The effects of wall stretch on ventricular conduction and refractoriness in the whole heart

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Background
Many studies have linked volume overload or increased myocardial strain (wall stretch) with atrial and ventricular arrhythmias\(^1\). Although the mechanisms for these mechanical load-induced rhythm disturbances remain unclear, they generally involve either triggered activity or re-entrant conduction\(^2\). Though both of these mechanisms may contribute, re-entry is the predominant mechanism underlying ventricular arrhythmias associated with mechanical dysfunction\(^3\). Stability of a re-entrant circuit depends on the constant presence of excitable tissue ahead of the activation wavefront. Slowed conduction and decreased refractoriness can therefore promote and sustain re-entry, while increased dispersion of conduction velocity and refractoriness can provide a substrate for its initiation\(^4\). Observations on the acute effects of myocardial strain on conduction velocity have varied widely with experimental preparation, mechanical loading conditions and measurement techniques, while findings on the stretch-dependence of effective refractory period have been somewhat more consistent. Given the inherent difficulties in measuring regional myocardial mechanics and conduction velocity in the whole heart without disturbing mechanical or electrical properties, many of the discrepancies between experimental reports are probably attributable to differences in experimental methods or definitions.

The effects of myocardial stretch on conduction velocity
Factors influencing speed and path of conduction
Intracellular conduction is determined by passive membrane capacitance, longitudinal resistances and voltage-dependent membrane conductances\(^5\). Membrane capacitance is a function of the dielectric properties of the membrane and the membrane surface area to cell volume ratio. Increasing membrane capacitance slows the initial depolarization from resting potential to threshold and reduces conduction velocity. Intracellular and extracellular longitudinal resistances determine how rapidly ions travel in the direction of conduction, and are affected by the effective internal and external cross-sectional areas available for ion transport.

The voltage-gated fast sodium current usually determines how rapidly the local membrane depolarizes (action potential phase 0), creating the electrochemical gradient that drives longitudinal ion diffusion. The conductance of these channels is influenced by channel kinetics and other regulatory processes, such as ligand gating and autonomic stimulation\(^6\). Altering resting membrane potential influences conduction speed in a biphasic manner\(^7\). Model studies suggest that this effect acts through membrane excitability\(^8\), as depolarization of the resting membrane potential decreases the charge required to reach threshold, but increases sodium channel inactivation, decreasing conduction velocity.

Intercellular conduction is primarily regulated by the conductance of gap junction. Gap junction open probability is sensitive to ischaemia, pH, intracellular cation concentrations and transjunctional voltage\(^9\). Activation wavefront curvature also affects propagation speed; a convex wavefront must stimulate an expanding volume of resting tissue, imposing a greater electrical load and slowing conduction\(^10\).

The path of conduction through the myocardium is a function of gap junction distributions, myocyte branching, fibre angle dispersion, the laminar sheet organization of the myofibres and the presence of connective tissue septa within the interstitium. The direction of fastest propagation follows the myocardial fibre direction due to the distribution of gap junctions within the cell\(^9\), with fibre conduction speed typically being two- to tenfold faster than...
the cross-fibre direction. Within the ventricle, fibre angles follow a left-handed helix in the epicardium and smoothly transition to a right-handed helix in the endocardium. Therefore, as activation spreads transmurally the principal axis of fastest in-plane propagation rotates and the wave front changes shape. Transverse to the fibre direction, cardiomyocytes are stacked into branching laminar sheets about 4–6 cells thick surrounded by a perimysial collagen fascia. A model reconstruction of tissue microarchitecture showed that this organization results in conduction across the sheet planes being slowed by 40% compared with cross-fibre conduction within the sheets.

**Determinants of regional wall stress and strain**

Myocardium and single myocytes are viscoelastic at rest, with a time constant for stress relaxation of about 1,000 s, and exhibit mechanical preconditioning behaviour and strain softening during repeated loading cycles. Myofilament activation also causes stress and strain to vary phasically throughout the cardiac cycle. In studies of the effects of mechano-electric feedback on conduction velocity in the normal heart, diastolic mechanics are the most important determinant as electrical activation precedes systolic contraction, but may be influenced by myofilament interactions when relaxation is incomplete at high heart rates. At lower heart rates, afterload alterations have little direct influence on conduction velocity, but may significantly affect repolarization and refractoriness. Finally, cardiac geometry and fibre architecture results in significant mechanical anisotropy and heterogeneity. Changes in conduction path are determined by strain, but whether cellular responses are determined primarily by stress or strain depends fundamentally on the relative compliance of the molecular mechanotransducer, such as a stretch-activated ion channel.

**Measuring conduction velocity during myocardial stretch**

Conduction velocity in one direction is usually calculated as the distance between recording electrodes aligned perpendicular to the wavepath divided by the interelectrode conduction time. In this case, the distance between recording sites and the number of myocytes per unit physical length can change during loading. Therefore, conduction velocity has been defined with both a spatial and material reference. Spatial conduction velocity is defined with a constant interelectrode distance before and during stretch. Material conduction velocity is defined between the same two physical points on the myocardium; thus material conduction velocity describes mechano-electric coupling effects over a constant segment of tissue.

However, to account for potential changes in conduction path with stretch in studies of two- or three-dimensional conduction, a highly resolved spatial description is most appropriate. In practice, to determine the two-dimensional conduction direction, the conduction time is sampled from an array of positions, and the gradients of activation time are used to calculate local conduction velocities. High spatial resolution sampling is achieved using contact electrode arrays or high speed optical mapping of action potential propagation with a fluorescent voltage-sensitive dye. An advantage of optical mapping being that it is non-contact, diminishing the chance of mechanical artifacts during measurement.

**Effects of stretch on conduction velocity in the heart**

Early investigations into the effects of myocardial stretch on conduction speed were limited to one-dimensional propagation. In studies of ventricular and atrial strips of myocardium from various species, stretching from slack length to the length of maximal developed tension caused a proportionate increase in spatial conduction velocity while material conduction velocity remained nearly unchanged, but additional stretch caused slowing of both measures. A similar biphasic relationship between spatial conduction velocity and stretch has been observed in more recent studies in isolated rabbit papillary muscle. Faster spatial conduction during stretch was also observed in sheep Purkinje fibres. In canine Purkinje fibres, both spatial and material conduction velocity initially increased with stretch and then decreased, but conduction in cat trabeculae was not similarly affected. Conversely, spatial conduction velocity in rat papillary muscle decreased with stretch, while papillary muscle from several other species showed no effect. Despite the varied effects of stretch across different structures, tissue types and species, most of these studies imply that conduction velocity may increase with stretch. However, these studies mostly concentrated on specialized structures, and after being excised these tissues were not subject to the same multiaxial constraints as in vivo.

Other studies have focused on the effect of physiological loading on whole chamber activation times. The dog heart in vivo, QRS duration correlated with acute increases in left ventricular pressure, while left atrial dilation increased atrial activation time, and ventricular volume inflation in isolated rabbit hearts increased maximal activation times. In contrast, one study of volume load in canine ventricle in vivo reported no change in spatial conduction time, while cellular conduction velocity increased during volume load of rat atrium. These whole chamber studies indicate that stretch slows material conduction; however, they do not directly compare measures of conduction speed or path.

More recent studies have investigated the effects of stretch on two-dimensional epicardial surface conduction using electrode arrays, in order to directly study the path of propagation. In volume-loaded left ventricle of isolated rabbit hearts after ventricular cyaoblation of all but a thin epicardial layer, neither graded load nor changes in pacing cycle length significantly alter fibre or cross-fibre conduction velocities. The authors acknowledged that the cyaoblation procedure stiffened the ventricle, possibly protecting the viable layer from mechanical stimulus. Conversely, studies in the isolated rabbit atria displayed decreased spatial conduction velocity as well as increased dispersion of conduction velocity, altered direction of propagation and increased occurrence of local conduction block during myocardial stretch.

Sung et al. used non-contact optical mapping during left ventricular volume loading in isolated rabbit heart, and employed a model-based analysis technique that accounted for epicardial curvature and changes in conduction path and allowed comparison of conduction velocity with local fibre direction. An increase of intraventricular pressure from 0 to 30 mmHg resulted in heterogeneous epicardial fibre and cross-fibre strains on the order of 3% and 1.5%, respectively, and a 16% decrease in transverse spatial conduction velocity. Figure 25.1 shows an example of the increase in activation times due to ventricular volume loading and the resulting velocity vector field. It also illustrates that application
of stretch can alter the path of conduction. Conduction velocity returned to baseline when load was removed. Similar results were obtained by our group in a more recent study in the isolated rabbit ventricle(31). Additionally, we see comparable conduction slowing in the freely beating isolated mouse heart during pressure loading of the left ventricle. In an alternative preparation, optical mapping revealed a 7.5% slowing of conduction velocity in monolayers of neonatal rat ventricular cardiomyocytes cultured on an elastic membrane during stretch(32) (see Chapter 20 for more details on mechano-electric coupling in cell cultures). These two-dimensional studies that also include changes in conduction path more consistently show conduction slowing due to stretch.

**Potential interactions of stretch with factors that influence conduction velocity**

Myocardial stretch may affect conduction speed in a number of ways. Stretch has been reported to depolarize the resting membrane potential(33), and this may be sufficient to slow conduction through fast sodium channel inactivation. Inward current through cation non-selective stretch-activated channels (SAC_Na) may depolarize the resting membrane during stretch. Several of the effects of mechanical coupling in myocardial preparations have been seen to be blocked by non-specific inhibitors of SAC_Na, including gadolinium or streptomycin(34). However, Mills and colleagues found that conduction slowing during ventricular filling was likely due to changes in excitability and not attenuated in the presence of gadolinium(31) or streptomycin(29). Sustained stretch may also increase resting membrane potential through altered cellular calcium handling(35). Myofilament binding sensitivity to calcium increases with stretch, and prolonged stretch results in a slow increase in the calcium transient, which subsequently interacts with other currents. However, increased resting potential should lower pacing threshold, but ventricular filling has been reported to have no effect on(23,36) or to increase threshold(26). SAC_Na altered calcium handling or stretch-sensitive cellular signalling could also regulate the conductances associated with phase 0 of the action potential, thus decreasing the maximum rate of rise of membrane potential(6).

Alternatively, stretch may result in changes in tissue and cell geometry, changing the path of conduction or altering distributed electrical properties of the myocardium. Penevsky and Hoffman(15) postulated that increased one-dimensional spatial conduction velocity was the result of increased fibre alignment during stretch. Rosen et al.(19) observed that stretch caused significant membrane unfolding, decreased cell diameter and slightly increased packing of the extracellular space. Unfolding of ‘slack’ membrane and integration of caveolae into the surface sarcolemma has also been observed in the loaded intact rabbit ventricle in a more recent study(37). Cell stretch would result in an increased surface area to volume ratio and a reduced cell cross-sectional area, increasing the effective longitudinal intracellular resistance. Others suggested stretch might result in a decrease in specific membrane capacitance (capacitance/area)(17,18), possibly due to membrane unfolding, accounting for increased spatial conduction velocity during moderate stretch. However, recent data indicate that increased membrane tension results in an increase in capacitance(38).

Myocardial stretch might also alter conduction through changes in intercellular coupling. Gap junction permeability is regulated by several factors that might be regulated by stretch, including intracellular cation concentrations and cell signalling pathways (primarily through connexin phosphorylation)(9). Additionally, it has been demonstrated that connexin hemichannel permeability is sensitive to both shear strain(39) and membrane stretch(40), so mechanical forces at cell–cell junctions might directly affect gap junction conductance. However, it has been shown that rate of propagation is not very sensitive to changes in intercellular conductance at normal levels of cellular coupling(41).

Mills et al. implemented a bidomain model analysis of the membrane potential response to a non-excitatory stimulus to investigate changes in these myocardial electrical properties during ventricular volume loading(31). The results suggested that wall stretch resulted in a 21% increase in cross-fibre space constant, indicating reduced intercellular resistance that would increase conduction velocity, and a 56% increase in membrane capacitance, which would slow conduction (Fig. 25.2). Computational simulations implementing these counteracting changes were consistent with experimental findings of a 15% slowing of conduction during ventricular loading. This explanation of stretch-induced changes in conduction velocity might account for the varying results obtained in different experimental preparations if the interaction between these two changes in tissue electrical properties varies in different tissue preparations and loading conditions. Further studies are required to investigate the cellular mechanisms behind these changes in passive electrical properties of the myocardium and the resulting change in conduction velocity. Development of similar experimental techniques in the isolated mouse heart provides a platform to conduct such studies, taking advantage of the availability of genetically modified murine models.
Effects of stretch on effective refractory period in whole heart

A number of studies have investigated the effect of stretch on ERP and APD in the atria. *In vivo* studies have had varied results, primarily attributed to differences in timing and duration of stretch, as seen in model studies\(^\text{[42]}\). In isolated preparations, ERP more consistently decreases with acute myocardial stretch\(^\text{[27,43-46]}\), but insignificantly increased in the isolated canine right atrium\(^\text{[47]}\). Despite these variations, studies have consistently shown that acute atrial stretch increases the dispersion of effective refractory periods (mechano-electric coupling in the atria is covered more thoroughly in Chapter 23 and in the review by Ravelli\(^\text{[41]}\)).

Studies on the effects of stretch on ventricular ERP have been more consistent across different species and experimental methods. In swine heart *in vivo*, an increase in afterload caused a greater decrease in ERP at the apex than the base, thus increasing dispersion of refractoriness\(^\text{[48]}\). Similarly, left ventricular loading in isolated rabbit\(^\text{[23,49]}\) and guinea pig\(^\text{[50]}\) hearts also decreased ERP. Increased preload decreased ERP in a manner that correlated better with increased diastolic wall stress, as estimated from end-diastolic pressure and ventricular geometry, than with increased systolic wall stress or diastolic circumference. Dilation also shortened ERP more significantly on the endocardial than epicardial surface\(^\text{[36]}\). This indicates that ERP may correlate better with cross-fibre than fibre stress or strain, since myocardial residual stress and torsion during filling allow a more uniform transmural distribution of fibre strain\(^\text{[13]}\) under load at the expense of cross-fibre strain gradients.

In rabbit, ventricular stretch caused by increasing preload also caused greater shortening of ERP as the drive cycle length decreased\(^\text{[26]}\). This finding was later corroborated in isovolumically contracting rabbit ventricles\(^\text{[36]}\). Reiter et al.\(^\text{[26]}\) suggest that this rate dependency may follow dependency on the stretch-sensitive delayed rectifier potassium current.

Those studies that also examined the effect of stretch on APD observed that mechano-electric feedback on late APD (typically analyzing APD\(_{90}\)) reflected the observed effects on ERP, decreasing with stretch in the ventricles\(^\text{[23,36,48,50]}\) and did not alter the ratio of ERP to late APD\(^\text{[36]}\). Several other studies have reported a decrease in late APD with stretch in various species including lamb, swine, guinea pig, rabbit, rat and human. However, some investigators report an increase in APD during stretch, primarily at late repolarization levels. Volume loading of the isolated rabbit ventricle caused an increase in both APD\(_{20}\) and APD\(_{80}\) (at 20% and 80% repolarization, respectively), suggesting ERP may increase with stretch\(^\text{[29]}\). Left ventricular APD\(_{90}\) increased during loading of the *in situ* canine heart\(^\text{[24]}\) and the isolated rat heart\(^\text{[51]}\). Chen et al. reported a regional decrease in APD\(_{25}\), APD\(_{50}\) and APD\(_{70}\) (APD at 25%, 50% and 70% repolarization, respectively) with increased local sarcomere length in the loaded right ventricle of sheep, but heterogeneously increased APD\(_{90}\) and incidence of early

The effect of myocardial stretch on effective refractory period

**Determinants and measurement of effective refractory period**

Effective refractory period (ERP) is the time interval following activation during which tissue is unable to activate again in response to the same stimulus, and thus is a measure of late-repolarization membrane excitability. ERP is determined by the voltage-regulated transition of fast sodium channels from the inactivated to the resting state, and is thus dependent on the time course of repolarization. Consequently, ERP is closely related to action potential duration (APD) and is also cycle-length dependent. One study observed that the ratio of ERP to monophasic APD at 90% repolarization (APD\(_{90}\)) remains nearly constant despite a 60% decrease in APD as cycle length decreases\(^\text{[36]}\).

ERP is typically measured after continuous pacing at a single cycle length to minimize dynamic variations in APD and other cellular kinetics. After this stabilization period, a stimulus is delivered at a shortened time delay (coupling interval). ERP is typically defined as the longest possible coupling interval that does not elicit a propagating action potential. Experimentally, action potentials are recorded directly with microelectrodes using a contact monophasic action potential electrode, or optically with a potentiometric fluorescent probe.
after-depolarizations. Similarly, several studies by Franz and colleagues also observe late APD lengthening in canine and rabbit, but see shortening at earlier levels of recovery. Franz suggests that depending on the stimulus strength used, mechanoelectric feedback on ERP may simply reflect changes in APD at varying levels; thus ERP could shorten during stretch reflecting shortening of early APD or lengthen with late APD (the effect of stretch on APD is covered in more detail in Chapter 37.)

**Potential interactions of stretch with factors that influence effective refractory period**

$SAC_{NS}$ and altered calcium handling have both been implicated in stretch-induced APD shortening, and consequently could influence ERP. Model studies indicate that the effect of stretch can have various influences on action potential shape depending on the relative contributions of $SAC_{NS}$ and calcium handling, both of which are sensitive to timing and intensity of stretch. Zabel et al. showed that including a length-dependent non-specific cationic conductance with a reversal potential near -30 mV could reproduce several experimental observations including early APD shortening and late APD lengthening during stretch, while Kohl et al. showed that a more moderate stretch can lead to an overall shortening of APD. Kohl further showed that stretch in a model that included sarcomere length-dependent calcium handling would produce an overall prolongation of APD if applied early or sustained throughout the action potential, but an overall shortening if applied during late repolarization.

Investigations into the mechanisms of stretch-induced ERP shortening by pharmacological interventions have yielded inconsistent results. The non-specific $SAC_{NS}$ blockers streptomycin and gadolinium attenuated or had a limited effect on acute stretch-induced changes in ERP and APD in some studies, but had no effect in others. The more specific $SAC_{NS}$ blocker GsMtx-4 did not block stretch-induced shortening of rabbit atrial ERP. This may be due to resistance of the potassium-selective $SAC_{NS}$ to these compounds, or conductance changes of other mechano-sensitive channels during load. Zarse et al. found that the L-type calcium channel blocker verapamil inhibited stretch-induced shortening of atrial ERP, suggesting the contribution of a length-dependent calcium handling mechanism, but others have observed no effect of verapamil on guinea pig and rabbit ventricular ERP shortening.

Increased dispersion of effective refractory period with stretch may follow inhomogeneous stress or strain distributions. A ventricular model study that coupled physiological fibre and cross-fibre strains to a stretch-dependent current within an action potential model predicted a nearly doubling of the dispersion of late APD. Finally, changes in electrotonic coupling could alter heterogeneity of repolarization in the myocardium, so changes in tissue architecture and gap junction conductivities may play a role in these arrhythmogenic effects of stretch on ERP.

**Summary**

Increased preload, afterload or sustained load typically decrease ERP; however, ERP follows APD, and some have observed APD prolongation at all levels of recovery. How stretch affects ERP is likely a result of the balance of several competing effects including $SAC_{NS}$ altered calcium handling and the timing and intensity of stretch, which could have varying levels of activation in different species and manners of stretch.

**Conclusions and outlook**

Recent evidence provides a basis to explain why stretch is associated with arrhythmias, particularly re-entrant forms. Both atrial and ventricular stretch slow spatial conduction, while atrial stretch increases conduction velocity dispersion and increases the occurrence of local functional conduction block, all of which promote re-entrant arrhythmias. These stretch effects may correlate with the application of diastolic mechanical load, but correlation with stress or strain is less clear owing to regionally heterogeneous cardiac geometry, structure and time-dependent material properties. Stretch may affect conduction velocity through altered effective cellular coupling, tissue and cellular level geometric changes, changes in intracellular resistance and membrane capacitance or alterations in excitability, particularly an increase in resting membrane potential due to the activity of $SAC_{NS}$ or altered calcium handling.

In general, both atrial and ventricular stretch decrease ERP, but stretch consistently increases dispersion of ERP, both of which are associated with increased incidence of re-entrant arrhythmias. The effects of stretch on refractoriness parallel effects on APD, which may vary as a function of the relative activation of competing mechanisms, $SAC_{NS}$ and altered calcium handling. Future studies should aim to identify the exact underlying cellular mechanisms responsible for these arrhythmogenic stretch-induced changes in conduction velocity and refractoriness. New experimental techniques and development of genetically modified animal models can provide a platform for these investigations in hopes of identifying potential therapeutic targets to prevent arrhythmias associated with mechanical dysfunction.

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**References**


