Intracellular calcium levels are unchanged in the diabetic heart

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1. Introduction

Diabetes mellitus has been associated with cardiac disease that has been suggested to occur secondary to atherosclerosis of the coronary arteries, macroangiopathy and autonomic neuropathy [1]. However, a cardiac disease particular to diabetes has also been demonstrated to occur in the absence of the above factors. This ‘diabetic cardiomyopathy’ that includes cardiomegaly, left ventricular dysfunction and clinically overt congestive heart failure has been strongly suggested by epidemiological, clinical and experimental studies [2–7]. The etiology of diabetic cardiomyopathy is complex and a number of factors have been suggested to be involved in the development of this disease state. These include: (a) changes in cardiac metabolism, (b) abnormal vascular sensitivity and reactivity to various ligands, (c) increased stiffness of the ventricular wall associated with perivascular thickening of basement membrane and interstitial accumulation of glycoprotein and insoluble collagen, and (d) abnormalities of various proteins which control ion movements, specifically intracellular calcium [6].

Streptozotocin (STZ) induction of diabetes in rats is the most commonly used animal model of diabetes. The chemical structure of STZ comprises a glucose molecule with a highly reactive nitrosourea side-chain that is thought to initiate its cytotoxic action. The glucose moiety directs this agent to the pancreatic β-cell [8]. STZ selectively destroys pancreatic β-cells and produces a diabetic state, the severity of which can be varied by altering the dose of STZ. With time (4–6 weeks), rats so treated develop biochemical and functional myocardial abnormalities which are the result of the drug-induced metabolic changes rather than a direct effect of the drug itself [6]. Thus, rats made diabetic with STZ exhibit depressed left ventricular contractility, diminished ventricular compliance and decreased inotropic and chronotropic responses to certain drugs in isolated cardiac preparations [4,9,10]. Changes in contractile properties of the heart have also been found in vivo in both anaesthetised [11] and conscious [12,13] STZ-diabetic rats. Considering that the diabetic rat does not develop atherosclerosis, the in vivo and in vitro abnormalities in contractility and metabolism reflect changes in the function of the myocardial cell [14]. Hence, similar to the human condition, the STZ diabetic rat is a useful model to elucidate the pathogenesis of diabetic cardiomyopathy. In this review, evidence is presented that basal calcium ([Ca^{2+}]) levels in both quiescent and electrically-stimulated cardiac cells from STZ diabetic rats is unchanged and may not reflect the cardiac abnormalities observed in STZ diabetes.

2. [Ca^{2+}]i mobilization in ventricular myocytes

It is well known that the integrity of the circulatory system can be influenced by changes in the flux of intracellular calcium ([Ca^{2+}]) in heart. Ca^{2+} movements are closely related to cardiac electrophysiological events, membrane integrity, energy metabolism and contractile...
function. Maintaining the intracellular free Ca\(^{2+}\) concentration requires a fine balance in the co-ordinated function of many organelles. Intracellular organelles which incorporate Ca\(^{2+}\) from and return Ca\(^{2+}\) to the cytosol play a central role in determining [Ca\(^{2+}\)] in the cell [15]. In a mammalian ventricular cell, Ca\(^{2+}\) moves across the sarcotubular system (SL), sarcoplasmic reticulum (SR), and mitochondrial membranes with the help of L-type Ca\(^{2+}\) channels, Na–Ca exchangers, Ca\(^{2+}\)–ATPase, and Ca\(^{2+}\) binding sites located within the SL, Ca\(^{2+}\) release channels and Ca\(^{2+}\)–ATPase within the SR and Na–Ca exchangers and Ca\(^{2+}\)–binding proteins within the mitochondrial membranes. It is assumed that the cytosolic concentration [Ca\(^{2+}\)] and spatial distribution of the [Ca\(^{2+}\)] transients are uniform within the cell [16]. During cardiac excitation, extracellular Ca\(^{2+}\) moves into the cells through activated Ca\(^{2+}\) channels and reverse Na–Ca exchange [17,18]. This Ca\(^{2+}\) current (I\(_C\)) may in turn control SR Ca\(^{2+}\) release via the SR Ca\(^{2+}\) release channel,ryanodine receptor [19] and contraction ensues. For relaxation to occur, Ca\(^{2+}\) must be removed from the cytoplasm, either by transport into the SR (by SR Ca\(^{2+}\)–ATPase) or out of the cell (by sarcolemmal Ca\(^{2+}\)–ATPase or Na–Ca exchange). These transport systems are thus in competition for cytoplasmic Ca\(^{2+}\) and act in concert, both to regulate the intracellular Ca\(^{2+}\) concentration and to modulate Ca\(^{2+}\)–dependent events [15,20].

3. Intracellular Ca\(^{2+}\) in the diabetic heart

Evidence for abnormal myocardial cell function in diabetes mellitus, influenced by metabolic changes, has appeared in recent years. Experimental studies at the cellular level have provided data for several possible explanations for this cardiac dysfunction. Among these, an abnormal intracellular Ca\(^{2+}\) homeostasis and trans-sarcolemmal receptor signalling defects have been suggested [5,21]. For example, during chronic diabetes intracellular Ca\(^{2+}\) homeostasis in the heart is altered, possibly as a result of an impairment in SR which acts as a Ca\(^{2+}\) store and takes up and releases Ca\(^{2+}\) on a beat-to-beat basis [22]. Additionally, SL Ca\(^{2+}\) pump and Na–Ca exchange activity, together with the efflux of Ca\(^{2+}\) through the SL are depressed in the diabetic heart [23,24]. Mitochondrial Ca\(^{2+}\) abnormalities have also been reported in the chronically diabetic rat heart [5]. Among the receptor defects, cardiac β-adrenoceptor function is altered in diabetic animals. These alterations include a depressed β-receptor number, a reduced response to β-adrenergic agonists, and a significant alteration in the response and sensitivity to perfuse Ca\(^{2+}\) stimulation [10,25–27]. These results collectively support the hypothesis that diabetes mellitus leads to an alteration in Ca\(^{2+}\) movements in the heart.

In more recent years, intracellular Ca\(^{2+}\) mobilization and contractility in the diabetic heart have been studied using isolated cardiomyocytes that have the responses of an intact heart but are not affected by non-myocardial tissues [28]. As single cells, they are suitable for measuring contractility, ion activities and Ca\(^{2+}\) mobilization, and an increasing number of reports have documented intracellular Ca\(^{2+}\) transients and ion channel and transporter activities in parallel with single cell contraction in diabetic heart cells [29–34]. Utilization of fluorescent dyes in isolated myocytes allows for the analysis of [Ca\(^{2+}\)] and the study of the mechanism by which stimulation causes [Ca\(^{2+}\)] changes. Moreover, the restoration of [Ca\(^{2+}\)], following stimulation-induced elevation can be observed, especially with rapidly desensitizing agonists that produce a brief increase in Ca\(^{2+}\) in the cytosol [35–39]. Using these methods, it has been repeatedly reported that cardiomyocyte contraction is impaired in diabetic hearts [29,31,40]. Cell shortening and velocities of contraction (+dL/dt) and relaxation (−dL/dt) were significantly lower in diabetic myocytes than controls, whereas time to peak shortening was prolonged in diabetic cells [34]. These observations agree with the results from intact hearts and tissue preparations of diabetic rats which show decreased peak ventricular pressure development and rates of ventricular pressure development and decline (±dP/dt).

The data on basal levels of [Ca\(^{2+}\)] in diabetic myocytes are more inconsistent. Early studies have reported an increase in resting [Ca\(^{2+}\)], an effect that appeared to support the hypothesis of Ca\(^{2+}\) overload in diabetic rat hearts [30,41]. However, the animals used in the above studies were claimed to be a model of non-insulin-dependent diabetes, and different from STZ-diabetic rats, often referred to as a model of poorly controlled insulin-dependent diabetes. Subsequently, Noda et al. [31,42] reported a reduced basal [Ca\(^{2+}\)] in the diabetic heart and suggested a relationship between depressed basal [Ca\(^{2+}\)], and reduced cardiac contractility. However, their measurements were made at an extracellular Ca\(^{2+}\) concentration of 0.5 mM that is not physiological for rats. In our laboratory, a large amount of data has been collected that demonstrates no differences in basal [Ca\(^{2+}\)], levels between diabetic and control cardiomyocytes [32,34,40]. Hence, under normal conditions for isolated cardiomyocytes, basal [Ca\(^{2+}\)], (both resting and electrically stimulated) does not necessarily reflect the depressed contraction in the diabetic heart and [Ca\(^{2+}\)], transients in response to different physiological/pharmacological stimulants may be better parameters to represent cardiac dysfunction.

4. Ca\(^{2+}\) mobilization in the diabetic heart

4.1. Sarcolemmal Ca\(^{2+}\) channels and Ca\(^{2+}\) and K\(^{+}\) currents in diabetic cardiomyocytes

Ca\(^{2+}\) channel activity in sarcolemmal membrane of diabetic hearts has been extensively studied. Unfortunately, the data are controversial. Increased PH200-110 (a dihydropyridine derivative) binding sites in diabetic car-
4.2. Na–Ca exchange activity in SL of diabetic myocardium

Depressed Na–Ca exchange activity without change in affinity to Ca$^{2+}$ has been previously reported in diabetic rats [24]. These changes may be due to compositional modifications in the SL membrane, possibly as a result of diabetes-induced hyperlipidemia [7]. In this regard, unpublished observations from our laboratory have indicated that the Na–Ca exchange in hypertensive-diabetic rats demonstrates a more significant increase in the affinity for Ca$^{2+}$. Interestingly, the hypertensive-diabetic rat model is known for its severe hyperlipidemia [7,52].

4.3. SR Ca$^{2+}$-ATPase in diabetic cardiomyocytes

It has been well documented that SR function in diabetic hearts is depressed. The early report of SR dysfunction in the diabetic rat heart came from isolated cardiac membrane vesicles whereby the uptake of $^{45}$Ca$^{2+}$ into SR vesicles (a function of SR Ca$^{2+}$-ATPase) was reported to be reduced [22]. More recently, Ca$^{2+}$ transients have been measured to elucidate SR function. In this regard, a depressed rapid-cooling contracture ($\sim 1^\circ$C, an indirect measure of SR releasable Ca$^{2+}$) was reported in papillary muscles [53] and isolated cardiomyocytes, accompanied by a reduction in intracellular Ca$^{2+}$ transients [34]. Caffeine can also induce Ca$^{2+}$ release from the SR, and the resulting contracture can be used as an indirect measure of SR Ca$^{2+}$ available for release. Caffeine-induced Ca$^{2+}$ transients and contracture are both depressed in diabetic myocytes [32,34].

4.3.1. Rapid-cooling and caffeine contracts

The Ca$^{2+}$ content of the SR that is available for release is clearly an important determinant of the contractile state. Two approaches have been used to study SR Ca$^{2+}$ in cardiomyocytes: rapid application of (a) cold solution (RCC, $\sim 1^\circ$C) or (b) caffeine to induce SR Ca$^{2+}$ release. The resulting contracture can be used as an index of SR Ca$^{2+}$ available for release. The advantage of using the above approaches is that they can be done ‘on line’ with contracted living cells [15]. Rapid cooling of cardiac muscle results in a contracture that can be attributed to the quick release of SR Ca$^{2+}$ to the cytoplasm. The amplitude of the contracture is indicative of the amount of Ca$^{2+}$ available for release from SR at the time of cooling. During the cooling process, the probability of the SR Ca$^{2+}$ release channel opening increases [54], the action potential duration is increased [55], peak Ca$^{2+}$ current decreases, myofilament Ca$^{2+}$ sensitivity is reduced [56], and Na–Ca exchange activity is attenuated [56]. The duration of [Ca$^{2+}$$]_r$, elevation during an RCC in unloaded conditions prolongs the active state and may allow the myocyte to shorten progressively. Due to inhibition of membrane Ca$^{2+}$ transport (Ca$^{2+}$ pump and Na–Ca
exchange), cell shortening declines slowly and the rise in \([\text{Ca}^{2+}]\) during an RCC is less transient than that observed during caffeine-induced contractures. As \([\text{Ca}^{2+}]\) is much higher during an RCC than during a normal twitch, it appears that rapid cooling can release all of the available SR \(\text{Ca}^{2+}\) [15], while only a fraction of the SR \(\text{Ca}^{2+}\) available for release is discharged during a normal twitch.

Caffeine increases SR \(\text{Ca}^{2+}\) channel opening and hence promotes a \(\text{Ca}^{2+}\) leak into the myoplasm. This process effectively prevents the SR from accumulating \(\text{Ca}^{2+}\). \(\text{Ca}^{2+}\) pumped into the SR is immediately reintroduced into the myoplasm via the open SR channels, and can then be removed from the cell by Na–Ca exchange. Hence, caffeine can inhibit SR \(\text{Ca}^{2+}\) uptake without directly effecting SR \(\text{Ca}^{2+}\) pump. One caveat is that caffeine can cause myofilament sensitization and phosphodiesterase inhibition that can increase cAMP and cAMP-dependent protein kinase and both these effects can complicate interpretation of the results [56]. Moreover, Indo-1 fluorescence is strongly quenched by caffeine. However, this occurs in a wavelength-independent manner so that the fluorescence ratio used to estimate \([\text{Ca}^{2+}]\) is unaffected.

### 4.3.2. Caffeine-induced calcium transients in quiescent myocytes

The SR content of \(\text{Ca}^{2+}\) can also be assessed by measuring \([\text{Ca}^{2+}]\), response to caffeine with the fluorescence \(\text{Ca}^{2+}\)-indicator, fura-2. Brief exposure to caffeine induces a transient inward current which reflects the electrogenic extrusion of \(\text{Ca}^{2+}\) across the membrane by the Na–Ca exchange [57]. With this method, it was demonstrated that the peak \([\text{Ca}^{2+}]\) transient in response to caffeine was significantly decreased in diabetes, suggesting a reduction in \(\text{Ca}^{2+}\) storage in the SR [34]. Insulin treatment prevented this effect [34] and this was probably related to the fact that insulin treatment of STZ diabetic rats reverses the depression of Ca-ATPase activity and \(\text{Ca}^{2+}\) uptake by the SR [22].

In diabetic hearts, there has been some debate as to whether \(\text{Ca}^{2+}\) overload or \(\text{Ca}^{2+}\) underload occurs, and whether the decrease in cardiac contractility in the diabetic rat is accompanied by reduced or excessive loading of the \(\text{Ca}^{2+}\) into SR. Our results, documenting the decline in SR \(\text{Ca}^{2+}\) store and release, as assessed by RCC and caffeine-induced [\(\text{Ca}^{2+}\)], transients [34], agree with the results of Bouchard and Bose [53] who reported a reduction in SR \(\text{Ca}^{2+}\) stores and decreased fractional release of \(\text{Ca}^{2+}\) during stimulation of papillary muscles from STZ-treated rats. Thus, the marked reduction of developed tension in diabetic tissues is suggested to be a consequence of depleted SR \(\text{Ca}^{2+}\) stores, rather than a result of chronic SR \(\text{Ca}^{2+}\) overloading. In this regard, the reduced \(\text{Ca}^{2+}\) uptake by the SR [22] could diminish \(\text{Ca}^{2+}\) stores and hence impair \(\text{Ca}^{2+}\) release, with a consequent reduction in cardiac contraction.

### 4.4. Mitochondrial function in diabetic hearts

The current view of \(\text{Ca}^{2+}\) accumulation by heart mitochondria is that it may act as a sink for \(\text{Ca}^{2+}\) under pathological conditions. Therefore, mitochondria may act as a potent and important buffering component of \(\text{Ca}^{2+}\) in the heart. A generalized depression of mitochondrial function exists in hearts from diabetic rats. Mitochondrial Ca-ATPase activity and \(\text{Ca}^{2+}\) transport capacity are significantly impaired in hearts from chronically diabetic rats [58]. Although the alteration in \(\text{Ca}^{2+}\) accumulation by mitochondria may have little effect on cardiac function on a beat-to-beat basis, any stimulus to the heart that results in elevated \([\text{Ca}^{2+}]\), may not be adequately buffered in diabetic hearts. Direct measurement of mitochondrial calcium concentration in diabetic cardiomyocytes has not been made, although a technique for such a measurement is available and has been applied in other pathological models such as anoxia and reoxygenation [59,60]. In a report using isolated cardiomyocytes, mitochondrial uptake of \(\Delta\text{Ca}^{2+}\), transmembrane potential gradient across the inner membrane of mitochondria, and cell respiration were significantly decreased in diabetic myocytes compared to control [61]. These changes were restored to normal by insulin treatment [61].

### 4.5. Myofilaments and \(\text{Ca}^{2+}\) sensitivity in diabetic cardiomyocytes

Two kinds of \(\text{Ca}^{2+}\)-dependent alterations may affect contractility of the diabetic heart: (a) changing the availability of \(\text{Ca}^{2+}\) to the myofilaments, and (b) modifying the responsiveness of the myofilaments to activation by intracellular \(\text{Ca}^{2+}\). The availability of intracellular \(\text{Ca}^{2+}\) is regulated by the SL and SR, and \(\text{Ca}^{2+}\) responsiveness is controlled by the myofilaments and the regulatory tropin–tropomyosin complex [62].

There has been no consistent finding regarding the sensitivity of the myofilament to \(\text{Ca}^{2+}\). The sensitivity of isolated myofibrils to \([\text{Ca}^{2+}]\), has been reported to be unchanged [63] or increased [64] during diabetes. The increased sensitivity has been suggested to contribute to the slow time-course of relaxation. More recently, two studies have suggested that the sensitivity of myofilaments to \(\text{Ca}^{2+}\) was decreased during diabetes [65,66]. Although these experiments were performed under different conditions, the data appear to suggest that altered intracellular \(\text{Ca}^{2+}\) transients are not the only cause of cardiac dysfunction during diabetes.

It has been reported that the lower activity of myosin ATPase and abnormal myosin isoenzyme distribution could be responsible for the depressed contractile function in diabetic myocardium [67]. Additionally, \(\text{Ca}^{2+}\)-sensitivity of skinned diabetic myocytes is significantly enhanced with unchanged maximal activated tension [64]. In con-
trast, increased expression in cardiac β-myosin heavy chain and changes in troponin T expression may contribute to the decrease in Ca\(^{2+}\)-sensitivity of myofilaments at pH 7.0, and decreased maximum tension-generating ability at pH 6.6 in diabetes [66]. A decreased Ca\(^{2+}\)-sensitivity of isometric tension in skinned cardiomyocytes from diabetic rats suggests that decreased cardiac output in the whole heart can occur independently of alterations in Ca\(^{2+}\)-handling by the SR or SL. During in vivo acidic conditions such as ischemia and hypoxia, a substantial decrease in ventricular pressure may occur, in part due to changes in myofilament protein expression [66]. The apparent Ca\(^{2+}\)-sensitivity was greatly diminished at a sarcomere length of 1.9 μm but was not affected at a longer length (2.4 μm). Another possibility is that diabetic Ca\(^{2+}\)-sensitivity changes in the myocardium are coupled with troponin T alterations [65].

### 4.6. \([Ca^{2+}]_i\) responses to β-adrenergic stimulation

Diabetic rat hearts are characterized by diminished responses to β-adrenergic stimulation [10,68]. This has been suggested to be due to a reduction in β-adrenergic receptor [25]. As electrically stimulated diabetic cardiomyocytes demonstrate a depressed maximum [Ca\(^{2+}\)] \(\rightarrow\) response to isoproterenol and 8-bromo-cAMP without a change in sensitivity [31,40], it suggests that in addition to alterations in β-adrenoceptor function there are postreceptor defects in the diabetic myocardium. The response of [Ca\(^{2+}\)], to isoproterenol can be blocked by thapsigargin, suggesting that the β-agonist-induced [Ca\(^{2+}\)] changes are mediated, in part, by SR Ca\(^{2+}\)-ATPase [40]. Insulin treatment of diabetic rats reversed the depressed response of [Ca\(^{2+}\)], to isoproterenol [40].

### 4.7. \([Ca^{2+}]_i\) responses to different pharmacological agents

As stated earlier, the peak [Ca\(^{2+}\)] transient induced by caffeine may be used as an indirect index of SR releasable Ca\(^{2+}\). The maximum [Ca\(^{2+}\)] response to ouabain is reduced in diabetic cells while the sensitivity of diabetic myocytes to ouabain was enhanced [32]. KCl-induced [Ca\(^{2+}\)] increase was enhanced in diabetic cells accompanied by a decreased caffeine and dichlorobenzenimid blockade of the KCl effects [32]. Nitrendipine blockade effects were similar in diabetic and control cells [30,32]. The maximum responses of [Ca\(^{2+}\)] \(\rightarrow\) exogenous ATP was increased in diabetic cells [32].

#### 4.7.1. Isoproterenol and 8-bromo-cAMP

In addition to the depressed response to isoproterenol, diabetic myocytes also demonstrate an attenuated [Ca\(^{2+}\)] response to 8-bromo-cAMP at \(10^{-5}\) M and higher concentration [32]. This suggests that at steps distal to the β-adrenoceptor and adenylyl cyclase, diabetic myocytes exhibit a deficiency. Hence, there may be an alteration between cAMP and [Ca\(^{2+}\)], increase such as PKA activation and the phosphorylation of proteins. The phosphorylation process in the SR of diabetic myocytes is as yet unclear.

#### 4.7.2. Ouabain

The response to ouabain in papillary muscle and left atria from hearts of diabetic rats was reported to be markedly depressed [69,70]. It was also reported that the maximum number of high- and low-affinity ouabain binding sites in membrane preparations obtained from chronically diabetic rats was significantly reduced to 60 and 49% of controls, respectively. These results suggest that the decreased inotropic response of ouabain in the intact cardiac tissue obtained from diabetic rats may be related to a decreased number of ouabain binding sites [71]. The altered \(K_d\) could be due to an alteration in the ouabain binding sites or an altered composition of the membrane in diabetes. The activity of Na-K ATPase is dramatically reduced in the diabetic heart [72]. As this enzyme indirectly regulates [Ca\(^{2+}\)], levels through its modulation of [Na\(^{+}\)], the diabetes-linked decrease in Na-K ATPase activity would be expected to mediate a net increase in both [Na\(^{+}\)], and [Ca\(^{2+}\)]. Myocardium from diabetic rats is susceptible to Ca\(^{2+}\) loading by ouabain incubation (as measured by afterdepolarizations) [73]. These data suggest a decline in the reserve capacity of the sarcolemmal Na\(^{+}\) pump in the diabetic heart. The decrease in Na-K ATPase could enhance the sensitivity to digitalis-like compounds by reducing the reserve capacity of the Na-pump and hence, the extent of digitalis-induced pump inhibition required before the onset of toxicity (in the case of single myocyte, hypercontraction). A reduction in reserve capacity may lower the tolerance to ouabain by decreasing the number of pump sites that the glycoside would have to inhibit before eliciting a marked [Na\(^{+}\)], accumulation and the resulting toxic effects believed to be mediated by Ca\(^{2+}\)-overload. Cardiac arrhythmias are a frequent and serious complication of the clinical use of digitalis glycosides, and it is possible that the tolerance to these cardiotoxic effects and the margin of safety for cardiotonic steroids is reduced in diabetic patients.

#### 4.7.3. KCl and ATP

The influx of [Ca\(^{2+}\)], is a prerequisite of the KCl-induced [Ca\(^{2+}\)], transient because EGTA abolished the transient. The [Ca\(^{2+}\)], influx is triggered by membrane depolarization and thus activation of L-type Ca\(^{2+}\)-channels and ‘reversed’ mode operation of Na–Ca exchange. Ca\(^{2+}\) influx via Ca\(^{2+}\)-channel and Na–Ca exchange may directly contribute to the [Ca\(^{2+}\)], increase and, more importantly, induce Ca\(^{2+}\) release from SR. In accordance with our results, an enhanced [Ca\(^{2+}\)], response to KCl (30 mM) in a myocyte suspension from diabetic rats has been documented [30]. Increased L-type Ca\(^{2+}\)-channel activity determined by \([^{3}]\)H]PN-200-110 binding sites in cardiac mem-

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brane has been reported in the diabetic heart [43]. Interestingly, the [³²H]PN-200-110 binding to control cardiac membrane was dose-dependently inhibited by verapamil, but this was not the case in diabetic cardiac membranes [43]. This suggests that L-type Ca²⁺-channels are quantitatively and qualitatively altered in diabetes, and may be related to the nifedipine effects found in this study. The effects of ATP in modulating Ca²⁺-channel and intracellular Ca²⁺ store are very similar to KCl [74]. They are both sensitive to BAY K 8644, nifedipine, and EGTA and both are partially inhibited by ryanodine and caffeine. These similarities suggest that the ATP mechanism is similar to the KCl effects of membrane depolarization [74] and direct activation of Ca²⁺-channels.

5. Summary

The quality control indices of myocyte isolation (viability, yield, survival time, cell response, etc.) suggest that the adult rat myocyte model is stable and useful in [Ca²⁺], measurements and functional studies at the cellular level. Moreover, diabetic cardiomyocytes are a valuable model for studying cellular functions of the diabetic heart as they retain most of the features of cardiac dysfunction of intact rat. Data from our studies indicate that the basal Ca in i the adult rat myocyte model is stable and useful in Ca i

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function of major organelles which handle Ca²⁺, in

myocytes is depressed, which in turn causes the alteration of [Ca²⁺], mobilization in myocytes. Different second messenger systems involved in E-C coupling may also be altered due to the metabolic impairments. The rapid increase in our understanding of the pathophysiology of calcium homeostasis in cardiomyocytes will be forthcoming as the powerful new tools of molecular and structural biology are used to investigate the regulation of the Ca²⁺ transport system.

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