Ph.D. Theses

Frequency-dependent properties of mammalian cardiac muscle

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Summary

The mechanism of electrical restitution and the effect of action potential duration and elevated cytosolic sodium concentration on the force-frequency relationship were studied in various mammalian cardiac muscle preparations.

Multicellular preparations and ventricular cardiomyocytes isolated from rabbit, guinea pig, dog, rat and human were studied using conventional microelectrode and patch clamp techniques combined with measurement of cytosolic calcium concentration.

In guinea pig and rabbit multicellular ventricular preparations the shortening of action potential duration to values characteristic of rat reduced the force of contraction and converted the positive force-frequency relationship into negative one. This conversion was greatly enhanced by inhibition of the Na-K pump. Prolongation of action potential duration in rat multicellular ventricular preparation increased the force of contraction and abolished the negative force-frequency relationship. It is concluded that both action potential duration and cytosolic sodium concentration are major determinants of the force-frequency relationship in mammalian myocardium.

The characteristics of electrical restitution in rabbit, guinea pig, dog, rat and human myocardium was determined by recording action potential duration following gradually increasing diastolic intervals (evoked after a train of stimuli). Action potential duration increased monotonically in canine, guinea pig and human ventricular preparation with increasing diastolic interval. In rabbit and rat papillary muscles the restitution process was biphasic: action potential duration first lenghtened, then shortened with increasing diastolic interval.

In all preparations studied the kinetics of restitution were intimately related to the recovery process of the L-type calcium channel, whereas it was little influenced by time-dependent changes of the various potassium currents. In addition, interventions leading to changes in intracellular calcium concentration (chelation of intracellular calcium or application of caffeine) characteristically modified the restitution process.

It is concluded that electrical restitution in mammalian myocardium is strongly influenced by changes of intracellular calcium concentration, which may be important to understand the frequency-dependent electrical changes under health and disease.

Introduction

Electrical restitution (restitution of action potential duration as a function of the coupling interval or proximity of an extra action potential) is a general feature of cardiac tissues. In multicellular mammalian preparations, as well as in single cardiomyocytes, the restitution process is believed to be governed by the time-dependent changes of those ion currents which control cardiac action potential duration. According to the diversity in types, density and kinetics of the ion channels in various cardiac membranes, significant interspecies differences in the restitution kinetics have been reported. There are, however, relatively few data published on the electrical restitution in dog, rabbit, guinea pig and human ventricular muscle. In addition, the mechanism of restitution is poorly understood in these preparation. In this study, therefore, electrical restitution was analyzed in multicellular and single cell ventricular muscle preparations using intracellular recording techniques.

Comparing to other mammalian species, the rat heart has unique features, like the well-known negative force-frequency relationship, its extremely short action potential duration and an unusually high intracellular Na^{+} activity. In addition, the sarcoplasmic reticulum (SR) is well developed in this preparation, and the activator Ca originates largely from the release stores of the SR. Due to these properties, the ventricular myocardium of the rat is an excellent preparation for studying the role of transient changes in $[Ca^{++}]_i$ in the restitution process. Contribution of the Ca transients to the restitution was studied in single ventricular cardiomyocytes, and the possible underlying mechanisms are speculated upon and discussed.

Methods

Determination of restitution relation in ventricular muscle

Isolated pieces of right ventricular free wall with trabeculae were excised from adult mammalian hearts. The preparations were individually mounted in a Plexiglass chamber allowing for continuous superfusion. The superfusate contained (in mmol l^{-1}) NaCl, 153; KCl, 5.4; $CaCl_2$, 2.7; $MgCl_2$, 1.05; HEPES, 5.4; and glucose, 11.0. The pH of this superfusate was 7.4 ± 0.05 at 37 ^{o}C when gassed with 100% 0_2. The preparations were

stimulated using 1 ms wide isolated constant current pulses having an amplitude of twice the diastolic threshold. These pulses were provided by a computer-controlled stimulator at a constant rate of 1 Hz, and delivered to the preparation through a pair of platinum electrodes. Conventional 3 M KCl filled glass microelectrodes having resistances between 10 and 20 MOhm were used to record action potentials. After amplification, the evoked potentials were digitized using a 100 kHz A/D converter and analyzed on a 20 MHz 386 microprocessor based personal computer. Microelectrode impalements were continuously maintained throughout each experimental protocol. In some experiments, mechanical responses were simultaneously recorded under isometric conditions using a capacitive mechano-electronic transducer, fixed to the free end of the trabecula. These preparations were stretched so as to produce maximum force of contraction at basic cycle length.

Restitution of action potential duration was studied in the following manner. The preparations were continuously paced using a train of 20 basic stimuli delivered at a cycle length of 1000 ms. These trains of basic stimuli were interrupted by a single extra stimulus applied at successively longer coupling intervals. In this way, each 20th basic action potential was followed by a single extra action potential occurring at successively longer diastolic intervals. The diastolic interval (DI) was defined as a time elapsed from the APD_{90} of the last basic action potential of the train to the upstroke of the extra action potential. Restitution curves were generated by plotting the duration of each extra action potential (APD_{50}) against its DI. A similar pattern of stimulation was applied to develop mechanical restitution curves. In this case, only those DI - twitch tension data pairs were included where relaxation of the last basic twitch was complete before the beginning of the test contraction.

The restitution curves were fitted as the sum of 3 exponentials according to the following equation:

$$APD=APD_p*(1-(A_1*\exp(-t/Tau_1))-(A_2*\exp(-t/Tau_2))-(A_3*\exp(-t/Tau_3)))$$

where: APD represented APD_{50} values at t = DI; APD_p was the asymptotic value of APD at long DI's; Tau_1, Tau_2, Tau_3 were estimated time constants of the individual

components; and, A_1, A_2, A_3 were their respective estimated relative amplitudes expressed as a fraction of APD_p.

Recording of action potentials, ion currents and Ca transients in single mammalian myocytes

Single ventricular myocytes were isolated from hearts of adult animals, using a collagenase and protease dissociation technique. The dispersed cells were resuspended in Tyrode solution. The electrical measurements were performed at room temperature (24-25 ^oC) using a whole cell configuration of the patch clamp technique in either a current clamp or voltage clamp mode. The patch pipettes, coupled to the headstage of an Axopatch 1B patch clamp amplifier, were filled with internal solution containing (in mmol l^{-1}) K-glutamate, 140; NaCl, 7.0; $MgCl_2$, 5.0; HEPES, 3.0; K_2ATP, 5.0; EGTA, 0.1; titrated to pH 7.2. When the Na/Ca exchange current ($I_{Na/Ca}$) was measured, the internal solution contained 120 mmol l^{-1} K-glutamate plus 30 mmol l^{-1} CsCl, while 5 mmol l^{-1} 4-aminopyridine and 0.1 mmol l^{-1} $BaCl_2$ was added to the external solution to block K currents. The filled patch pipettes had DC resistance of 2-4 MOhm. The electrode potential was adjusted to zero prior to establishing a high resistance (1-2 GOhm) seal. Membrane action potentials were elicited by application of brief depolarizing current pulses having amplitudes of 1-2 nA. $I_{Na/Ca}$ current tails were recorded at -40 mV after a 0 mV step lasting for 50 ms. Current and voltage signals were digitized using a 100 kHz A/D converter under software control on an IBM compatible PC. Records thus obtained were analyzed with the aid of softwares developed by Axon Instruments.

To measure $[Ca^{++}]_i$, the cells were loaded with the acetoxymethylester of the fluorescent dye, indo-1. Fluorescence was excited at 340 nm and collected at 400 and 500 nm. $[Ca^{++}]_i$ was calculated from the ratio of emission at 400 and 500 nm. The indo-1 signals were calibrated directly in terms of $[Ca^{++}]_i$, using the equation of Grynkiewicz et al. with a dissociation constant (K_d) of 220 nmol l^{-1}. This procedure requires the knowledge of both the minimum and maximum fluorescence ratios (R_{min} and R_{max},

respectively), as well as the value of ß, the ratio of the 500 nm signal obtained in nominally zero and in saturating Ca^{++} solutions. In order to estimate the mean value of R_{min}, in some cells the patch pipette was filled with a Ca^{++}-free solution containing either 10 mmol l^{-1} BAPTA. or 30 mmol l^{-1} EGTA. In these experiments measuring R_{min}, the external solution was first Ca^{++}-free, then it was replaced by external solution containing 1 mmol l^{-1} Ca^{++} and the cells were damaged with the patch pipette. This intervention raised $[Ca^{++}]_i$, to a stable high level without a significant loss of the indicator (at least during the first minute).

Statistics

All results are reported as arithmetic means ± SEM's and the significance of differences was determined using Student's t-tests for unpaired data. The differences were considered significant when $P < 0.05$.

Results

The effect of action potential duration and elevated cytosolic sodium concentration was studied on the force-frequency relationship in isolated rabbit, guinea pig and rat papillary muscle preparations. Shortening of action potential duration in guinea pig and rabbit from 150-200 ms to values characteristic of rat (20-40 ms), using the K(ATP) channel activator levkromakalim (15 µmol/l), markedly reduced the force of contraction and converted the positive force-frequency relationship into negative one at longer pacing cycle lengths. This conversion was greatly enhanced in the presence of acetylstrophanthidin (0.2-1 µmol/l), an inhibitor of the Na-K pump. Acetylstrophanthidin (1 æmol/l) alone, however, had no effect on the force-frequency relationship. Prolongation of action potential duration in rat with inhibitors of cardiac K channels (4-aminopyridine [10 mmol/l] plus tetraethylammonium [2 mmol/l]), increased the force of contraction and abolished the negative force-frequency relationship, observed in rat at longer pacing cycle lengths. It is concluded that both action potential duration and cytosolic sodium concentration are major determinants of the force-frequency relationship in mammalian myocardium.

The mechanism of electrical restitution was studied in isolated rat ventricular muscle using drugs that inhibit specific ion currents. The effect of transient changes in

cytosolic Ca concentration and Na/Ca exchange in relation to the restitution process was also studied in single ventricular cardiomyocytes. Conventional microelectrode techniques were applied to record action potentials having gradually increasing coupling intervals, each evoked following a train of stimuli with a frequency of 1 Hz. Ion currents were recorded from enzymatically isolated cells using the whole cell patch clamp technique. Ca transients were monitored in myocytes loaded with the fluorescent dye, indo-1. The electrical restitution process in multicellular rat ventricular preparations at 37 ^{o}C was described as a sum of 3 exponential components: an early positive component, a subsequent fast negative component and a late negative component, having time constants of 21.9±1.9, 73.1±6.0 and 1053±61 ms, respectively, (n=9). Inhibition of the transient outwrd K current, the delayed rectifier K current, or the chloride current did not substantially alter these time constants. The early positive and fast negative components were fully abolished by nifedipine or $MnCl_2$. In the presence of caffeine, the fast negative component was absent, while the time constant of the early positive component increased to 39.5±5.8 ms (n=5). In single myocytes loaded with indo-1, the Ca transients decayed with a time constant of 151±12 ms at room temperature (n=5). These Ca transients were accompanied by inward current tails, identified as a Na/Ca exchange current, having a decay time constant of 140±4.5 ms. It is concluded that electrical restitution in rat ventricular muscle is relatively little affected by recovery from voltage-dependent inactivation of ion channels, it is rather governed by transient changes in cytosolic Ca concentration possibly *via* Ca-dependent inactivation of the L-type Ca current and activation of the Na/Ca exchange current.

The steady-state frequency-dependent properties and restitution kinetics of action potential duration (APD) was studied in isolated human atrial and ventricular cardiac muscle preparations. Conventional microelectrode techniques were used to record action potentials under steady-state conditions or after abrupt change in cycle length, i.e. following increasingly longer diastolic intervals (DI). Restitution relations were generated by plotting action potential duration against the respective diastolic interval. Restitution relations were fitted to multiexponential functions. Restitution of APD-90 in ventricular preparations, paced at a basic cycle length (BCL) of 750 ms, were fitted as the sum of 4 exponentials, having time constants of 42.8 ms, 139 ms, 1.34 sec and 16 sec,

respectively. Similar results were obtained for restitution of APD-50 values. In atrial preparations restitution kinetics could be characterized by 3 exponentials with time constants of 154 ms, 1.52 sec and 25.5 sec (BCL=750 ms). The very fast component of restitution, observed in ventricular muscle, was apparently missing in atrial fibers. When the atrial preparations were paced at a longer BCL of 5000 ms, no change in time constants of restitution was observed, however, the amplitudes of the 2nd and 3rd atrial components were significantly decreased comparing to preparations paced at a BCL of 750 ms. Comparing the time constant values estimated for restitution with the reported kinetic parameters of cardiac ion channels it may be speculated that the 1st component of restitution in ventricular muscle may be attributed to the recovery of L-type Ca current from inactivation. The lack of this very rapid component in atrial muscle can be explained by the parallel recovery of the Ca current and the transient outward K current, prominent in atrial myocardium. The 2nd ventricular component (first in atrium) may be due to the time-dependent deactivation of the delayed rectifier K current. The 3rd and 4th ventricular components (2nd and 3rd components, respectively, in atrium) may probably be related to electrogenic Na/Ca and/or Na/K exchange, both more intensive at faster driving rates.

The role of the calcium current and changes in intracellular calcium concentration ([Ca2+]i) in regulation of action potential duration (APD) during the electrical restitution process was studied in mammalian ventricular preparations. Properly timed action potentials were recorded from multicellular preparations and isolated cardiomyocytes using conventional microelectrodes and EGTA-containing patch pipettes. APD increased monotonically in canine and guinea pig ventricular preparations with increasing the diastolic interval, while in rabbit papillary muscles the restitution process was biphasic: APD first lengthened, then shortened as the diastolic interval increased. When the restitution process was studied in single cardiomyocytes using EGTA-containing patch pipettes, the restitution pattern was similar in the three species studied. Similarly, no difference was observed in the recovery time constant of calcium current (ICa-L) measured under these conditions in voltage clamped myocytes. Loading the myocytes with the [Ca2+]i-chelator BAPTA-AM had adverse effects in rabbit and canine cells. In rabbit myocytes steady-state APD lengthened and the late shortening component of

restitution was abolished in BAPTA-loaded cells. In canine myocytes BAPTA-load shortened steady-state APD markedly, and during restitution, APD decreased with increasing the diastolic interval. The late shortening component of restitution, observed in untreated in rabbit preparations, was greatly reduced after nifedipine treatment, but remained preserved in the presence of 4-aminopyridine or nicorandil. Beat to beat changes in APD, peak ICa-L and [Ca2+]i, measured using the fluorescent dye, Fura-2, were monitored in rabbit ventricular myocytes after 1 min period of rest. In these cells, the shortening of APD was accompanied with gradual reduction of the peak ICa-L and elevation of diastolic [Ca2+]i during the initial 8 post-rest action potentials. It is concluded that elevation of [Ca2+]i shortens, while reduction of [Ca2+]i lengthens APD in rabbit, but not in canine ventricular myocytes. These differences may probably be related to different distributions of [Ca2+]i-dependent ion currents and/or to differences in calcium handling between the two species.

Publications

In extenso publications

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