Diabetes mellitus has been shown to be associated with heart failure of unknown origin, which is termed ‘diabetic cardiomyopathy’ [1]. Regan et al. [2] have shown that the small vessel lesions in diabetes mellitus have little or no relation to diabetic cardiomyopathy, suggesting that the contractile function of the diabetic cardiomyopathy was primarily defective. Although many studies have been performed to reveal cellular and subcellular derangement in diabetic cardiomyopathy [3–6], the precise mechanism is still unknown.

It has been reported that Ca$^{2+}$ metabolism in diabetic myocardium has several abnormalities, including a decreased activity of Ca$^{2+}$-ATPase in sarcoplasmic reticulum (SR) [7,8] and sarcolemma [9,10]. It has also been reported that the activity of Na$^+$/Ca$^{2+}$ exchange is lower in diabetic myocardium [10]. These reports have suggested that Ca$^{2+}$ overload may be involved in the pathogenesis of diabetic cardiomyopathy [3]. Although it has been shown that certain mechanical and electrophysiological features in diabetic rats correlate well with conditions of intracellular Ca$^{2+}$ overload [11,12], direct measurement of the cytosolic Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) has not been made previously. The most popular method for measuring [Ca$^{2+}$]$_i$ uses fluorescent probes such as fura-2 and indo-1 [13]. We have reported that [Ca$^{2+}$]$_i$ of unstimulated rat myocytes isolated from insulin-dependent diabetes mellitus (IDDM) was lower than that of normal myocytes using fura-2 (53 ± 3 nM in diabetic myocytes and 75 ± 5 nM in normal myocytes, mean ± s.e.; $P < 0.01$) [14]. Recently, Lagadic-Gossmann et al. [15] also reported that basal [Ca$^{2+}$]$_i$ level was lower in diabetic myocytes using indo-1. Horackova and Murphy [16] have reported that the Ca$^{2+}$ content is decreased in diabetic rat myocytes using the radioisotope method. However, measurements of basal [Ca$^{2+}$]$_i$ in diabetic heart are controversial. There was no significant change in basal [Ca$^{2+}$]$_i$ in one study in which fura-2 was used to measure [Ca$^{2+}$]$_i$ [17]. Another study reported high [Ca$^{2+}$]$_i$ in diabetic rats using Ca$^{2+}$-selective microelectrodes [18]. Schaffer’s group reported that total myocardial Ca$^{2+}$ content [19] or [Ca$^{2+}$]$_i$ in cardiomyocyte suspensions measured by fura-2 [20] was increased in non-insulin-dependent diabetes mellitus (NIDDM). However, the model of diabetes in these studies is different from other studies, and it was shown that differences in cardiac metabolism and Ca$^{2+}$ handling exist between IDDM and NIDDM [21]. For example, hearts from NIDDM rats exhibit a dramatic decrease in diastolic compliance, suggesting the activation of actomyosin caused by Ca$^{2+}$ overload [21]. It is possible, therefore, that there could be a difference in [Ca$^{2+}$]$_i$ of myocytes isolated from IDDM and NIDDM rats.

There are not sufficient data, and the reports on basal [Ca$^{2+}$]$_i$ in diabetic heart are not consistent. In summary, most reports indicate that [Ca$^{2+}$]$_i$ of myocytes isolated from IDDM rats is either decreased or not significantly different, while [Ca$^{2+}$]$_i$ of NIDDM rats is elevated. Therefore, it is possible that there could be differences in Ca$^{2+}$ regulation and [Ca$^{2+}$]$_i$ of cardiomyocytes between IDDM and NIDDM. The other data of IDDM rats will be interpreted in the following section.

Although the activity of sarcolemmal Ca$^{2+}$-ATPase has been reported to be depressed in diabetic myocardium [9,10], it has been reported that the Ca$^{2+}$ net influx is significantly reduced in diabetic rat myocardium [16,22]. The capacity of sarcolemmal Ca$^{2+}$-ATPase to transport Ca$^{2+}$ from the cell is extremely limited and the contribu-
tion to the maintenance of [Ca\(^{2+}\)], is negligible [23]. Alterations in Ca\(^{2+}\) binding and phospholipid composition of the sarcolemmal membranes of diabetic rat hearts may have some influence on transmembrane Ca\(^{2+}\) flux [24]. The lower Ca\(^{2+}\) uptake could explain the lower [Ca\(^{2+}\)], in diabetic myocytes.

In the rat heart, Ca\(^{2+}\) of the SR accounts for \(\sim 90\%\) of Ca\(^{2+}\) transient [25]. The decreased activity of Ca\(^{2+}\)-ATPase in SR [7,8] could reduce Ca\(^{2+}\) loading of the SR, causing the depressed peak of Ca\(^{2+}\) transient [26]. However, it is possible that [Ca\(^{2+}\)], might not increase, since cytoplasmic Ca\(^{2+}\) could be extruded from the cell by Na\(^{+}\)/Ca\(^{2+}\) exchange and/or sarcolemmal Ca\(^{2+}\)-ATPase. If Na\(^{+}\)/Ca\(^{2+}\) exchange is assumed to be implicated in the efflux of Ca\(^{2+}\) under physiological conditions, then the reported depression of Na\(^{+}\)/Ca\(^{2+}\) exchange [10] could cause the elevation of [Ca\(^{2+}\)]. However, this is equivocal, since the mode of Na\(^{+}\)/Ca\(^{2+}\) exchange is bidirectional (forward mode and reverse mode) and it was reported that in rat myocardium the high intracellular Na\(^{+}\) concentration ([Na\(^{+}\)],) in unstimulated myocytes is in favor of Ca\(^{2+}\)-entry via Na\(^{+}\)/Ca\(^{2+}\) exchange [27].

There are other possible reasons for the low [Ca\(^{2+}\)], in diabetic myocytes. First is the duration of the diabetic state, since it has been reported that the positive and negative dp/dt of left ventricle were reduced in diabetic rats more than 30 days after the injection of streptozotocin [28]. Various parameters such as [Ca\(^{2+}\)], may be different according to the duration of the diabetic state. However, the lower [Ca\(^{2+}\)], levels were reported either 3–4 weeks [15] or 8 weeks [14] after the injection of streptozotocin. Another possible reason is related to the isolated myocytes used in the study. If there was heterogeneous progression of diabetic cardiomyopathy, the rod-shaped cells obtained from the enzymatic method might have been obtained from the intact part of diabetic myocardium. There was, however, no difference in the percentage of rod-shaped cells after cell isolation between control and diabetic rats in the study [14]. The difference in intracellular environment such as the activities of esterases or the viscosity of cytosol could be the cause of different fluorescence ratios of fura-2. However, there was no difference after the addition of 50 \(\mu\)M digitonin between control and diabetic myocytes [14]. Lagadic-Gossmann et al. [15] have also reported that there were no differences in the degree of indo-1 loading, or the relative proportion of indo-1 in the cytoplasm. [Ca\(^{2+}\)], in myocytes has been shown to be influenced by intracellular pH (pH\(_i\)) [29]. It is possible, therefore, that changes in pH\(_i\), in diabetic myocytes could affect [Ca\(^{2+}\)], since the activity of Na\(^{+}\)/H\(^{+}\) exchange has been reported to be lower in diabetic myocardium [30,31]. However, the value of pH\(_i\) was not different between diabetic and control myocytes (7.06 \(\pm\) 0.02 in diabetes and 7.07 \(\pm\) 0.02 in control) [14]. It is likely, therefore, that the lower [Ca\(^{2+}\)], of diabetic myocytes is not an artifact.

[Ca\(^{2+}\)], is regulated by [Na\(^{+}\)], via Na\(^{+}\)/Ca\(^{2+}\) exchange. The regulation of [Na\(^{+}\)], in unstimulated myocytes is mediated mainly through two membrane transport systems, namely Na\(^{+}\)/H\(^{+}\) exchange and the Na\(^{+}\)/K\(^{+}\) pump. A marked decrease in the activity of the amiloride-sensitive Na\(^{+}\)/H\(^{+}\) exchange has been shown in hearts from diabetic rats [30,31]. The inhibition of Na\(^{+}\)/H\(^{+}\) exchange could cause a decrease in [Na\(^{+}\)], in diabetic hearts. We have measured [Na\(^{+}\)], using the fluorescent indicator, sodium-binding benzofuran isophthalate (SBFI), and have reported that the level of [Na\(^{+}\)], in diabetic myocytes was significantly lower than that in normal myocytes (9.2 \(\pm\) 0.4 mM in diabetic myocytes and 12.0 \(\pm\) 0.3 mM in control myocytes; \(P < 0.01\)) [32]. The lower [Na\(^{+}\)], in diabetic myocardium could lead to decreased [Ca\(^{2+}\)], via Na\(^{+}\)/Ca\(^{2+}\) exchange. Thus, there may be a direct link between the activity of the depressed Na\(^{+}\)/H\(^{+}\) exchange and [Ca\(^{2+}\)], and therefore cardiac contractility. It has been reported that transient force recovery after acidosis was suppressed in the presence of amiloride, suggesting the contribution of Na\(^{+}\)/H\(^{+}\) exchange to the force development [33].

Conflicting results have been reported by other investigators regarding [Na\(^{+}\)], in diabetic myocardium. Kjeldsen [34] reported increased Na\(^{+}\) content in streptozotocin-induced diabetic rat ventricular muscle using flame photometry. Warley [35] demonstrated increased Na\(^{+}\) concentration in diabetic myocytes by using X-ray microanalysis. In these experiments, however, [Na\(^{+}\)], was measured not from living myocytes or muscles but from frozen or homogenized samples. Lagadic-Gossmann and Feuvray [36] have reported that intracellular Na\(^{+}\) activity was elevated in papillary muscle from diabetic hearts using Na\(^{+}\)-sensitive microelectrodes. It has been reported that the activity of the Na\(^{+}\)/K\(^{+}\) pump is decreased in diabetic myocardium [34,37] and the increase in the Na\(^{+}\) content of the diabetic heart was explained mainly by the decreased activity of the Na\(^{+}\)/K\(^{+}\) pump. It has been suggested that the increase in [Na\(^{+}\)], could lead to the elevation of [Ca\(^{2+}\)], via Na\(^{+}\)/Ca\(^{2+}\) exchange. However, [Na\(^{+}\)], of quiescent myocytes is determined by the balance between Na\(^{+}\) influx via Na\(^{+}\)/H\(^{+}\) exchange and Na\(^{+}\) extrusion via the Na\(^{+}\)/K\(^{+}\) pump. Therefore, the inhibition of Na\(^{+}\)/H\(^{+}\) exchange [30,31] could cause a decrease in [Na\(^{+}\)], in spite of the decreased activity of the Na\(^{+}\)/K\(^{+}\) pump.

The role of low [Ca\(^{2+}\)], in diabetic myocytes remains to be solved [15]. One possibility is that low [Ca\(^{2+}\)], could serve to offset the diabetes-induced increase in myofila-
ment Ca\(^{2+}\) sensitivity [38,39]. Our hypothesis is that low [Ca\(^{2+}\)], could be an adaptation to protect against cell contracture, since we have shown that the values of [Ca\(^{2+}\)], when diabetic myocytes were contracted during metabolic inhibition or during the perfusion of high [Ca\(^{2+}\)], solution was significantly lower than those of control rats [14].

Clinical and experimental studies have shown that there is depressed ventricular function in diabetic myocardium, in the form of a diminished developed tension and a
diminished velocity of contraction [40]. The impairment of force generation in diabetic myocardium could be due to the derangement of 3 elementary parameters in the excitation-coupling process [41]: (1) the pulse of the cytosolic Ca$^{2+}$ concentration that occurs during each cardiac cycle (Ca$^{2+}$ transients), (2) the sensitivity of myofilament to [Ca$^{2+}$], (3) maximal Ca$^{2+}$-activated force. Hearts from diabetic animals have been shown to have decreased sensitivity to external Ca$^{2+}$ [42]. The finding of a decreased Ca$^{2+}$ sensitivity of isometric tension in skinned cardiac myocytes from diabetic rats suggests that decreased cardiac output in the whole heart can occur independently of alterations in Ca$^{2+}$ handling. Changes in β-myosin heavy chain (MHC) [43,44] and troponin T expression [44] may contribute to the lowered Ca$^{2+}$ sensitivity of myofilaments. There has been, however, no consistent finding regarding the sensitivity of the myofilament; e.g., a slight but significant increase in Ca$^{2+}$ sensitivity was observed in skinned cardiac fibers [38,39]. It has been reported that the lower activity of myosin ATPase and abnormal myosin isozyme distribution could be responsible for the depressed contractile function in diabetic myocardium [43,45]. However, there is no simple correlation between active developed tension and myosin ATPase activity [46], and other derangements in contractile activity such as a decrease in relaxation rate [40] indicate abnormal intracellular Ca$^{2+}$ handling. Therefore, the abnormal contractile function of diabetic myocardium may be primarily due to the abnormalities of Ca$^{2+}$ transients.

Since Ca$^{2+}$ transients of diabetic rat myocytes have not been measured directly, we measured Ca$^{2+}$ transients and cell shortening using high temporal resolution video imaging analysis [47]. Indo-1 was used as the fluorescent probes for [Ca$^{2+}$], because indo-1 is suitable for measuring rapid changes in [Ca$^{2+}$], without a cell movement artifact [13]. It was shown that the diastolic base and systolic peak of Ca$^{2+}$ transients were significantly lower than those in normal myocytes [47]. The cell circumferential shortening of diabetic myocytes was also significantly lower than that of normal myocytes. Lagadic-Gossmann et al. [15] reported that both diastolic and peak [Ca$^{2+}$], were reduced in diabetic myocytes, and that the relationship between stimulation frequency (0.2–1 Hz) and normalized peak systolic [Ca$^{2+}$], was the same for both normal and diabetic myocytes. They showed that the decay of systolic [Ca$^{2+}$], was slower in diabetes, leading to a lengthening of Ca$^{2+}$ transient duration. Both the decreased magnitude of the peak of Ca$^{2+}$ transient and the slowed decay of Ca$^{2+}$ transient could be explained by the inhibition of SR Ca$^{2+}$-ATPase [26]. The decreased peak of Ca$^{2+}$ transients could be responsible for the decreased contractile function in diabetic myocardium. The depressed peak of Ca$^{2+}$ transients could be due to the reduction of Ca$^{2+}$ current. However, the prolonged action potential duration of diabetic myocardium may be due to enhanced Ca$^{2+}$ current [48]. Although it was reported that L-type Ca$^{2+}$ current was not modified by diabetes [49], it was recently shown that Ca$^{2+}$ current decreases in later stages of diabetes (24–30 weeks after streptozocin) [50]. It is also possible that diabetic myocardium may depend more on neurohormonal factors to maintain Ca$^{2+}$ transients and contraction. Previous studies have shown that the number of β-adrenergic receptors was reduced in diabetic myocardium, and that diabetic myocardium was less sensitive to isoproterenol than normal myocardium [51]. Therefore, the depressed Ca$^{2+}$ transients of diabetic myocytes may reflect a tight dependence on β-stimulants to maintain Ca$^{2+}$ transients. Noda et al. [47] and others [17] have shown that isoproterenol did not restore Ca$^{2+}$ transients of diabetic myocytes to the levels seen in normal myocytes.

It has been reported that rapid cooling contracture, which causes Ca$^{2+}$ release from the SR, is significantly depressed in diabetic myocardium [52] and myocytes [53], and that the reduction of developed tension in diabetic myocardium was a consequence of diminished Ca$^{2+}$ stores in the SR. It has been also reported that the caffeine-induced Ca$^{2+}$ release, which was used as an index of SR Ca$^{2+}$ content, was significantly reduced in diabetic myocytes [53]. Therefore, it is possible that the diminished SR Ca$^{2+}$ stores and Ca$^{2+}$ release during contraction are involved in the diminished peak of Ca$^{2+}$ transients and decreased contractility in diabetic myocytes.

In conclusion, it is most likely that basal [Ca$^{2+}$] and peak level of Ca$^{2+}$ transients are decreased in insulin-dependent diabetic myocytes. The lower Ca$^{2+}$ levels could be caused by the decreased SR Ca$^{2+}$ stores, and would be, at least in part, related to depressed cardiac contractility in diabetic heart. However, there could be a difference in Ca$^{2+}$ regulation in different types of diabetes (e.g., non-insulin-dependent diabetes mellitus). Further studies are required to substantiate the exact mechanism of diabetes-induced changes in intracellular Ca$^{2+}$ regulation and contractile function.

References


