Investigation of mechano-electric feedback and the Frank-Starling relationship in the heart.

Abstract

To date, the precise relationship between mechanical stretch and changes in the electrophysiology of the heart remain unclear. This relationship, termed mechano-electical Feedback (MEF), is thought to underlie many cardiac arrhythmia associated with pathological conditions. These electrophysiological changes are observed not only in the whole heart, but also at the single cardiomyocyte level, and can be explained by the presence of stretch-activated ion channels (SACs). Most investigations of the actions of stretch have concentrated on these sacrolemmal ionic currents thought responsible for the proposed MEF-induced changes in contractility. While these studies have provided some useful insight into possible mechanisms, due to the inappropriate use of solutions and unphysiological degrees of stretch, the results may be somewhat misleading. Currently, little is known about the involvement or contribution of non-selective or K^+ selective SACs to the normal cardiac cycle. Here, we present a new conception that stretch-induced changes in cardiac electrophysiology (MEF) are important in normal cardiac cycle. This will be achieved by demonstrating the effects of stretch on the Frank-Starling mechanism (stretch induced increases in cardiac contractility) while pharmacologically manipulating stretch-activated ion currents. Experiments will be conducted using a number of agents known to influence stretch-activated channels either in a positive or antagonistic manner. Secondly, studies based upon human atrial tissue will be used to demonstrate the presence and function of similar SACs in the human heart, providing a connection between SACs and MEF in the mammalian heart.

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The heart's function is primarily to maintain a constant circulation of blood throughout the body by regular rhythmic contractions. It is not yet fully understood, however, how the heart is able to adapt its rhythmic contractions to changes in blood flow through the body, and why this adaptability is altered in certain pathophysiological conditions.

Frank-Starling Curve:

In 1866 Leipzig Cyon first described the influence of diastolic filling of the isolated perfused heart on cardiac output. These observations, later published by Joseph Coats (1869) demonstrated the negative inotropic effects of decreasing ventricular filling pressure and the positive inotropic effects of increasing ventricular filling pressure on cardiac contractility (***Figure 1***). Later, (1895-1914), Otto Frank and Ernest Starling demonstrated that greater diastolic volumes of the heart result in an increase in cardiac performance during subsequent contractions.[18, 50, 55] More recently, this increase in contractility has been shown to be biphasic in nature,[48] being composed of both a primary response (within a couple of heart beats) and a secondary, delayed response that develops over several minutes.[9, 62]

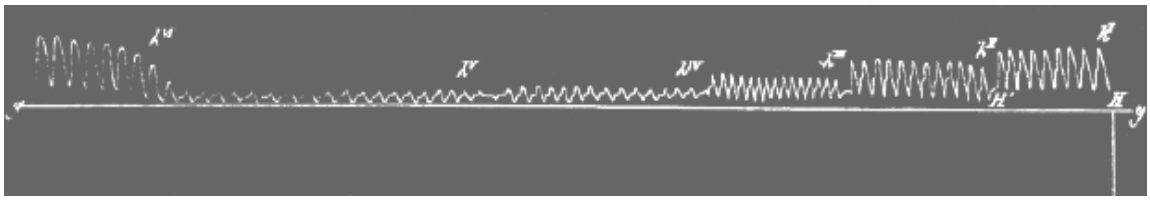

Figure 1 *Effect of lowering the filling pressure on diastolic pressure (H) and amplitude of contraction (h) of the isolated frog heart. Restoration of amplitude when original filling pressure was applied (from right to left) is shown. Recording made by H. P. Bowditch. Reprinted from* ***13****.*

Initially it was thought that, like striated skeletal muscle, the ascending limb of the Frank-Starling curve could be described in terms of decreasing double overlap of the actin myofilaments.[4, 26] However, the relationship between cardiac distension and contractile force is far too steep to be adequately explained by such a mechanism.[4, 32] The first, rapid increase in contractility has since been related to an increase in the sensitivity of the myofilaments to intracellular calcium ($[Ca^{2+}]i$),[3, 33] and more loosely to the length-dependence of intracellular Ca^{2+} release (see 4 for review).

Changes in lattice spacing and myofilament Ca^{2+} sensitivity

Increases in contractility have also been suggested to result from a reduction in the lattice spacing of the thick and thin filaments and a concurrent increase in myofilament Ca^{2+} sensitivity.[3, 9, 23, 32, 45] When a muscle is stretched, not only does it lengthen, but also its diameter decreases and the thick and thin filaments are brought closer together. The result is a decrease in filament spacing and a corresponding increase in the number of active cycling cross-bridges being formed, resulting in an increase in contractility.[4, 30, 58] However, this model does not account for the observed decrease in contractility on the descending portion of the Frank-Starling curve. Furthermore, Wannenburg *et al* (1997) demonstrated that there was no change in the rate of cross-bridge detachment over the ascending portion of the Frank-Starling relationship, suggesting that there was no change in the rate of active cycling cross-bridges being formed.[57] More recently, the theory of decreasing filament spacing was further discredited by Konhilas *et al* (2002) when mimicking the reduction in myofilament lattice spacing with 1-1.5 % dextran. Unexpectedly, the associated decrease in myofilament spacing was found not to affect myofilament Ca^{2+} sensitivity.[38] These observations, among others,[36, 57]

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suggest the Frank-Starling mechanism is complex and likely to be influenced by more than one factor.

Changes in the intracellular Ca^{2+}

In contrast to the mechanism that initiates the immediate increase in contractility, the gradual increase in myocardial contractility has been attributed to a slow rise in the $[Ca^{2+}]i$ transient.[3, 8, 33] In addition, biphasic responses have been demonstrated at the level of single cardiomyocytes.[59] However, while these studies suggest that changes in the $[Ca^{2+}]i$ transient are not responsible for the immediate effects of the Frank-Starling response, this inference is questionable for the following reason. The changes in contractility associated with the Frank-Starling response occur over several heartbeats.[42] Furthermore, the steep rise in contractility associated with the Frank-Starling mechanism reflects the effect of stretch observed over several beats rather than the instantaneous effect seen in a single heart beat.[42] In support of this observation, studies conducted in isolated cardiomyocytes from atria and ventricles demonstrate that diastolic $[Ca^{2+}]i$ changes immediately with stretch[23,56] while the $[Ca^{2+}]i$ transient changes over a short period (~4 seconds). Therefore, the changes in diastolic $[Ca^{2+}]i$ and the $[Ca^{2+}]i$ transient may account for the observed increase in cardiac contractility in Frank-Starling's experiments.[25, 56]

Electrophysiological effects of myocardial stretch

As well as causing increased contractility, myocardial stretch also induces electrical responses in cardiac tissue. These mechano-electric changes, termed mechano-electric feedback (MEF), have been demonstrated in both the Langendorff perfused whole heart as well as isolated tissue and cardiomyocytes.[20, 40, 54, 61] In Langendorff experiments, inflation of a small balloon inserted into the left ventricle induces depolarisations in the ventricular tissue during diastole[20,60] (*Figure 2*). In single ventricular myocytes, these stretch-induced depolarisations of the membrane potential are associated with a large rise in diastolic $[Ca^{2+}]_i$, and an increase in intracellular sodium concentrations ($[Na^{+}]_i$).[9, 25, 29, 61] Furthermore, the size of the ventricular depolarisations increased with the amount of ventricular stretch until a threshold was reached, above which a ventricular action potential (AP) was generated with each stretch pulse. This threshold is associated with the initiation of extrasystoles.[16, 19, 20, 40] Thus, if stretch of the ventricles during diastole can alter the electrophysiology of the heart, then it is likely that the same effects occur throughout and may therefore contribute to the Frank-Starling phenomenon.

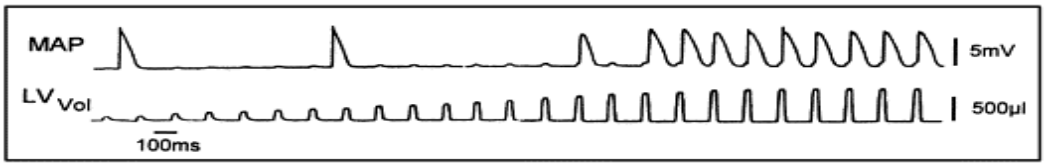

Figure 2: *Stretch-induced effects on the whole heart. A monophasic action potential recording from the epicardial surface of a rabbit ventricle in response to volume pulses (change in volume is shown in lower trace) applied to a balloon inserted into the left ventricle. Volume pulses induced a transient membrane depolarization, which increased amplitude parallel to increases in volume pulse amplitude. Above a certain volume, the transient depolarisations produced premature ventricular excitations (extrasystoles). Diagram from Franz e.t al., (1992).*

Other Effects of Stretch

Many mechanical heart disorders are now thought to alter the heart's electro-physiological properties through these stretch-induced changes (MEF). Experiments based upon sudden chest impact[35] and volume or pressure changes in whole hearts[20] have demonstrated changes

in heart electrophysiology. In general, cardiac-muscle stretch has been shown to shorten the AP, induce membrane depolarisation during diastole and promote early/delayed after-depolarisations and extrasystoles.[7, 14, 15, 19, 20, 20, 28]

Stretch and Ventricular Arrhythmia

These studies demonstrate that abnormal degrees of stretch or pressure are pro-arrhythmic in whole hearts and in isolated preparations.[27] In pathological conditions where there is altered mechanical loading of the heart, extrasystoles are one of the most likely outcomes of cardiac stretch during diastole or repolarisation.[20] Extrasystolic beats arise when the magnitude of these stretch-induced depolarisations are sufficient to induce premature action potentials (APs)[20]. Extrasystolic beats also have the ability to induce fibrillation or runs of tachycardia, which are promoted by the associated reduction in AP duration. This is because, with a shorter AP duration, subsequent stretch-induced APs (or extrasystoles) can be induced earlier and more frequently throughout the cardiac cycle.

SACs in The Heart:

Thus, mechanical stretch of the heart induces electrical responses or changes in the heart via MEF. These electrical responses have also been observed in isolated cardiomyocytes indicating the stretch-sensitive mechanism exists at the cellular level. Stretch activated ion channels (SACs) were first demonstrated in ventricular myocytes in 1988,[12] and can explain mechanically-induced electrical responses or mechano-electric feedback (MEF) in isolated cardiomyocytes as well as in the whole heart.[29, 40, 61] Moreover, the open probabilities/current of these channels in membrane patches directly reflects changes in membrane stretch.[54, 31]

Types of SACs found in the heart

To date, all of the SACs found in cardiomyocytes of the heart are cation-selective,[29] and fall broadly into two category types. The first of these channels, commonly referred to as stretch-activated non-elective cation channels (NSACs), is weakly selective among monovalent cations (Na^+) and selective towards the passage of divalent cations (like Ca^{2+}).[25, 29, 53] The other cardiac SACs are K^+-selective (SAPCs) and include the TRAAK and TREK family of K^+ channels.[31, 29]

During the normal cardiac cycle, Na^+ ions diffuse into the cardiomyocyte causing depolarisation. The resultant change in membrane potential causes L-type Ca^{2+} channels to open, initiating cardiomyocyte contraction via calcium-induced calcium release (CICR). This process is terminated by the activation of voltage-gated K^+ channels, which allow K^+ ions to diffuse out of the cell and repolarise the cell membrane. Thus, due to the contrasting movement of K^+ ions and Na^+/Ca^{2+} ions across the cell membrane, SAPCs may have different effects to those of NSACs on cellular electrophysiology.[40] The reversal potential for NSACs is between 0 and -40 mV,[8, 12, 53] so their activation during diastole (resting membrane potential), or during the repolarisation phase of the cardiac AP, results in cardiomyocyte depolarisation.[20, 34] By contrast, activation of NSACs during systole or during the plateau of the cardiac AP may bring about cardiomyocyte repolarisation.[29, 34]

In contrast to NSACs, the reversal potential of SAPCs in the heart is around resting membrane potential (-90 mV).[35] Thus, opening of stretch-activated potassium channels during systole will cause potassium ions to diffuse out of the cell and result in repolarisation of the cardiomyocyte. By contrast, activation of SAPCs during diastole should have little impact on the resting membrane potential, because there is little driving force for the diffusion of K^+ out of the cell.[35] Taking into account both types of SACs in the heart, it has been demonstrated that stretch applied during diastole induces depolarisation.[20, 60, 61] This

depolarisation is likely to be caused by the influx of primarily Na^{+}[61] and Ca^{2+} ions[9, 53] and may therefore initiate changes in CICR, indirectly by activation of the voltage-activated L-type Ca^{2+} channels,[24, 44] or directly via the increase in diastolic $[Ca^{2+}]i$ and loading of Ca^{2+} into the SR. Furthermore, Akay & Carelius (1993) demonstrated that the activation of SACs in membrane patches could provide sufficient depolarising current to directly trigger cardiomyocyte contractions. In addition, these effects and the increase in diastolic $[Ca^{2+}]i$ are sharply attenuated by both streptomycin[24] and gadolinium (Gd^{3+}),[54] two non-specific blockers of SACs and SAPCs.[5, 24, 31]

Systolic stretch has also been demonstrated to reduce the amplitude of the AP plateau and to shorten the AP duration.[14, 19, 29, 60] By contrast, Zeng *et al* (2000) demonstrated a lengthening of the APD in isolated cardiomyocytes from Sprague Dawley rat hearts. Either way, the mechanism by which cardiac stretch alters membrane potential may be due to the activation of the two types of cardiac SACs, namely the NSACs and SAPCs. The resultant effects on membrane potential are then also governed by the period of the cardiac cycle during which they are activated. This conclusion implies that both NSACs and SAPCs are involved in the normal function of the heart (including the Frank-Starling mechanism), and their effects are dependent on the period of the cardiac cycle. However, to date, no experiments have been performed on the whole heart in which these two types of stretch-activated current have been pharmacologically manipulated during the normal cardiac cycle.

Lab *et al* (1994) and, more recently, Nicolosi *et al* (2001) have demonstrated that gadolinium (10-20 μM) partially suppresses the Frank-Starling response. Both studies demonstrate that, with increasing cardiac muscle stretch, there is a larger reduction in contractility along the ascending limb of the Frank-Starling curve. However, Nicolosi *et al* (2001) failed to consider that this observation may be due to the inhibitory effect of Gd^{3+} on SACs. Rather, they suggest the results demonstrate the partial blocking of L-type Ca^{2+} channels by Gd^{3+}. This partial block would appear unlikely because nifedipine produces a much more consistent reduction in contractility throughout the Frank-Starling curve.[39] It would appear that the increasing reduction in contractility reflects the increasing involvement of SACs, which in turn corresponds with increasing degrees of stretch.[20, 31, 54]

Even more importantly, many previous studies involving the use of Gd^{3+} as a blocker of SACs have used inappropriate solutions and thus may have produced misleading results.[27, 39, 40, 61] Caldwell *et al* (1998) and Linda *et al* (1991) pointed out that:

(a) not all SACs are sensitive to Gd^{3+} (which can result in false negative results),
(b) anions present in biological solutions (viz. phosphate, carbonate, EGTA, sulphate, as well as carboxylic acids, and albumin) avidly bind free Gd^{3+} and effectively remove it from the experimental solution.

The involvement of SACs in Beat-to-Beat Regulation

Due to the different effects of SAC activation by stretch throughout the cardiac cycle, we anticipate that MEF plays an important role in the beat-to-beat regulation of the heart (although this has been largely discounted by others). During the cardiac cycle, normal contraction of the intact ventricle is made possible by individual cardiomyocytes contracting in an almost synchronous manner. SACs may play a role in coordinating the cardiomyocytes of the ventricle to contract at the appropriate time and evenly distribute ventricular wall stress. However, in cardiac pathology (e.g, infarction) these MEF interactions between cardiomyocytes can be disrupted or exaggerated resulting in abnormal changes to the AP as it propagates through the heart. The resultant uncoordinated contraction of cardio-myocytes results in “dispersion” of the propagating AP between adjacent cardiomyocytes in the depolarised and repolarised states. As a result, depolarised cardiomyocytes in regions of the

heart may induce an early depolarisation of adjacent cardiomyocytes that are in the process of repolarising **(*Figure 3*).**[40,41]

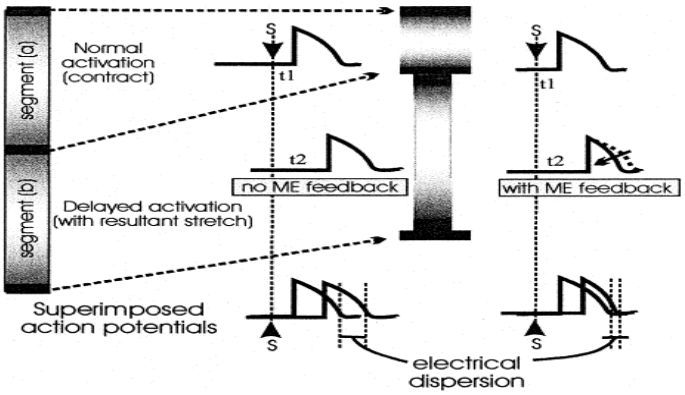

Figure 3: *Intact heart regulation involving mechano-electric feedback. Action potentials of two segments in series during mechanical interaction, with one segment contracting abnormally. Segment (a) stimulated at (S), produces an action potential after a delay of t1 ms (top action potential). It contracts normally. Segment (b), for some reason, has a conduction delay (t2). This means a later action potential and later mechanical activation, so segment (a) stretches segment (b). Superimpositions of action potentials show the repolarisations of the action potentials with a wide time difference between them- large electric dispersion. If, however, the stretched segment involves mechano-electric feedback, stretched segment (b)'s action potential shortens, and this reduces the potentially dangerous electrical dispersion. Diagram obtained fromLab (1999).*

Stretch and Atrial Arrhythmia

Mechano-sensitive channels may have an important role in normal regulation of the heart and coordination of cardiomyocyte contraction. However, MEF and SACs also have the potential to disturb cardiac rhythm in pathophysiological conditions.[20, 51]

Atrial fibrillation is the most common arrhythmia, being associated with high blood pressure, and is present in people with atrial dilation. Moreover, atrial dilation is associated with an increase in heart rate and susceptibility to atrial fibrillation (AF).[1, 43, 51] Recent work suggests that AF results from an abbreviation of AP duration, a phenomenon concurrent with the activation of SACs.[52, 56] This chronotropic and pathological effect of atrial stretch is now thought to be caused by the presence of SACs in the sino-atrial node of the right atria.[34, 46, 56] Thus, in a similar mechanism to that observed in the ventricles, MEF and the activation of SACs may contribute to the susceptibility and initiation of AF. Moreover, SACs have been demonstrated in atrial cells, and as a result of their activation by acute atrial dilation, they may facilitate the induction and maintenance of AF.[9, 22] However, the number of studies based upon the role of MEF in human atrial tissue is limited.[46]

As with the ventricles, diastolic stretch of atrial tissue results in depolarisation, which is partially due to an influx of Ca^{2+} ions.[35, 56] In addition, dilation of the atrium in the Langendorff model also results in a reduction in AP duration and amplitude.[51] Gd^{3+} and the most specific SAC blocker, Grammastola toxin (GsTx4) have been demonstrated to inhibit stretch-induced AF[6] in a similar manner to that observed in ventricular myocytes.[6, 27] However, to date, few experiments have been carried out on human atrial tissue. Thus, it is not currently known if blockers of SACs in animal cardiac tissue induce the same results in human atrial tissue, although stretch of human atrial tissue induces similar results to those observed in other animal models.[11]

General Aims & Hypotheses:

Phase I: The involvement of MEF & SACs in the Frank-Starling mechanism

The current project will aim to determine the contribution of MEF to the normal cardiac cycle and the Frank-Starling (length-tension) relationship in the heart. We will investigate the Frank-Starling curve and the effects of various pharmacological agents known to affect both SAPCs and NSACs. These experiments will give insight into the basic function of SACs and MEF in the heart. It is hypothesised that the SACs are tonically active during the normal cardiac cycle, and thus important to the normal functioning of the heart.

Phase II: The involvement of MEF & Specific SACs in the normal cardiac cycle

The second set of experiments aim to determine which of the SACs underlie the responses seen in the Frank-Starling experiments. It is hypothesised that NSACs are most active and important during diastole (as has been proposed) while SAPCs are more specifically involved in the early termination of the cardiac AP. It is also expected that these experiments will demonstrate that the importance of the two different populations of SACs and that their effects are cardiac phase-dependent (ie dependent on timing and the cardiac cycle).

Phase III: The functional presence of MEF & SACs in human atrial tissue

A third group of experiments will seek to ascertain the effects of SACs on the intracellular current recordings in human atrial tissue. In addition, the conclusions drawn from the previous two experimental projects will be extrapolated to humans. It is expected that the same pharmacological agents used to manipulate SACs in the animal models will influence intracellular current recordings in the human atrial tissue. It is hypothesised that there are both populations of SACs (SAPCs and NSACs) are present in human atrial tissue.

Expected Outcomes:

Currently, the exact role of MEF in the heart is not known and it remains to be demonstrated that MEF participates during the normal cardiac cycle. MEF has been proposed to play a pivotal role in the induction and maintenance of arrhythmia during unphysiological and pathophysiological conditions. However, it is not known which of the SACs are important and how they contribute to normal cardiac function. It is expected that the 1st phase of the experimental work will demonstrate the involvement of MEF in normal physiological conditions (via the Frank-Starling response). The experiments will help to determine which population of SACs is primarily involved during physiological pressure changes in the heart. The second phase of experiments will demonstrate which of the populations of SACs are involved in MEF during the different periods of the cardiac cycle. This is of great importance when determining the role of MEF (and SACs) in the generation and maintenance of cardiac arrhythmia.

By contrast, the electrophysiological characteristics of human atrial tissue and how they are altered by stretch remains to be explored. It is expected that this series of experiments will demonstrate the presence of stretch-activated currents and provide a connection between pathology and susceptibility to atrial arrhythmia. The use of the various SAC blockers and activators should aid in clarifying the population(s) of SACs involved in human atrial MEF.

Together, the individual phases of the project will provide insight into the electro-physiological changes that occur in the heart and human atria in response to stretch. In addition, it will provided a physiological basis for the initiation and maintenance of many types of cardiac arrhythmia. Most importantly, however, it will demonstrate the importance of MEF and SACs and their contribution to the normal cardiac cycle.

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Research Plan:

Whole Heart Experiments

Langendorff experiments will aim to demonstrate the role of MEF in the normal cardiac cycle. Pharmacological agents known to affect the function of SACs will be added to the perfusate of Langendorff heart preparations, and any resultant changes in the Frank-Starling (length/tension-force) relationship and MAPs investigated. In addition to these initial experiments, phase 2 of the project will be approached through a separate series of Langendorff experiments where the left ventricle is subject to a series of cardiac-cycle timed sudden ventricular stretches.

Protocols

Preparation of Langendorff Heart:

Experiments will be conducted on hearts isolated from rats. In brief, male Sprague Dawley rats (300-350 g) are fully anaesthetised with 0.6–0.7 ml sodium pentabarbitone in 2000 units of heparin. The heart will then be quickly excised and mounted on a glass cannula attached to a Langendorff perfusion system. The heart will then be perfused retrogradely through the aorta at the rate of 15ml/min with a suitable physiological solution at 37^0C.

A small latex balloon will be tied onto the end of a steal catheter attached to a fluid filled transducer and callibrated syringe. The balloon, catheter and transducer are filled with a 50:50 distilled water-ethanol solution (free of air bubbles). The left atrium is then cut from the heart. The balloon is then inserted into the left atrium, and passed through the atrial-ventricular valve into the left ventricle. A purpose made electrode will then be gently pressed against the outer wall of the left ventricle. The electrode will be used to deliver a 3 volt, bipolar stimulus at a frequency of 5 pulses per second and of 0.5 ms duration for the duration of the experiments.

A third electrode, the MAP electrode (type to be decided upon) will be set up in a similar manner to that of the stimulus electrode. A metal clip placed around the aorta of the heart will form the ground electrode for MAP recordings.

Role of MEF in the Frank-Starling Relationship:

The will be allowed to stabilise with the LV balloon in place for 20 minutes (until electropphysiological and mechanical stability is achieved) prior to experiments. For each heart, a series of 3-4 repeat Starling curves are then to be generated for each of the experimental conditions as follows:

1. Control Starling curves
2. 1^{st} concentration of SAC blocker/activator.
3. Re-control for Starling curves
4. 2^{nd} concentration of SAC blocker/activator
5. 3^{rd} concentration of SAC blocker/activator
6. Re-control following 10-15 min washout

Each Frank-Starling curve is obtained through 2.5 mmHg step-wise increments in the diastolic pressure (balloon volume) from 0 to 25 mmHg and down again to 0 mmHg. Between each addition or washout of a drug, 15 minutes perfusion time is allowed to ensure uniform distribution or washout of the drug in the heart between each experimental condition.

Rapid Ventricular Stretch Experiments:

These experiments will be conducted in a similar manner to that used in the Frank-Starling curves. However, rapid balloon volume changes will be precisely controlled and delivered via a servo-motor system whose timing will be dependent on the cardiac cycle (similar to Zabel, Koller, Sachs & Franz 1996). The computer and Powerlab software will be used in series with a delayed trigger driven by the stimulator (used to pace the heart) to time balloon inflations (during both systole and diastole). Various physiological diastolic pressures, like those used for the Frank-Starling curves, will be used to determine servomotor-injected balloon volumes.

Drugs and Concentrations:

1. Streptomycin (20, 50 and 100 μM, to block all SACs[5, 24])
2. Gadolinium (1, 10 and 20 μM to block all SACs[31,54])
3. GsTx-4 (10, 200 and 500 nM, to block all SACs selectively[6])
4. Chlorpromazine (0.1, 0.5 and 1 μM, to block SAPCs[49])
5. Chloroform (0.1, 0.4 and 0.8 mM, to stimulate SAPCs[49])
6. Riluzole (10, 50 and 100 μM, to stimulate SAPCs[17])

Human Atrial Tissue Experiments:

These experiments will aim to demonstrate the presence of MEF and SACs in human cardiac tissue. Human right atrial tissue will be obtained from the Cardiac surgery theatre at the Royal Adelaide Hospital, under existing collaborative arrangements. The tissue will be mounted on a purpose made electro-mechanic set-up and the tissue stretched with known amounts of tension using special micro-manipulators normally used in patch clamp experiments. The tissue will be placed in a purpose made perfusion chamber, with one end of the tissue connected to a force transducer attached to a rigid post. The other end of the tissue sample will be connected to the micromanipulator. Electrophysiological recordings will be made using fine intracellular electrodes filled with 3M KCl. The changes in the action potential morphology with stretch will be used as an index of MEF. The same pharmacological agents known to affect the function of SACs in animal models will then be added to the perfusate, and any resultant changes in action potential morphology during stretch investigated. This technique of intracellular impalement has been used successfully in the lab in the past and recently by Dr Daniel Ninio when assessing the effects of antriarhythmic drugs.

Data Collection:

Changes in Left Ventricle diastolic, systolic pressurse, rate change of force of contraction, perfusion pressure and MAP for Langendorff and AP morphonolgy for human tissue experiments are to be monitored and recorded real-time on computer using Chart recorder version 4 (Powerlab, ADInstruments, 2001) .

Data Analysis:

Changes in pulse pressure (systolic pressure – diastollic pressure), and rate of force of contraction for Langendorff experiments will be compared between control and drug exposed conditions for each heart. Concurrently, the amplitude, duration and rate of decay will be analysed for all MAP recordings. All measurements will be determined using chart recorder analysis tools. Each of these parameters are expected to demonstrate and aid in the characterisation of MEF and the SACs involved in various conditions in both animal and human atrial models.

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Budget Justification:

For the majority of the above Langendorff experiments, the equipment is freely available in either the department or David Saint's laboratory. However, for the studies involving small, abrupt, timed inflations of the left ventricular balloon, apparatus able to trigger these inflations in synchronisation with the cardiac cycle is required. It is likely that a solenoid driven calibrated syringe controlled by a triggerable amplifier with adjustable delay will be suitable. In addition, a suitable MAP electrode will be required (if one cannot be made).

With regard to the use if human atrial appendage, preliminary experiments conducted by Dr Daniel Ninio were conducted using borrowed equipment which has since been returned. Thus, it has become necessary to purchase the equipment involved. Equipment to be purchased is primarily the high impedance input amplifier and a force transducer to measure tissue stress.

Thus, other than a small salary request for a part-time laboratory technician, the main requests for the budget are with regard to replacing equipment that has been borrowed from other laboratories. In addition, other items such as a calibrated syringe and suitable servo-motor/amplifier combination form the third largest expense.

Expected Time Table:

Year	2003		2004	2005	
Period	**January - July**	**July - December**	**January - July**	**July - December**	**January - July**
Phase I: Involvement of MEF in the Frank-Starling (length-tension) mechanism	Implament Techniques, Examine MEF/SAC involvement in Frank-Starling mechanism - how the mechanism is altered by pharmacologically induced changes in MEF/SACs	Preparation of data regarding involvement of MEF and SACs in Frank-Starling mechanism to be published	Publish data regarding involvement of MEF and SACs in Frank-Starling mechanism, complete any additional experiments required		
Phase II: Involvement of MEF in the isolated heart		Begin establishment of technique for transiently inflating left ventricular balloon	Establish technique for rapidly and accurately inflating balloon in left ventricle - Examine involvement of MEF/SACs in normal cardiac cycle	Preparation of data regarding involvement of MEF and SACs in normal cardiac cycle to be published	Attempt to publish data regarding involvement of MEF and SACs in normal cardiac cycle, complete any additional experiments required
Phase III: MEF in Human Atrial Tissue				Manufacture of a purpose made tissue bath for human atrial tissue experiments. Establish technique for accuratelyrecording MAPs in human atrial tissue during stretch	Examine and define components of MEF in human atrial tissue, and the involvement of SACs

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