Integration of Functional and Biochemical Biomarkers for Verapamil-Induced Cardiac Dysfunction

ABSTRACT #0172
POSTER #0107

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ABSTRACT

Voltage-gated calcium channels have a significant role in the physiologic function of the heart, affecting both the electrical and mechanical processes. Blockade of the L-type Ca channel in the heart has been associated with cardiac dysfunction. We performed experiments utilizing isolated perfused rat hearts and isolated cardiac myocytes to investigate the effects of verapamil on several functional and biochemical cardiac biomarkers. Verapamil (0.001- 0.1μM) was perfused for 20 minutes at each concentration in isolated rat hearts. 0.1μM was perfused through four hearts positioned within a ^{31}P NMR for 60 minutes measuring contractility, developed pressure, ATP, phosphocreatine (PCr) and inorganic phosphate (Pi). The L-type calcium current was measured in acutely isolated cardiac myocytes over a range of 0.001-100μM. In isolated hearts, 0.01 μM verapamil was the threshold concentration for LV dP/dt_{max} and DP reductions with only slightly more observed at 0.1 μM. In isolated myocytes, 0.01μM to 0.1 μM was associated with a ~12-20% reduction in calcium current amplitude. Isolated hearts perfused with 0.1 μM verapamil within the NMR decreased in dP/dt_{max} and DP with little or no change in ATP or PCr. However, Pi began to decrease and saturated on a timeframe concomitant with the functional changes. In conclusion, we evaluated several functional and biochemical biomarkers of cardiac activity in the presence and absence of verapamil. Blockade of the L-type calcium current provides a molecular mechanism for the observed functional changes with a decrease in Pi resulting from a reduced myocardium workload and subsequent need for energy metabolism.

INTRODUCTION

Voltage-gated calcium channels have a significant role in the physiologic function of the heart, affecting both the electrical and mechanical processes. Blockade of the L-type Ca channel in heart has been associated with negative inotropic effects, heart rate reduction and AV conduction disturbances. Verapamil, a phenylalkylamine, has a high affinity for heart tissue, specifically the nodal cells (sinoatrial and atrioventricular) that are rich in calcium channels, and is used as a class IV antiarrhythmic. Calcium channels are also present in smooth muscle, therefore verapamil acts on the smooth muscle resulting in vasodilation of the blood vessels.

We performed a series of experiments to determine the effects of verapamil on several functional and biochemical cardiac biomarkers utilizing isolated perfused rat hearts and isolated cardiac myocytes. Additionally, the effects of verapamil were characterized in isolated cardiac myocytes to define a molecular mechanism for the observed functional effects.

METHODS

Ex-Vivo Studies

This ex-vivo studies were conducted after approval of the Institutional Animal Care and Use Committee (IACUC) of Battelle and in compliance with USDA regulations.

Twenty-five male Sprague Dawley rats were anesthetized and hearts were quickly removed and submerged in 50mL chilled cardioplegic solution. Hearts were secured to the cannula of the emka© isolated heart apparatus via suture and then reanimated (after a 10 minute arrested state) by retrograde perfusion of modified Krebs solution at 37°C. A fluid-filled balloon attached to a pressure transducer was inserted into the left ventricle and slowly inflated to a preload of approximately 8-10 mmHg.

Fourteen hearts were equilibrated to the Krebs solution perfusion for 20 minutes and then Krebs containing 0.1% DMSO (vehicle) for an additional 20 minutes. Baseline parameters of contractility (dP/dt max and min, left-ventricular developed pressure, end diastolic pressure), heart rate (HR) and coronary flow parameters were collected for at least 20 minutes following equilibrium. Five hearts were then perfused with 5 increasing concentrations of verapamil in consecutive 20 minute durations and nine hearts were perfused with vehicle as time matched controls.

Whole heart ^{31}P content was assessed by inserting the perfused isolated heart (n=11), prepared as described above in heart preparation, in a proton/phosphorus dual tune volume RF coil (30 mm diameter and 50 mm length) and placed into an 11.7T wide bore vertical superconducting magnetic. ^{31}P frequency was 81.01 MHz as determined by using a positive control sample of ATP dissolved in krebs at pH 7.4. First and second order shims where optimized using the proton signal arising from the water at 500.1 MHz. Then, ^{31}P spectra at 202.4 MHz were acquired every four minutes for 60 minutes post dose (plus 20 minutes for isoproterenol challenge). Each spectrum is the average of 480 acquisitions. Acquisitions were collected every 500 ms after the transmission of a 50 μs square RF pulse. Peak height before and after drug exposure was used to determine changes in inorganic phosphate (Pi), phosphocreatine (PCr), and adenosine triphosphate (ATPγ). Hearts were perfused with vehicle (n=6) for time matched control or 0.1 μM verapamil (n=5) for 60 minutes. After 60 minutes, all hearts were perfused with 0.1 μM isoproterenol for 20 minutes.

In-Vitro Studies

The details of the experimental method have been described previously (Roca et al., *Ped Res*, '96 40:462-8). Myocytes were isolated from right atrial specimens obtained from patients undergoing cardiopulmonary bypass surgery. Currents were measured using the whole-cell variant of the patch clamp method (36 ± 1ºC). The L-type Ca current was elicited by a pulse to 0mV (200ms) from a holding potential of –70mV. Cells were exposed to a concentration range of 0.001-100 μM.

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RESULTS

During the dose escalation study performed on isolated perfused rat hearts, verapamil decreased left ventricular dP/dt_{max} and developed pressure starting with perfusion of 0.01 µM with greater decreases for 0.1 and 1 µM as compared to controls (Figures 1 – 5). In isolated cardiac myocytes, 0.01 to 0.1 µM was associated with a ~12 to 20 % reduction in calcium current amplitude (IC_{50} = 1.2 µM) (n=4-6).

Based on the heart's functional response during the escalating dose study, 0.1 µM was chosen as the concentration of verapamil to perfuse for 60 minutes while collecting whole heart energetics via NMR. Isolated hearts perfused with 0.1 µM verapamil within the NMR decreased dP/dt_{max} and developed pressure within the first 10 minutes of perfusion and maintained for the remaining 50 minutes (Figures 6 and 7). There was little or no change in ATP or PCr occurring when dP/dt_{max} decreased or for the remaining 50 minutes of observation (Figures 8 and 9). However, Pi began to decrease and saturated on a timeframe concomitant with the functional changes (Figure 10).

Over a therapeutic concentration range, addition of verapamil was associated with reductions in developed pressure (dp) and dP/dt_{max} (dP max), and the L-type Ca current (Figure 11).

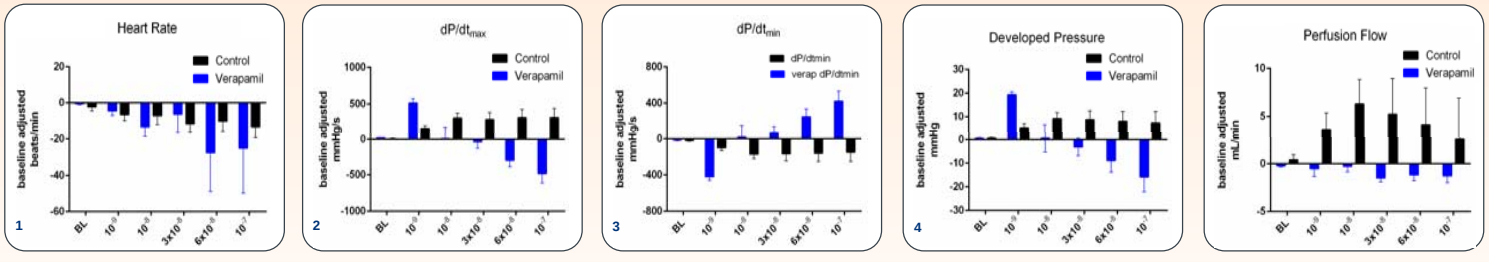


Figure 1 – 5. The effects of escalating doses of verapamil on heart rate, dP/dt_{max}, dP/dt_{min}, developed pressure (DP) and flow in isolated perfused rat hearts.

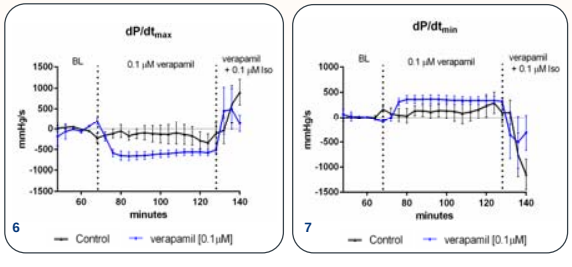
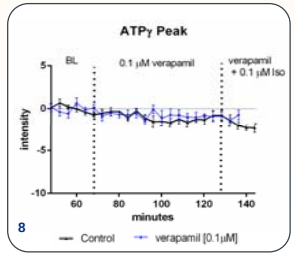
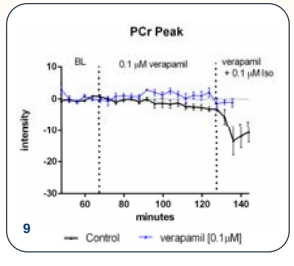


Figure 6 – 7. Effects of 0.1 µM verapamil perfused for 60 minutes on left ventricular function in isolated rat hearts while positioned within NMR.





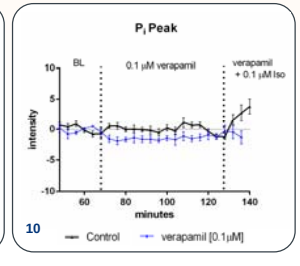


Figure 8 – 10. Effects of 0.1 µM verapamil perfused for 60 minutes on whole heart energetics in isolated rat hearts while positioned within NMR.

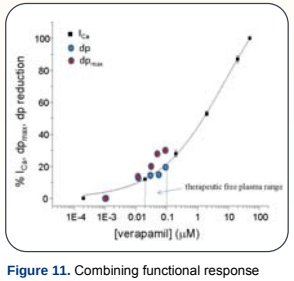


Figure 11. Combining functional response with L-type Ca block.

CONCLUSION

In conclusion, we evaluated several functional and biochemical biomarkers of cardiac activity in the presence and absence of verapamil. Blockade of the L-type Ca current in cardiac myocytes provides a molecular mechanism for the observed functional changes with a decrease in inorganic phosphate (Pi) resulting from a reduced myocardium workload and subsequent reduced need for energy metabolism.