The Origins of Heart Rate Variability in Isolated Hearts

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Introduction

• Heart rate variability (HRV) refers to the phenomenon of beat-to-beat changes in spontaneous consecutive cycle lengths in runs of ECG recordings
• It is an important feature of the healthy heart beat
• Loss of, or decrease in, HRV is seen in many pathological conditions, including heart failure and following myocardial infarction, where it predicts a poor prognosis
• Traditionally, HRV is ascribed to the varying of the contradictory arms of the autonomic nervous system for overall control of the heart beat
• However, a number of authors have demonstrated HRV in cardiac preparations devoid of any extrinsic autonomic control (including Langendorff perfused hearts, isolated sino-atrial node (SAN) preparations and isolated nodal cells (both alone and in small clusters))
• The fundamental physiological causes of HRV in such preparations remain poorly characterised

Methods

• We investigated the effects of numerous pharmacological agents on the HRV in Langendorff-perfused isolated male New Zealand white rabbit hearts (weight 1.5 kg), to investigate the underlying physiological basis of the witnessed phenomenon
• Hearts were initially perfused with bicarbonate-buffered Tyrode's solution, before being switched to one of the following agents...
  1. Atropine (3.45 µM, n=4)
  2. Propranolol (1 µM, given with atropine, thus n=4)
  3. Isoprenaline (50 nM, n=8)
  4. Carbachol (2x10⁻7 M, n=9)
  5. Ryanodine (2 µM, n=6)
  6. Thapsigargin (2 µM, n=7)
  7. Caesium chloride (2 mM, n=8)
• Sequences of 500 consecutive beats were recorded from the epicardial surface of the right atrium and left ventricle using spring loaded platinum surfaced electrodes (Harvard Apparatus, Holliston MA, USA)
• We went on to study single freshly isolated SAN myocytes (n=6) from female New Zealand white rabbits (0.8-1.1 kg) isolated using enzyme digestion and subsequently exposed to whole cell patch clamping at 37°C
• Shorter sequences of 60-175 consecutive cell cycles were recorded from these cells
• The ECG data from these isolated SAN myocytes was compared to 100 beat long strings of consecutive stationary data from freshly harvested spontaneously beating Langendorff perfused whole hearts from female New Zealand white rabbits (n=8, weight 1.5 kg)
• Time-domain characteristics, including mean, standard deviation and standard error of the cycle lengths recorded, were calculated for each cardiac preparation
• Variability was calculated in the time- (normalised standard deviation), frequency- (power spectral analysis) and non-linear domains (Poincaré plots)
• We went on to perform computer modelling using a biophysically detailed model of the SAN cell in order to try to better interpret our findings

Results – Langendorff heart

The combination of propranolol and atropine does NOT have a significant effect on measured HRV, suggesting that isolated heart HRV is not due to constitutive action of intrinsic autonomic neurons within the wall of the heart

Isoprenaline decreases HRV, mimicking the effect witnessed in vivo with sympathetic overactivity

Ryanodine decreases HRV, suggesting that variability of Ca²⁺ release from the sarcoplasmic reticulum is intrinsically important to generating HRV

Thapsigargin also decreases HRV, suggesting that variability of Ca²⁺ refilling into the sarcoplasmic reticulum is intrinsically important to generating HRV

Results – single SAN cells vs Langendorff heart

• Isolated single SAN cells beat with a faster and markedly more irregular intrinsic rhythm than do Langendorff perfused whole hearts

Results – Computer Modelling

• Variability directly measured from single cells was 'injected' into a biophysically detailed model of a single SAN cell, to reproduce experimentally witnessed variability (shown on Poincaré plot, right)

Conclusions

1. A significant amount of variability exists in cardiac preparations devoid of significant autonomic influences
2. In the Langendorff heart, this variability can be modulated by substances that replicate the effects of the autonomic nervous system (isoprenaline, carbachol) and also that interfere with Ca²⁺ cycling within cardiac cells (ryanodine, thapsigargin)
3. The effects of ryanodine and thapsigargin strongly implicate calcium cycling within SAN cells as being a likely cause for such ‘autonomic-independent’ variability
4. The effect of caesium would suggest a stabilising role for the funny current, It, in beat-to-beat heart rate control
5. There is more variability and a faster intrinsic rate in single SAN cells than in whole Langendorff hearts
6. The increased variability can be explained by computer modelling as being due to a ‘variability smoothing’ effect that occurs when cells are coupled together
7. The heart rate in the isolated Langendorff perfused heart is slower compared to the spontaneous beating rate of isolated nodal cells. This can be explained, because in the intact heart the SAN cells are in contact with non-pacemaking atrial muscle cells and these are expected to act as an electrotonic load on the SAN cells and slow their pacemaking rate
8. Further studies are underway at the SAN cellular level to further elucidate the fundamental physiological causes of HRV, specifically those related to Ca²⁺ cycling in SAN cells