The causes and the modulators of short term variability in ventricular repolarization

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Although short term beat-to-beat variability of action potential duration (SBVR) is considered as a predictor of imminent cardiac arrhythmias, the underlying mechanisms were not clear.
The specific aim of our work was to explore the causes and the modulators of SBVR. We supposed that the mechanism of SBVR was multifactorial. The action potential duration of canine ventricular cells were lengthened and shortened in different ways and their effects on SBVR were studied. The role of alteration of intracellular calcium concentration was also investigated on the development of SBVR. To explore the possible role of electrotonic interactions among the cardiac cells in short term variability in ventricular repolarization, action potential were measured on multicellular cardiac preparations as well.

Experiments were carried out on single cell and multicellular mammalian ventricular preparations. Sharp glass microelectrodes filled with 3 M KCl were used to record transmembrane potential. The action potential duration at 90% repolarization (APD) were used to draw Poincaré plots and calculate SBVR.

Our results show that APD of midmyocardial (MID) cells were the longest while the shortest APD values were obtained on subepicardial (EPI) cells. SBVR was the greatest in MID cells, smallest in EPI cells, and had an intermediate level in subendocardial (ENDO) myocytes. These transmural differences in APD and SBVR were statistically significant. When the transient outward potassium current (I_{to1}) of EPI cells was suppressed by 4-aminopyridine, their action potential became similar to the action potential of ENDO cells. The spike-and-dome action potential morphology disappeared but SBVR decreased slightly. This suggests that transmural differences in APD and SBVR are not related to the spike-and-dome configuration of action potentials observed in EPI or MID cells.

We determined the relationship between SBVR and APD. APD was modified by inward and outward current injections. Our results show that SBVR is an intrinsic property of mammalian myocardium, and SBVR is an exponential function of APD. This exponential relationship refers to our standard curve. On the basis of this observation we propose the concept of relative SBVR, where changes in SBVR are normalized to changes in APD (ΔSBVR/ΔAPD), namely any change in SBVR and APD caused by the different experimental conditions is compared to our control SBVR-APD curve.

Relative SBVR was increased by nisoldipine, HMR 1556, dofetilide, 9-anthracene carboxylic acid and veratridine. These molecules are the specific blockers of the L-type Ca^{2+} current ($I_{Ca\text{-}L}$), the slow (I_{Ks}) and rapid delayed rectifier (I_{Kr}) potassium currents; calcium-activated chloride current ($I_{Cl(Ca)}$) respectively, while the veratrdine is the activator of sodium current (I_{Na}).
SBVR was reduced by BAY K8644 (enhancement of $I_{Ca\text{-}L}$), tetrodotoxin, lidocaine (block of I_{Na}), chromanol 293B in the presence of HMR 1556 (relatively selective blockade of I_{to1})
These data proves that ionic conductance which increases the membrane current (I_{net}), determined from the action potential waveform at the middle of the plateau stabilizes SBVR, therefore SBVR is inversely proportional to I_{net}.
The action potential duration was decreased by activation of the ATP-sensitive K+ current (IK-ATP) using the various concentrations of lemakalim, and it was lengthened by $BaCl_2$ which blocks I_{K1}. Lemakalim decreased both SBVR and APD, while $BaCl_2$ increased both of these parameters. The data points obtained in the presence of lemakalim and $BaCl_2$ remained on the standard APD-SBVR curve. This indicates that in contrast to the above mentioned ionic currents I_{K1} and I_{KATP} have little specific influence on SBVR.

Similar results were obtained by computational modeling study. An experimentally-calibrated stochastic canine action potential model was used. This is based on the model of Decker et al. (Decker et al. Am J Physiol Heart Circ Physiol 2009; 296, 1017–1026.) and our experimentally recorded canine ventricular action potentials. The model of Decker is the most up-to date electrophysiological model of the AP in a canine ventricular myocyte.
The contribution of four main ionic currents (I_{Kr}, I_{Ks}, I_{to1}, $I_{Ca\text{-}L}$) to SBVR was investigated in control condition and after pharmacological ionic channel inhibition.
The computational model shows that fluctuations in these four currents reproduce a large part of SBVR measured in canine ventricular myocytes and the contributions of individual ionic currents to SBVR combine in a non-additive way.
Under physiological conditions I_{to1} is the largest contributor to SBVR; I_{to1} alone able to reproduce 62% of SBVR. I_{Kr} is the next largest contributor to SBVR, while $I_{Ca\text{-}L}$ and I_{Ks} contribute the least.
In case of complete I_{Kr} inhibition, simulation results show that stochasticity in I_{Ks} and $I_{Ca\text{-}L}$ are the largest contributors to SBVR. Regarding complete inhibition of I_{Ks}, no significant differences between the contributions of stochasticity in the currents before and after inhibition were found.

The number of ion channels responsible for I_{to1} (Nto1) strongly negatively influences SBVR. NCa-L and NKr are non-significantly correlated with SBVR, meaning that they do not independently influence SBVR substantially. NKs is non-significantly (positively) correlated with SBVR, also indicating a weak independent influence on SBVR.

Our preliminary experiments suggested the possible role of alteration of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) on SBVR. Exposure of myocytes to the Ca^{2+} chelator BAPTA-AM decreased, while Ca^{2+} ionophore A23187 increased the magnitude of relative SBVR. Both effects were primarily due to the concomitant changes in APD.
The influence of Ca^{2+} released from the sarcoplasmic reticulum on SBVR was studied using ryanodine and cyclopiazonic acid. Ryanodine blocks Ca^{2+} release from SR, while cyclopiazonic acid is a selective inhibitor of the SR Ca^{2+} pump (SERCA) resulting in a depletion of sarcoplasmic reticulum Ca^{2+} release pool. Relative SBVR was reduced by both agents. Inhibition of the Na^{+}-Ca^{2+} exchanger by SEA0400 increased relative SBVR, too.
These results suggests that systolic Ca^{2+} release, experienced by the surface membrane, is a relevant signal for SBVR modulation.

Beta adrenergic-stimulation is known to cause activation of L-type Ca^{2+} and various K^{+} currents and to increase intracellular calcium ion levels $[Ca^{2+}]_i$ as well.
A non-specific beta adrenergic receptor agonist isoproterenol effect was studied at various pacing cycle lengths (0.3 sec - 5 sec). Although both the isoproterenol induced shortening of APD and reduction of SBVR increased with increasing the cycle length of stimulation, the relative SBVR progressively decreased at longer cycle lengths.
Our results proves that isoproterenol-induced action potential plateau shift and $I_{Ca\text{-}L}$ increase developed faster than the shortening of APD and stimulation of I_{Ks} and I_{Kr}, but these currents decrease the relative SBVR. Since beta adrenergic stimulation increases $[Ca^{2+}]_i$, and we have shown that it increases SBVR therefore we studied the effect of beta adrenergic stimulation on Ca^{2+} chelator BAPTA-AM incubated ventricular cells. The effect of isoproterenol on relative SBVR was largely similar in the presence and absence of BAPTA-AM, indicating that the effect of beta adrenergic stimulation on SBVR was not related to the concomitant changes in $[Ca^{2+}]_i$, it was rather caused by the isoproterenol-induced augmentation of $I_{Ca\text{-}L}$. I_{Ks}, and I_{Kr}.

The effects of temperature, pH, redox potential, osmolarity were examined on SBVR.
Both SBVR and APD were increased by cooling, while the opposite change was observed on warming (between 34 °C and 41°C). Changes of pH within 1 pH unit had no significant effect on relative SBVR. Neither SBVR nor APD was changed by shift of pH from 7.4 to 6.4. On the other hand both SBVR and APD were reduced significantly by an alkaline shift of from 7.4 to 8.4. The ventricular cells were incubated in oxidative and reductive solutions to change their redox state. In oxidative environment the relative SBVR was increased significantly while reductive environment decreased it. The osmotic concentration of the bathing solution was set either to 250 mOsm or to 350 mOsm. Changing the osmotic concentration of the medium had no effect on SBVR or APD.

Experiments were carried out on multicellular preparations as well. Trabecular muscle of left and right ventricle of canine and pig heart was isolated and the action potentials were measured. Our results show that APD of pig and dog ventricle cells are similar, 237±16 ms and 228±3 ms at 1 Hz stimulation rate, respectively. SBVR of multicellular preparation was significantly smaller (0.98±0.27 ms) than what was obtained in isolated cells (2.93±0.07 ms). Our results show that coupling of cardiomyocytes significantly decreases SBVR due to the electrotonic current flow among neighboring cells. The frequency-dependent changes in APD and SBVR were observed on multicellular preparations as well. The APD and SBVR values were 235±12 ms and 1,25±0,71 ms at 0.5 Hz stimulation rate while at 2 Hz we measured 172±4 ms and 0,67±0,44 ms, respectively.

During this project we studied and explored the possible causes and the modulators of SBVR which can be a predictor of imminent cardiac arrhythmias. The experimental results of this project prove our hypothesis, namely that the mechanism of SBVR is multifactorial and our data may contribute to understand the development of ventricular arrhythmias.