

impedance and EFP signals. In addition to the pharmacological effects, dynamics of the cell growth and development in culture were monitored to derive an analytical expression for the system. Detailed investigation of the cellular beat signal facilitated multi-parameter evaluation allowing integrative assessment of cardiomyocyte behavior. The resulting multiplex signatures can be used as a fingerprint tool to highlight changes in cardiac function and potentially to categorize drugs based on their mechanisms of action.

The hybrid impedance-EFP measurements in human SC-CMs exemplify a novel screening method for early and sensitive multi-parameter profiling in vitro. This integrative approach meliorates the screening pipeline by bridging single cell-based assays and ECG measurements for translational assessment.

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Room Temperature vs Ice Cold - Temperature Effects on Cardiac Cell Action Potential

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The effects of temperature on cardiac cells have been previously explored. It is well characterised that on single cell level the action potential measured at room temperature has longer duration compared with that obtained at body temperature. The duration of the AP (APD) is a parameter which is related to the ability of cells to cope with faster pacing rates. APD prolongation by drug or disease condition may lead to arrhythmia. However so far, the comparison has been done mostly on data obtained at body and room temperature. One common explanation for the prolongation of the APD at room temperature is the slower kinetics of underlying ion channels, exchangers and pumps (please check for pump).

We measured AP from live cardiac ventricular slices after incubating in ice cold solution for over 60min. The AP from rabbit cardiac tissue slices, were measured shortly after transfer into body temperature solution. These AP exhibit a very short APD (e.g. 89.6ms APD, 6min after warming up). Only after incubation in body temperature of about 1h or more, the AP shape and duration are back to the values under control conditions (205±/-21ms, at 2Hz pacing). The mechanisms for the changes in AP are still not clear however an impaired ion gradient control and subsequently increased intracellular sodium concentration could be responsible for these changes.

The temperature effects of ice cold solution are of different origin compared to the reduction to room temperature settings. This could be one of the main reasons why implanted hearts can adapt to the new environment without exhibiting prolonged AP, once coming from cold incubation. However, further investigations are needed to explain the effects in total.

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Postnatal Development of Calcium Signaling in Rat Cardiomyocytes

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During development, cardiac myocytes undergo rapid growth and structural remodeling. With increased cell size and increased requirements for calcium for contraction, excitation-contraction coupling becomes increasingly dependent on calcium-induced calcium release (CICR). Extending into the interior of the cell, t-tubules form a network securing signal transduction into the whole volume of the cell.

We studied the development of L-type calcium currents and of t-tubules in ventricular myocytes of neonatal and young rats (ages 2-18 and 28 days). Calcium currents were recorded in isolated ventricular myocytes (80 ms depolarization from -50 to 0 mV) using patch-clamp. Plasma membrane-specific fluorescent probe FM 4-64 was used to observe formation of t-tubules in myocytes using isolated cells and intact myocardial tissue by laser-scanning confocal microscopy. Formation of t-tubules started around day 9 (D9) as small membrane invaginations. Invaginations formed a loose network with predominating longitudinal elements around D14, and after D17 developed into a semi-regular tubular network. Cell size, area and membrane capacitance was increasing throughout the observed age interval and around D28 reached values typical for cells from adult rat hearts. During early development, calcium current density increased, saturating around D11. The rate of Ca-current decay increased with age, reaching values typical for adult myocytes after D10.

These data suggest that functional local calcium signaling is present already at D11, when the t-tubules only start to form. This is supported especially by the

dynamics of calcium current decay, which at D11 becomes as fast as in adult cardiac myocytes.

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Determination of the Upper Bound of Intracellular [Na⁺] by Electrophysiological Method: Probing the Subsarcolemmal [Na⁺]

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Rationale:

Intracellular Na⁺ concentration [Na⁺]_i plays an important role in cardiac Ca²⁺ cycling, contractility, and metabolism. Previous measurements of [Na⁺]_i used methods ranging from sodium selective electrodes to fluorescent sodium indicators. These techniques have significant limitations during physiological conditions and none of them are able to measure the subsarcolemmal Na⁺ concentration ([Na⁺]_s). The goal of this study was to estimate [Na⁺]_s from the magnitude of the peak of the action potentials (APs) in isolated ventricular myocytes.

Method:

Rabbit ventricular APs were recorded in current-clamp mode of whole-cell patch-clamp technique. To maximize physiological relevance, measurements were performed at body temperature, while the ionic compositions in the internal and external solutions were kept to be physiological and intracellular Ca²⁺ cycling was also preserved.

Results:

(1) Most accurate estimate of [Na]_s occurred under conditions that maximize peak voltage of AP, namely increasing the external [Na⁺].

(2) Under these approximately ideal conditions the peak voltage of the AP was only about 4 mV less than the Nernst potential for Na⁺, assuming that [Na⁺]_s is about 11 mM.

(3) [Na⁺]_s remained fixed at about 11 mM over the range of pipette [Na⁺] of 5 to 15 mM, which indicates a range of autoregulation of [Na⁺]_s.

(4) Elevated [Na]_s was found at increased pacing frequency and in heart failure.

Conclusion:

We applied a method for estimating the [Na⁺]_s during the rising phase of the AP. [Na]_s was found to be about twice as high than what had been measured in the bulk cytoplasm. Importantly, our data suggest the existence of a Na⁺ autoregulatory mechanism that can maintain an almost constant [Na⁺]_s despite wide perturbations introduced by the pipette.

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Age-Related Changes in Electrical Activities and MicRNAs of Left Ventricular Cardiomyocytes Isolated from Rat Heart

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Hearts from subjects with different ages have different Ca²⁺ signalling and cardiac function. Although earlier studies have reported significant changes in contractile performance of the heart during aging, some studies provide evidences that aging process is associated with altered cardiovascular responsiveness to different stimuli. It is well accepted that release of Ca²⁺ from intracellular stores in response to an action potential initiates cardiac contraction while global increase in free Ca²⁺ concentration ([Ca²⁺]_i) consists of summation of Ca²⁺ release. Additionally, it is known that mutations or aberrant expression of various transcriptional and posttranscriptional regulators are correlated with cardiac diseases and microRNAs (miRNAs) are implicated in the pathogenesis of cardiac dysfunction. Therefore, in the present study, we hypothesized that there should be some important age-related differences in cardiac electrical activity including action potential and voltage-dependent K⁺-channel currents (such as I_{to} and I_{K1}) besides changes in intracellular Ca²⁺ handling of cardiomyocytes. Action potential duration was found to be significantly prolonged with significant existence of afterdepolarizations while the K⁺-channel currents were depressed in 12-month-old rats compared to 3-month-old rats. Basal [Ca²⁺]_i was significantly higher and Ca²⁺-transient amplitudes were significantly smaller in 12-month-old rats. Using microarray analysis, we were able to identify changes in expression in several miRNAs out of total examined 351 miRNAs. Among them, 3 miRNAs were further validated by real-time PCR. The expression levels of miR-1, miR-133a, and miR-133b are found to be markedly depressed in the 12-month-old rats cardiomyocytes compared to the youngs. As summary, the present data suggest a possible association between altered heart function and some target miRNAs during aging in mammals, having become an intriguing target for therapeutic intervention. (Supported by grants from Tübitak SBAG-2124S254).