Altered Cardiac Myocyte Ca Regulation In Heart Failure

Ca is critical in both the electrical and mechanical properties of cardiac myocytes, and much is known about ionic currents and the normal excitation-contraction coupling process. In heart failure, there are significant alterations in how myocyte Ca is regulated, and these alterations are critical in dictating both contractile dysfunction and certain cardiac arrhythmias that are characteristic of heart failure.

The basic excitation-contraction coupling (ECC) process in ventricular cardiac myocytes (4, 6) begins with the action potential (AP). The initial depolarization (mainly due to Na channels) activates I-type Ca current (\(I_{Ca}\)), which triggers Ca release from the sarcoplasmic reticulum (SR) by local Ca-induced Ca release (FIGURE 1). This elevates cytosolic [Ca] ([Ca],) which causes Ca binding to the myofilament protein troponin C, which activates contraction. For relaxation (and refilling of the heart during diastole), Ca must be transported out of the cytosol, thereby allowing Ca dissociation from troponin and deactivation of the myofilaments. The [Ca]i decline is mediated by the 1) SR Ca-ATPase, 2) sarcolemmal Na-Ca exchange (NCX), 3) sarcolemmal Ca-ATPase, and 4) mitochondrial Ca uniporter. Under normal conditions (in mammals larger than rat), the SR Ca-ATPase takes up ~70 of the Ca involved in the Ca transient, whereas nearly 30% is extruded via NCX, leaving a tiny amount (~1% each via the two “slow” mechanisms). The amount of Ca that enters the cell at each steady-state beat must equal the amount that is extruded (and likewise for the SR). Thus the integrated Ca entry via \(I_{Ca}\) is about the same as that extruded via NCX, and the amount released by the SR is the same as that taken back up. Note that both Ca entry via \(I_{Ca}\) and Ca extrusion via NCX cause inward current (3 Na in coupled to 1 Ca out). This couples Ca movements strongly to electrophysiological effects. The following review of alterations in HF cannot be comprehensive (especially with respect to references), but I will try to give an overview of many important systems in a personal perspective, while acknowledging key areas where controversy exists as space permits.

Ca Handling Alterations in HF

Ca removal during relaxation is mainly due to SR Ca-ATPase and NCX (as above). In HF, there is typically a downregulation of SR Ca-ATPase and upregulation of NCX function (36, 42, 43). Both of these effects tend to shift Ca out of the cell and reduce SR Ca content (see below). That is, during [Ca]i decline, NCX and SR Ca-ATPase compete for Ca, and the more that is extruded from the cell (by NCX) the less SR Ca-ATPase can take up. This reduces the SR Ca available to be released during subsequent ECC. In most HF models, overall [Ca]i decline is slowed, and this contributes to diastolic dysfunction (hampering diastolic ventricular filling). However, we also find that a large increase in NCX function can compensate for a moderate decrease in SR Ca-ATPase function in HF such that the rates of [Ca]i decline and relaxation are relatively normal in our rabbit HF model (42, 43), despite the reduced SR Ca content that both effects cause. The balance may differ among different HF stages and etiologies. For example, in end-stage human HF, we found that the reduction in SR Ca content and rate of [Ca]i decline was primarily due to reduced SR Ca-ATPase function, with little alteration in NCX function (37). Hasenfuss et al. (20) found two groups of HF patients, one with relatively normal diastolic function where NCX expression was elevated and SERCA expression was reduced (like our rabbit model), and another group with diastolic dysfunction with lower SERCA expression but relatively unaltered NCX expression. Thus there is a spectrum of HF phenotypes with respect to Ca removal during relaxation by the SR Ca-ATPase and NCX. It is unknown whether these SR Ca-ATPase and NCX changes are progressive or orchestrated or indeed how they are controlled during HF progression.

Ca influx occurs mainly via \(I_{Ca}\) although a small amount of Ca can enter the cell via NCX during the cardiac AP (4). Most HF studies have found relatively unaltered \(I_{Ca}\) density in ventricular myocyte. However, some have suggested that there may be some reduction in the number of Ca channels, but with higher activity per channel (possibly via basal PKA-dependent phosphorylation), which shifts activation toward more negative membrane potential (\(E_m\)) (12, 49). However, since Ca transients are smaller in HF, there may be greater integrated Ca influx due to less Ca-dependent inactivation of \(I_{Ca}\). This can be seen as part of an intrinsic feedback system of Ca-dependent inactivation (wherein large Ca transients limit Ca influx, whereas small Ca transients encourage greater Ca influx). This intrinsic feedback of \(I_{Ca}\) is part of a bigger feedback system of Ca flux balance in myocytes, involving also NCX and SR Ca fluxes, as characterized by Eisner’s group (17).
NCX can work in either direction (Ca efflux or influx), depending on the intracellular and extracellular [Na] and [Ca], and also E_m (FIGURE 2). Under normal conditions, NCX works mainly in the Ca extrusion or forward mode (Na influx), and this is driven by both elevated [Ca]_i and negative E_m. Thus AP repolarization enhances Ca extrusion by NCX as [Ca]_i decline proceeds during relaxation. However, during the very early rising phase of the AP, positive E_m can drive Ca entry via NCX, especially before local [Ca]_i rises (which shifts NCX toward Ca efflux). Under normal conditions, this is a very small quantity of Ca as NCX is quickly reversed by rising [Ca]_i near the membrane (61). Indeed, the submembrane [Ca]_i rises earlier and faster than the global [Ca]_i, because I_{Ca} and SR Ca release are both near this region. In HF, the smaller Ca transient, the elevated intracellular [Na] ([Na]_i), and prolonged AP duration all tend to increase the amount of Ca entry via NCX during the AP (16, 61, 62). Thus, in HF, NCX can bring in a significant amount of Ca to support contraction, thereby limiting the extent of contractile dysfunction. Of course, all of the Ca that enters via I_{Ca} and NCX must still be extruded by NCX in HF, and this may explain why NCX expression and function is typically upregulated in most (but not all) HF models.

The enhanced Ca influx across the sarcolemma in HF must be balanced by greater Ca extrusion via NCX for the cell to be in steady state Ca balance. In principle, enhanced sarcoplasmal Ca-ATPase function could also occur, but there is little data to suggest that this is significant. Transporting more Ca back and forth across the sarcolemma at every beat carries an energetic expense as well, which may add to the limited energetic reserve in HF. This is because the net energetic cost for pumping Ca into the SR is lower (2 Ca/ATP) vs. sarcolemma (1 Ca/ATP, whether direct on the sarcoplasmal Ca-ATPase or indirectly in exchange for 3 Na, which require 1 ATP to be extruded by the Na-K-ATPase).

SR Ca content is typically depressed in HF (37, 43, 22), and three main factors contribute to this: 1) enhanced NCX function, 2) reduced SR Ca-ATPase function, and 3) enhanced SR Ca leak. The enhanced NCX function seems to be driven mainly by higher

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FIGURE 1. Ca And Na transport in ventricular myocytes
Transport pathways are indicated. Inset: example action potential (AP), Ca transient, and contraction for a rabbit ventricular myocyte at 37°C. ATP is ATPase (SR and sarcolemmal); other abbreviations as in text.
probability in single-channel lipid bilayer recordings (34). The first cellular demonstration of enhanced diastolic SR Ca leak in intact ventricular HF myocytes was made by Shannon et al. (52), who had developed a novel quantitative method to assess SR Ca leak in intact myocytes. Marks’ group (27, 34, 63, 64, 66) developed an elegant molecular hypothesis to explain this enhanced diastolic RyR2 opening in HF in a series of experimental tests. They propose that RyR2 is hyperphosphorylated at Ser-2809 by PKA (due to the hyperadrenergic HF state, lower RyR-associated phosphatases, and phosphodiesterases) and that this phosphorylation causes the RyR2 to release FKBP12.6 (an immunophillin that binds FK-506) with consequent enhancement of RyR2 opening. Although they have produced impressive data that all fits this hypothesis, several groups have been unable to confirm certain key aspects. This includes findings that PKA does not increase RyR2 phosphorylation in HF or cause FKBP12.6-RyR2 dissociation, that PKA activation does expression levels [mRNA and protein level (42)]. Indeed, the thermodynamic driving forces on NCX (high [Na], low [Ca], and longer AP duration) limit Ca extrusion at least during the AP plateau. The reduced SR Ca-ATPase function may be due in large part to reduced expression of SERCA2a in HF, but there may also be less downregulation of phospholamban (PLB; which inhibits SERCA unless phosphorylated) such that the SERCA-to-PLB ratio is reduced. PLB may also be less phosphorylated in HF, an effect that may be due to increases in global phosphatase expression in HF myocytes (32). This would result in even greater SR Ca-ATPase inhibition. Interesting also is the observation that functional PLB knockout in humans causes HF, whereas the gene-targeted PLB-knockout mice have no apparent cardiac problems (32). This raises a cautionary note about extrapolating mouse data to human HF.

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not increase steady-state RyR2 activation, and that there are additional PKA sites on RyR2 (26, 29, 55, 68). CaMKII is also part of the RyR2 macromolecular complex (which includes PKA, phosphatases 1 and 2A, calmodulin, and FKBP12.6), and CaMKII-dependent RyR phosphorylation can activate RyR2 and diastolic SR Ca release (1, 7, 33, 47, 65, 67). We found that in HF rabbits there is more CaMKII expression, association with RyR2, and activation state, and that the enhanced SR Ca leak was prevented by inhibiting CaMKII but not PKA (1). Notably, CaMKII blockade restored SR Ca content but only modestly enhanced Ca transient amplitude (1). This may be because CaMKII increases RyR Ca-sensitivity to cause both enhanced diastolic leak and enhanced fractional SR Ca release during systole (28). Thus CaMKII-dependent RyR phosphorylation may reduce SR Ca content, but because it also enhances fractional SR Ca release, it may contribute little to systolic dysfunction in HF (but see arrhythmogenesis below). Moreover, although there may be enhanced SR Ca leak in HF, the details of how it occurs and its functional consequences remain controversial.

SR Ca release is critical in ECC. The smaller Ca transients, despite enhanced Ca influx, must be due to lower SR Ca release. Both fractional SR Ca release during ECC and diastolic SR Ca leak have similar dependence on SR Ca content (FIGURE 3). That is, when SR Ca content is less than 40% of the normal steady-state level (or free intra-SR [Ca] ~400 nM), there is very little leak and normal \( I_{Ca} \) cannot trigger appreciable SR Ca release (3, 17, 51, 52). However, when SR Ca content increases above the normal range, SR Ca leak and fractional release increase very steeply. The steep rise in diastolic SR Ca leak means that the maximal SR Ca content is limited by this leak rather than by the SR Ca-ATPase approaching its thermodynamically limiting [Ca] gradient (i.e., \( \Delta G_{ATP} = RT \ln([Ca]_{SR}/[Ca]_i) \)). In HF, SR Ca leak is higher at any given SR Ca content. This results in a lower SR Ca content at which SR Ca leak and Ca-ATPase rates are in balance, and also limits the maximal SR Ca content attainable. When leak is enhanced in HF, this again has energetic consequence. That is, if the SR Ca-ATPase pump is engaged in futile cycling just to maintain SR Ca against a large leak, it will be costly in terms of ATP consumption and thereby influence the energetic reserve. Given the low SR Ca content in HF, one would expect that fractional SR Ca release would be depressed. However, in rabbit, canine, and human HF,
fractional SR Ca release is relatively normal (comparable reductions in SR Ca content and Ca transient amplitude; Refs. 22, 36, 37, 43). This may be a consequence of the enhanced CaMKII-dependent RyR phosphorylation that we see in HF, and this may minimize contractile dysfunction. On the other hand, some investigators have reported reduced ECC efficacy or gain in HF (18), although not all studies have measured all three key ECC parameters in HF (\(I_{\text{Ca}}\), SR Ca content, and twitch Ca transients). The reduced SR Ca release in some studies (even with comparable \(I_{\text{Ca}}\)) may simply be attributable to the lower SR Ca content and the intrinsic effect to reduce fractional release (e.g., in cases where RyR sensitization may not occur in HF). Some (but not all) groups who have examined RyR2 expression levels in HF have also found some reduction. Another intriguing possibility is that there could be structural changes in the ECC system that weaken the fidelity of ECC. Gómez et al. (18) proposed that there might be increased distance between the Ca channel and RyR in the junction. Although this has not been physically observed, it has not been ruled out.

There have been reports in HF of diminished density of transverse-tubules (21, 30, 31). Since 75% of the junctions for ECC in rat and rabbit ventricle are in the transverse tubules (vs. surface sarcolemma; Ref. 4), this could decrease the number of junctions activated by local \(I_{\text{Ca}}\). However, in other HF models, we and others have not observed major alterations in either transverse-tubule density, spatial drop-out in ECC, or cell surface-to-volume ratio in HF. Note that increases in transverse tubules would be required to prevent a reduction in surface-to-volume ratio in the much larger HF myocyte. Moreover, what looked like a spatial drop-out in ECC efficacy in feline HF myocytes (i.e., possibly missing transverse tubules) was shown to be attributed to the altered AP waveform with much less transient outward current \(I_{\text{to}}\) in HF (19). That is, without the early repolarization driven by \(I_{\text{to}}\), the early AP plateau is more positive and decreases the driving force for \(I_{\text{Ca}}\) and thus limits functional activation (48).

Overall, evidence seems to indicate that there is neither a reduction in \(I_{\text{to}}\) trigger nor in how the RyR responds to a given \(I_{\text{Ca}}\) (and SR Ca content). Rather, the main SR Ca release defect in HF seems to be reduced SR Ca content, which by itself would strongly depress fractional release for a given \(I_{\text{Ca}}\). In fact, it seems likely that the RyR sensitivity may even be enhanced (for a given \(I_{\text{Ca}}\) and luminal SR [Ca]), perhaps as the result of RyR phosphorylation. Although some structural alterations in ECC efficacy may occur in certain HF models or stages, this issue remains incompletely resolved.

**Altered Na Regulation in HF**

Ca extrusion in cardiac myocytes depends almost completely on the NCX system, and small perturbations in [Na\(^+\)] can greatly influence Ca transients and contractions. [Na\(^+\)], is elevated by about 3 mM in rabbit and human HF myocytes at rest or during regular stimulation (2, 16, 39, 44). As indicated above, the elevated [Na\(^+\)] shifts NCX to favor more Ca influx in HF, and this may better maintain SR Ca load and thus contractility in HF. If [Na\(^+\)] did not increase, then SR Ca content and systolic function would be even lower.

So why is [Na\(^+\)] elevated in HF? It must be due to either enhanced Na influx or reduced Na extrusion. The main Na influx pathways are NCX, Na current \(I_{\text{N}}\), and Na-H exchange (NHE), contributing about 66%, 20%, and <5%, respectively, to total Na influx in rabbit ventricular at 1 Hz (8). Na efflux is mediated mainly by the Na-K-ATPase (NKA), and NKA expression appears to be reduced in HF (10, 50). However, functional measurements show that the [Na\(^+\)] dependence of Na extrusion via NKA can be relatively unaltered in HF and that the reason [Na\(^+\)] is elevated is because Na influx is higher (2, 16). In our rabbit HF model, most of the extra Na influx was prevented by the very specific Na channel blocker tetrodotoxin (even at rest), indicating a role for Na channels (16). This may relate to observations that, in two canine HF models and human HF, there is a more prominent slowly inactivating Na current in ventricular myocytes (57, 58). Using almost the same rabbit HF model we used, Baartscheer et al. (2) showed that the elevated [Na\(^+\)] in HF was cariporide-sensitive, suggesting an important role for NHE. Since NHE was also upregulated in HF, this seems plausible, but the concentrations of cariporide used also inhibit slowly inactivating \(I_{\text{Na}}\) (11), leaving the role of NHE in [Na\(^+\)] elevation in HF somewhat equivocal. The role of slowly inactivating \(I_{\text{Na}}\) also merits further exploration, because this current can contribute to AP prolongation and triggered arrhythmias (see below).

If NKA expression is reduced in HF, why is NKA function in myocytes relatively normal? There are two logical explanations: 1) some NKA molecules are nonfunctional (e.g., due to internalization or inactivation), and 2) NKA activity is differentially regulated in HF. There is no information on the first possibility, but altered NKA regulation by the accessory protein pholemmman (PLM) in HF has emerged as a real possibility (10). PLM is a member of the FXYD protein family (56), of which there are 7 (FXYD-1 to -7, where PLM is FXYD-1). These FXYD proteins co-immuno-precipitate with NKA and seem to be tissue-specific modulators of NKA. For example, FXYD-2 (called NKA γ-subunit) and FXYD-4 are expressed differentially in kidney regions and alter NKA function differentially. PLM is highly expressed in heart and is a major target for PKA- and PKC-dependent phosphorylation (45). Crambert et al. (14) showed that coexpression of PLM with NKA in oocytes reduced the Na affinity of NKA. We used PLM knockout mice vs. wild-type littermates and found that PLM inhibits NKA, mainly by reducing its affinity for internal Na (15). Moreover, in wild-type mice, β-adrenergic stimulation
reversed this inhibition and activated NKA function but had no effect in PLM knockout mice (15). β-Adrenergic stimulation of wild-type myocytes made the [Na], dependence of NKA similar to that found in the PLM-KO mice. These data suggest that PLM affects NKA function much like the way PLB affects SERCA (inhibition that is relieved by phosphorylation).

In HF, we found that PLM expression was reduced by 42% (more reduced than for NKA), and a higher percentage of PLM was phosphorylated in HF (rabbit and human; Ref. 10). Having less PLM in HF and with more of it phosphorylated, there is less overall NKA inhibition by PLM. Thus downregulation of NKA expression in this rabbit HF model may be functionally compensated by less PLM-dependent inhibition. This scenario may not be universal because short-term postmyocardial infarction rat hearts show increased PLM expression (69).

**Potassium Current Alterations Influence Ca Handling**

K currents also change in HF, and these can modify the AP and influence Ca handling. Let us keep this complex area simple for the present context. Reduced levels in HF have been reported for $I_{K1}$, delayed rectifier K currents ($I_{Ks}$ and/or $I_{Kr}$), and the inward rectifying K current that stabilizes the resting $E_m$ ($I_{K1}$; 4, 23, 35, 43). Although all of these reductions in outward current can contribute to the prolonged AP duration observed in HF, the AP prolongation is especially apparent at low heart rates. In addition, the detailed effects on Ca handling for each K current class differ somewhat. The reduction in $I_{Ks}$ can reduce the early AP repolarization (notch) and decrease the amount of $I_{Ca}$ during the early part of the AP because of a decreased electrochemical driving force (19, 48). This can reduce the amount of SR Ca release, or at least reduce the synchronization of SR Ca release, which is important for synchronizing contraction. Reductions in $I_{Ks}$ or $I_{Kr}$ prolong the plateau phase of the ventricular AP and keep $E_m$ at a relatively positive value for excess time. This decreases the ability of NCX to extrude Ca and can even lead to progressive Ca entry via NCX during the AP (especially in HF where [Ca], is relatively low and [Na], is relatively high). That can result in enhanced cellular Ca content. This can be inotropic but can also set the stage for spontaneous SR Ca release and triggered arrhythmias via delayed afterdepolarizations (DADs; see below). It can also keep $E_m$ in the range where $I_{Ca}$ can be reactivated (after recovery from Ca-dependent inactivation), and this has been implicated in early afterdepolarizations (EADs; see below). Finally, reduction of $I_{K1}$ tends to destabilize the diastolic $E_m$ and increase the likelihood that any inward current (e.g., Ca activated during a DAD) can bring $E_m$ to threshold to trigger an AP. Thus altered K channel function in HF can influence cellular Ca regulation in multiple ways.

**Systolic Dysfunction in HF**

Although many factors surely contribute to systolic dysfunction in HF (including myofilament properties, fibrosis, energetics, geometry, etc.), a very major factor is the reduced myocyte Ca transient amplitude. Many factors discussed above can influence the amplitude and kinetics of the Ca transient, but the reduction in SR Ca content seems of paramount importance in mediating the reduced Ca transient amplitude. As discussed above, three factors contribute to the reduced SR Ca content (reduced SR Ca-ATPase activity, enhanced NCX function, and enhanced SR Ca leak), each to variable degrees in different HF models or stages. However, to the extent that enhanced RyR-dependent SR Ca leak is due to higher sensitivity to [Ca], it may also enhance fractional SR Ca release and have little intrinsic effect on steady-state Ca transient amplitude. Thus systolic dysfunction in HF is more attributable to SR Ca-ATPase and NCX changes. The prolonged AP duration and elevated [Na], (and lower SR Ca release) in HF tend to enhance Ca entry and also limit Ca extrusion, thereby minimizing the net systolic dysfunction.

Ca transients in HF also typically rise more slowly, which may limit synchronous contractile activation (and be caused by both the low SR Ca content and reduction in $I_{Ca}$). [Ca], decline is also typically slowed in HF, largely due to depressed SR Ca-ATPase. This can compromise diastolic filling and increase refractoriness of ECC at the next beat (especially at higher frequency), both of which would further decrease systolic function. These factors, along with inability to increase SR Ca content at higher heart rate, contribute to the well-known negative (or blunted) force-frequency relationship that is characteristic of HF (38).

**Arrhythmogenesis in HF**

Many late-stage HF patients also experience arrhythmias and sudden cardiac death. In all nonischemic HF patients and 50% of ischemic HF patients, ventricular tachycardia (VT) is initiated by non-reentrant mechanisms (i.e., by triggered mechanisms such as DADs, EADs, and abnormal automaticity; Refs. 40, 41). All three of these mechanisms involve altered Ca handling in the myocyte. DADs are initiated by spontaneous SR Ca release and consequent Ca-activated inward currents that depolarize $E_m$ from the diastolic value toward the threshold to trigger an AP. The word “spontaneous” in this context may be a misnomer because we now know that this release is triggered by intra-SR [Ca] and recovery of the RyR from the prior excitation. In HF, the higher SR Ca leak may increase the likelihood of such Ca release events propagating as Ca waves, which activates a transient inward current ($I_{Ca}$) that causes a DAD. Although there are three Ca-activated currents that could potentially contribute to $I_{Ca}$ ($I_{NCX}$, a chloride current, and a nonselec-
tive cationic current), at least in human and rabbit ventricular myocyte (including HF) NCX is almost entirely responsible for $I_{Ca}$ and DADs (9, 43). Moreover, the higher NCX levels in HF mean that any given diastolic SR Ca release will cause greater $I_{Ca}$ in HF myocytes. The lower $I_{K1}$ level in HF myocytes (like in pacemaker cells) also means that a given inward current will produce a larger depolarization, which is more likely to trigger an AP. This mechanism could also be considered abnormal automaticity in the sense that SR Ca release, inward $I_{NCX}$ and low $I_{K1}$ levels are known to contribute to spontaneous cardiac pacemaker activity (5, 59).

So if SR Ca content is reduced in HF, why would DADs occur? In HF before the real end stages, there is residual β-adrenergic responsiveness and high sympathetic tone. This sets up the scene for local and possibly regionally heterogeneous β-adrenergic stimulation in the heart. This may lead to local regions of cells where SR Ca content is elevated to the point of activating spontaneous DADs, inducing SR Ca release events that may be synchronized by the prior normal activation. Since the SR Ca leak at any level of SR Ca content is higher in HF myocytes (52), DADs could occur at a lower than normal SR Ca content. This is consistent with human RyR2 mutations that are linked with catecholaminergic polymorphic ventricular tachycardia (CPVT), and where the mutant RyRs have enhanced Ca sensitivity (25, 46).

It is thought that EADs are in large part mediated by reactivation of $I_{Ca}$, which has recovered from Ca-dependent inactivation during long APs near plateau $E_{Na}$ once $[Ca]_{i}$ has significantly declined (24, 53). Thus the longer AP duration and smaller Ca transient in HF increase the likelihood of EADs. It would seem then that EADs and DADs differ mechanistically as well as at what $E_{Na}$ level they initiate from, but both are mediated by Ca fluxes. On the other hand, it also seems that some subset of events that look like EADs are initiated by SR Ca release and $I_{Na}$ (13, 54, 60). The longer AP duration at the low heart rates where EADs occur would also foster more Ca influx via Ca entry through both NCX and $I_{Ca}$ (especially in HF). This could load the SR and initiate SR Ca release. Ca mishandling may even be involved in the initiation of reentrant arrhythmias, based on its central role in the development of cardiac alternans and refractoriness of $I_{Ca}$ and RyR. However, that is beyond the present scope.

In conclusion, much is known about Ca handling in normal and failing cardiac myocytes, with increasing quantitative and molecular detail. Indeed, we can now develop a rich working hypothesis about how altered myocyte Ca handling contributes to both systolic dysfunction and arrhythmias in HF. However, there are many areas where controversy exists, and further study is required to test these hypotheses.

References


