus laevis* oocytes at concentrations ranging from 3 to 30μM (Casis *et al.*, 2006). It is a "type 1" HERG channel partial agonist binding to residue Y652A on the α S6 subunit of the HERG channel (Grunnet *et al.*, 2011). Several analogues have been synthesized to investigate their effects on ion currents and contractility in cardiac muscle. Here we report the effects of two novel urea compounds LTUJH47B and LTUJH51B (Heppell and Al-Rawi, 2015).

Sheep hearts were purchased from Hardwick's Abattoirs (Kyneton, Victoria), transported back to the laboratory on ice and the right ventricle was enzymatically digested to produce single cardiac cells. Changes in potassium fluxes were investigated using the FluxOR assay (Life Technologies) on isolated sheep cardiomyocytes dispensed (10^4 cells per well) into 96-well micro-array plates and the resultant fluorescence was read using a Flexstation 3 (Molecular Devices). The FluxOR assay uses thallium as an indicator activating a fluorescent dye that has been loaded into the cells. When potassium channels are opened fluorescence increases. In potassium negative stimulus buffer, no current is expected while in potassium positive stimulus buffer, a depolarization step is introduced that will open hERG channels and fluorescence is observed.

When isolated sheep cardiomyocytes were exposed to 10μM NS1643 we confirmed that hERG channels were opened with increased fluorescence in the potassium positive conditions. In the presence of 10μM LTUJH47B and LTUJH51B a smaller current was observed in the potassium containing stimulus buffer, but there was also an increase in the potassium negative stimulus conditions. This suggested that another current was being activated by these novel compounds. To test whether a calcium current was being activated sheep cardiomyocytes were loaded with calcium sensitive probe Fluo4AM (Life Technologies) and using the same buffering conditions in the FluxOR assay for depolarization including the addition of 1mM calcium chloride. In the potassium free conditions there was a significant increase in fluorescence in the presence of LTUJH51B but not with LTUJH47B. In the potassium positive conditions there was no increase in fluorescence associated with calcium. These results suggest that LTUJH51B may be opening voltage dependent calcium channels in sheep cardiomyocytes.

Finally using isolated snail hearts (*Helix aspersa*) mounted onto an isometric force recording apparatus (PowerLab, AD Instruments), changes in heart rate and contractility were recorded. NS1643 had little effect on spontaneous heart rate or force production ($CaCl_2$ range 0 to 10mM). Both LTUJH47B and LTUJH51B decreased heart rate by 18% at 100μM but only LTUJH51B increased control force by 142%. It appears that both of these novel compounds exhibit weak hERG channel activity and LTUJH51B increases calcium fluxes in cardiac muscle.

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