Review

Regulation of intracellular Ca\(^{2+}\) in the heart during diabetes

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Abstract

Cardiovascular disease is a significant medical problem. The diabetic population is even more susceptible to cardiovascular complications and heart failure than non-diabetic patients. Atherosclerotic complications, a neuropathy and microvascular lesions have all been implicated causally in the accelerated cardiovascular disease during diabetes. However, one mechanism which may participate in the abnormalities in heart performance demonstrated during diabetes and may also contribute to heart failure in the diabetic is a derangement in the capacity of the myocardial cell to regulate its [Ca\(^{2+}\)]. The purpose of this treatise is to identify the current controversies and conclusions available regarding the specific defects in Ca\(^{2+}\) flux thought to contribute to these cardiac defects during diabetes mellitus.

Keywords: Diabetes; Calcium, intracellular concentration; Cardiomyopathy; Calcium fluxes; Contractile function

1. Introduction

It was approximately two decades ago that a defect in cardiac contractile performance was first identified in diabetic animals in the absence of vascular injury. Three studies, one from the lab of Timothy Regan \(^1\), another from James Scheuer’s group \(^2\) and the third from Edmund Sonnenblick’s lab \(^3\), were critical in providing comprehensive descriptions of the contractile abnormalities which were present in a chemically-induced diabetic animal model. These studies provided the framework and rationale for a large number of studies which subsequently confirmed and extended these original observations. These three investigations were important because they changed the direction of our understanding of cardiovascular disease in the diabetic population. Instead of cardiovascular disease and cardiac failure in diabetes being thought of as primarily a vascular problem \(^4\), these studies \(1\)–\(3\) documented the presence of a cardiomyopathy in animals. This diabetic cardiomyopathy has been extended over time to include a number of species including the human (see Refs. \(^5\) and \(^6\) for reviews on the topic). The original conclusion from these three seminal works remains valid: chronic diabetes mellitus induces cardiac performance defects which can occur in the absence of vascular complications. The data did not refute the presence of vascular defects or their importance in cardiovascular disease and morbidity. They simply identified the possibility that some of the cardiac failure and dysfunction associated with diabetes was due to problems inherent within the myocardium itself. Although the mechanisms responsible for the diabetic cardiomyopathy remain unresolved (see several reviews \(6\)–\(9\)), a disturbance in intracellular Ca\(^{2+}\) homeostasis was immediately proposed by several labs \(10\)–\(12\) as an important contributory factor. The purpose of the present article is to review and update this information in order to further our understanding of the significance of Ca\(^{2+}\) in the diabetic cardiomyopathy.

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2. The regulation of Ca\(^{2+}\) by the diabetic heart

2.1. Relationship of Ca\(^{2+}\) to the contractile response of the diabetic myocardium

The contractile defects identified in hearts from insulin-deficient diabetic animals by a number of laboratories include: slower shortening and relaxation kinetics, diminished peak tension or pressure, elevated end-diastolic pressure, and lower values for stroke volume, cardiac work, cardiac output and resting heart rate [6]. The vast majority of these data originate from chemically-induced rat models of diabetes. However, animals of other species with chemically-induced diabetes exhibit similar cardiac dysfunction [1,13]. Furthermore, strains of animals with genetic insulin-dependent diabetes also display similar defects in cardiac performance [14,15]. Thus, the problem is not limited to a particular animal species or mechanism leading to an insulin-deficient diabetic condition. The defect in cardiac function is clearly associated with an aspect of the diabetic state.

Cardiac function in diabetic animal models which are insulin-resistant, not insulin-deficient, has received far less research attention. One chemically-induced model of insulin resistance [16], a diet-induced model of glucose intolerance [17] and three genetic models of diabetes which are not insulin-deficient [18–20] have all shown evidence (although some is indirect) of cardiac contractile dysfunction. It is presently unclear if another model of diabetes (the JCR:LA-cp rat) which is glucose-intolerant in the presence of high circulating levels of insulin displays contractile performance abnormalities at rest or only under stressful stimuli [21,22]. Further work is obviously required to determine if cardiac performance is compromised in both insulin-deficient and hyperinsulinemic diabetic states. These studies would not be trivial because it would identify if insulin or glucose abnormalities were central to the functional integrity of the heart.

Regulation of the intracellular [Ca\(^{2+}\)] in the myocardium is a critical determinant of contractile performance. The contractile defects described above, therefore, may involve a change in the ability of the myocardium to regulate its [Ca\(^{2+}\)] during diabetes. If the diabetic myocardium has a compromised ability to regulate Ca\(^{2+}\), then one might hypothesize that the Ca\(^{2+}\) content or concentration of the diabetic heart would be altered. This concept has lead to controversial results. An increase in ventricular calcium content in both insulin-deficient and insulin-resistant diabetic animals has been reported [20,23,24]. However, others have found that the net influx of Ca\(^{2+}\) was reduced in hearts from insulin-deficient rats [25]. The study of isolated single cells from the heart instead of whole tissue preparations has not resolved the matter. In single cardiomyocytes from diabetic animals, an increased resting [Ca\(^{2+}\)] [26], no change in [Ca\(^{2+}\)] [27,28], a decreased resting [Ca\(^{2+}\)] [29], and a decreased calcium content [30] have been reported. It is impossible to make reliable conclusions from such a confusion of varying results. However, one might hypothesize that insulin status may be a factor since all of the studies which used an insulin-resistant diabetic animal model reported an increase in cell or tissue [Ca\(^{2+}\)] [20,24,26]. However, this issue will require further study.

Although we cannot make firm conclusions as to whether the resting diabetic heart exists in a Ca\(^{2+}\)-deficient or a Ca\(^{2+}\)-overloaded state, it is clear that its response to stimuli which alter the [Ca\(^{2+}\)], is defective. At a tissue level, a hypersensitive response to plasma [Ca\(^{2+}\)] has been documented in insulin-deficient and insulin-resistant diabetic rat hearts [21,30–35]. This is probably the simplest way to demonstrate a defect in the capacity of the myocardium to regulate its [Ca\(^{2+}\)]. However, other studies corroborate these initial conclusions. Diabetic hearts exhibit a supersensitivity to drugs which modify [Ca\(^{2+}\)]. These include BAYK 8644 [33,36], CoCl\(_2\) [37], Cd [31], verapamil [33], and ryanodine [37,38]. These data suggest that Ca\(^{2+}\) movements and their regulatory processes are altered in the diabetic myocardium. Other studies have employed varying stimulation frequencies and patterns to identify potential changes in Ca\(^{2+}\) flux within the myocardium. For example, post-rest contractions [38,39] and rapid cooling contractures [39] were depressed in diabetic hearts in comparison to control, suggesting a defect in Ca\(^{2+}\) release from the sarcoplasmic reticulum. Post-stimulation potentiation was also attenuated in the diabetic myocardium [38], again suggestive of changes in cellular Ca\(^{2+}\) flux.

2.2. Alterations in the Ca\(^{2+}\) transport pathways

Ca\(^{2+}\) movements and the [Ca\(^{2+}\)] in the myocardium are regulated in two manners: (1) in an active fashion by various enzymatic processes and, (2) passively by cellular buffering systems. Both appear affected by the diabetic condition.

Among the first subcellular organelles studied in the myocardium as a function of chronic diabetes were the contractile proteins. It is very clear that the Ca\(^{2+}\)-ATPase activity of the myosin protein is depressed during diabetes [12,40–44]. This depression is thought to contribute to the depressed force generation of the diabetic heart. Changes in contractile protein phosphorylation capacities may contribute to the altered ATPase activities and contractile protein function [45,46]. The issue which remains unresolved is the involvement of the myofibrillar components in the change in sensitivity of the myocardium to Ca\(^{2+}\). Two studies have indirectly implicated the myofibrillar proteins as responsible for the change in sensitivity of the diabetic myocardium to Ca\(^{2+}\) [32,34]. However, the initial work on isolated myofibrils directly demonstrated that the Ca\(^{2+}\) sensitivity of the myofibrillar complex was not altered during diabetes [12]. This has been corroborated by two other studies from different laboratories [30,43].
Another subcellular system involved in the regulation of Ca\(^{2+}\) and, therefore, tension in the heart is the sarcoplasmic reticulum (SR). The uptake of Ca\(^{2+}\) into the SR is depressed in the diabetic heart [11,47,48]. The SR Ca\(^{2+}\) pump is defective as well [11,47,48]. This does not appear to be as a result of a decrease in the pump density [49]. Instead, other factors like the SR membrane phospholipid environment [47], fatty acid profile [50,51], carnitine metabolism [48,51] and calmodulin activity [52] have been suggested to contribute to the SR defect. Insulin treatment of insulin-deficient animals will normalize SR activity [47,48] but correcting the thyroid status of the diabetic animals will not restore SR function [47]. Ca\(^{2+}\) release from the SR is also attenuated due to a depletion of SR Ca\(^{2+}\) stores [39] (Fig. 1).

The change in SR function may have two implications for cardiac performance. The depression in SR Ca\(^{2+}\) uptake may cause a slower rate of relaxation by the diabetic heart. The poor release of Ca\(^{2+}\) from the SR would result in less Ca\(^{2+}\) available for force generation and may partly explain the lower indices of tension generation in the diabetic hearts.

Transsarcolemmal Ca\(^{2+}\) flux pathways have received a great deal of research attention recently. The action potential duration is significantly prolonged in the hearts from insulin-deficient and insulin-resistant diabetic animals [18,31,37,53]. This is due to a decrease in the transient outward current and the steady-state outward current [54–56]. Several studies have documented no change in the L-type Ca\(^{2+}\) current in diabetic hearts [18,54,55]. A recent study found that longer durations of diabetes induce a decrease in this channel [56]. Biochemical investigations have found that Ca\(^{2+}\) channel density was decreased but the affinity of the channel for Ca\(^{2+}\) antagonist drugs was increased [57]. The former alteration may explain the depressed contractile force in the diabetic hearts whereas the latter may contribute to the increased sensitivity of the diabetic myocardium to Ca\(^{2+}\). It is noteworthy that the Ca\(^{2+}\) current was not altered in hearts from insulin-resistant diabetic animals [18].

Several other proteins important in Ca\(^{2+}\) homeostasis in the heart have been shown to be altered functionally during diabetes. Activities of the sarcolemmal Ca\(^{2+}\) pump [58,59], the Na\(^{+}/\)Ca\(^{2+}\) exchanger [58,60], the Na\(^{+},K^{+}\)-ATPase [61] and the Na\(^{+}/\)H\(^{+}\) exchanger [60] are all depressed during insulin-deficient diabetes. These proteins will have direct or indirect effects on cellular [Ca\(^{2+}\)] and, ultimately, cardiac performance. For example, the decreased Na\(^{+}/\)Ca\(^{2+}\) exchanger activity may contribute to the slower rates of relaxation in the diabetic heart. The depressed Na\(^{+}/\)H\(^{+}\) exchange will compromise the ability of the diabetic heart to recover from an acidic load [62]. Lower intracellular pH would be expected to result in poor force generation. The passive Ca\(^{2+}\) binding capacity of the sarcolemmal membrane is also decreased during diabetes [63]. If the sarcolemmal bound Ca\(^{2+}\) represents a pool of Ca\(^{2+}\) important in force generation, then this may also contribute to the depression in cardiac contractile performance.

2.3. The association of calcium with cell injury and death

The diabetic myocardium, as described above, is hypersensitive to Ca\(^{2+}\) and possesses defects in the function of many of the systems responsible for the regulation of intracellular Ca\(^{2+}\) in a normal physiological state. It is possible, therefore, that in pathological conditions where one would expect a stimulation of Ca\(^{2+}\) entry, defects in the Ca\(^{2+}\) regulatory systems may become accentuated. This may result in an inability to prevent excessive Ca\(^{2+}\) entry which could lead to serious injury to the myocardium. Intracellular Ca\(^{2+}\) overload has been associated with cell damage and death during pathological conditions like ischemia, hypoxia and exposure to free radicals [64,65].

Is the diabetic myocardium, therefore, hypersensitive to injury from pathological stimuli? The answer to this question appears to be dependent upon the type of stimulus, the duration of diabetes and the type of diabetes. For example, metabolic inhibition of diabetic rat myocytes induced hypercontracture and rounding up of the cells at lower intracellular Ca\(^{2+}\) concentrations than in control preparations [29]. KCl depolarization induced a greater accumulation of Ca\(^{2+}\) in diabetic myocytes than was observed in controls [26]. The diabetic heart may be hypersensitive to ischemic challenge as well; however, the duration of diabetes may be an important factor. At 3 months of age, insulin-resistant diabetic rats were less affected by an ischemic challenge [22]. However, hearts from rats which were 6 months of age or older were hypersensitive to ischemic reperfusion injury [22]. These results are consistent with findings in insulin-deficient diabetic rats which demonstrated a resistance to ischemic injury 2 weeks after the induction of a diabetic state followed by a deterioration of postischemic function after 8 weeks of diabetes [66].

There is evidence that calcium is involved in the damage to the diabetic heart. Long-term treatment with the calcium channel antagonists, nifedipine [67] and nisoldipine [68], are protective against myocardial ischemic damage in older rats with long-standing diabetes. These hearts were also more sensitive to the cardioprotective effects of
a Ca\textsuperscript{2+} antagonist applied during the ischemic challenge [69,70]. Indeed, Ca\textsuperscript{2+} antagonists have also been shown to be beneficial against the cardiac dysfunction exhibited under control conditions in insulin-deficient diabetic rats [71]. The data suggest that the diabetic heart allows the entry of an excessive amount of Ca\textsuperscript{2+}. The diabetic myocardium may also have less of a capacity to buffer this abnormally high [Ca\textsuperscript{2+}]. Mitochondrial Ca\textsuperscript{2+} uptake, which is thought to serve as a cytoplasmic buffering system during conditions of high [Ca\textsuperscript{2+}], is depressed in the diabetic heart [72].

It is important to recognize that the duration of diabetes may not be the only factor which may influence the susceptibility of the heart to ischemic insult. Although older insulin-resistant rat hearts were hypersensitive to ischemic challenge, many studies in insulin-deficient diabetic rats have found that these hearts were more resistant to ischemic challenge than control hearts [73–78]. The protective mechanism in this case may again involve Ca\textsuperscript{2+} but in an indirect manner. Activation of the Na\textsuperscript{+}/H\textsuperscript{+} exchange pathway is thought to play a central role in ischemic injury to the heart [64,79–84]. The acidic cell interior (which is generated by the ischemic insult) stimulates the Na\textsuperscript{+}/H\textsuperscript{+} exchanger to remove H\textsuperscript{+} from the cell in exchange for extracellular Na\textsuperscript{+}. The accumulation of intracellular Na\textsuperscript{+} is known to activate the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, which will result in excessive entry of Ca\textsuperscript{2+} into the cell. The diabetic heart exhibits a defective Na\textsuperscript{+}/H\textsuperscript{+} exchanger [60,74,85] and a defective Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger [58,60]. The depressed Na\textsuperscript{+}/H\textsuperscript{+} exchange activity will protect the diabetic heart from ischemic insult and result in less damage and dysfunction [74,85,86].

3. Conclusions

Although it is unclear if the resting levels of cardiac calcium are altered, changes in Ca\textsuperscript{2+} in response to a variety of stimuli are disturbed during diabetes. For example, cardiac contractile performance is abnormal in the presence of varying perfusate Ca\textsuperscript{2+} concentrations. The diabetic heart also responds differently to agents which alter [Ca\textsuperscript{2+}], Proteins which actively regulate [Ca\textsuperscript{2+}] in a direct or an indirect manner are defective. Passive buffering of [Ca\textsuperscript{2+}], is also depressed in the diabetic heart. These changes have important implications for cardiac performance not only during normal physiological states but also during pathological challenges like ischemia (Fig. 2). The dysfunctional regulation of Ca\textsuperscript{2+} in the myocardial cell (directly or indirectly), therefore, may be a prime determinant of injury to the heart in the presence of diabetes. Future studies should be designed with the objective of improving the regulation of [Ca\textsuperscript{2+}] in the diabetic heart and understanding the mechanism responsible for the defect. Temporal data would suggest that the sarcoplasmic membrane may be a critical site for study [87]. Although the SR has an important influence on cardiac function [88], several dissociations of SR function from contractile performance in the diabetic heart would suggest that it may not be the primary defect [48,89,90]. Furthermore, changes in protein expression may not always be the primary cause for the dysfunctional activities of the Ca\textsuperscript{2+} regulatory proteins [49,91,92]. Instead, lipid abnormalities accompanying the diabetic state may provide promising mechanistic answers to the study of Ca\textsuperscript{2+} homeostasis in the diabetic myocardium [8,47,48,50,51,61,63,93]. Interventions which lower circulating lipid levels improve cardiac performance in diabetic animals [8,94,95]. These treatments have also improved the function of many of the subcellular organelles thought to contribute to the abnormal contractile performance of the diabetic heart [50,51,96]. In addition to lipids, insulin also remains as an attractive suspect for three reasons. Firstly, as discussed above, there are important differences in cardiac function and integrity between diabetic models which are insulin-deficient and those which have normal or elevated insulin levels. Secondly, insulin has the capacity to directly influence Ca\textsuperscript{2+} movements in the myocardium [97,98]. Finally, the presence of insulin has also been shown to affect the severity of ischemic injury in the heart [99]. Specific experimental tests to identify the etiology of diabetic heart dysfunction and abnormal Ca\textsuperscript{2+} regulation are now possible and should provide new insight and strategies for the prevention of myocardial damage.

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