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Inhibition of Sarcoplasmic Reticulum Ca^{2+} Uptake by Pyruvate and Fatty Acid in H9c2 Cardiomyocytes: Implications for Diabetic Cardiomyopathy

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High extracellular glucose concentration was reported to suppress intracellular Ca^{2+} clearing through altered sarcoplasmic reticulum (SR) function. In the present study, we attempted to elucidate the effects of pyruvate and fatty acid on SR function and reveal the mechanistic link with glucose-induced SR dysfunction. For this purpose, SR Ca^{2+}-uptake rate was measured in digitonin-permeabilized H9c2 cardiomyocytes cultured in various conditions. Exposure of these cells to 5 mM pyruvate for 2 days induced a significant suppression of SR Ca^{2+}-uptake, which was comparable to the effects of high glucose. These effects were accompanied with decreased glucose utilization. However, pyruvate could not further suppress SR Ca^{2+}-uptake in cells cultured in high glucose condition. Enhanced entry of pyruvate into mitochondria by dichloroacetate, an activator of pyruvate dehydrogenase complex, also induced suppression of SR Ca^{2+}-uptake, indicating that mitochondrial uptake of pyruvate is required in the SR dysfunction induced by pyruvate or glucose. On the other hand, augmentation of fatty acid supply by adding 0.2 to 0.8 mM oleic acid resulted in a dose-dependent suppression of SR Ca^{2+}-uptake. However, these effects were attenuated in high glucose-cultured cells, with no significant changes by oleic acid concentrations lower than 0.4 mM. These results demonstrate that (1) increased pyruvate oxidation is the key mechanism in the SR dysfunction observed in high glucose-cultured cardiomyocytes; (2) exogenous fatty acid also suppresses SR Ca^{2+}-uptake, presumably through a mechanism shared by glucose.

Key Words: H9c2 cell, Ca^{2+}-uptake, High glucose, Pyruvate, Dichloroacetate, Fatty acid

INTRODUCTION

Cardiovascular complication is a major contributor to mortality and morbidity of chronic diabetes mellitus (Kannel & McGee, 1974; Palumbo et al, 1981). Recently, it was proved that diabetic heart might lead to a cardiomyopathy causing congestive failure independent of coronary vascular disease (Galderisi et al, 1991; Bell, 1995; Shehadeh & Regan, 1995). The major functional alterations in the early phase of diabetic cardiomyopathy include diastolic impairments, which progress to systolic dysfunction and eventually to ventricular failure. Animal studies indicate that experimental diabetes is associated with alterations in intracellular Ca^{2+} homeostasis in cardiomyocytes (Ganguly et al, 1983; Makino et al, 1987; Bouchard & Bose, 1991). As contraction and relaxation of cardiac myocytes are regulated primarily by cytosolic Ca^{2+} levels, altered intracellular Ca^{2+} homeostasis is presumably the primary mechanism of the diastolic impairment in diabetic cardiomyopathy (Mahgoub & Abd-Elhattah, 1998).

The most important machinery for the intracellular Ca^{2+} homeostasis in cardiomyocytes is the sarcoplasmic reticulum (SR). Ca^{2+} is released from SR for contraction and actively transported back into SR for relaxation (Barry & Bridge, 1993), and Ca^{2+} uptake into the SR is conducted by SR Ca^{2+}-ATPase (SERCA). Diabetic cardiomyopathy was previously reported to be associated with decreased SERCA activities (Ganguly et al, 1983). Similarly, a significant prolongation of the decay phase of calcium transient was observed in myocytes of diabetic heart, which was attributable to dysfunction of SR Ca^{2+} uptake machinery (Lagadic-Gossman et al, 1996). However, the mechanism of decreased SR Ca^{2+} uptake rates in diabetic cardimyocyte is largely unknown.

One of the major obstacles in the researches on the pathophysiology of diabetic cardiomyopathy lies in the complexity of diabetic state. In diabetic hearts, multiple metabolic and endocrine alterations concomitantly affect myocardium. In addition, mechanical overload to myocardium is usually superimposed by hypertension. Therefore, it is hard to elucidate the causal relation between any individual factor and cardiomyocyte dysfunction in *in vivo* diabetes models. In this regard, Davidoff and Ren tried to

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ABBREVIATIONS: BSA, bovine serum albumin; DCA, dichloroacetate; PDH, pyruvate dehydrogenase complex; SERCA, SR Ca^{2+} ATPase; SR, sarcoplasmic reticulum.

establish an *in vitro* cell culture model of diabetic cardiomyopathy by exposing isolated rat cardiomyocytes to high extracellular glucose concentration (Davidoff & Ren, 1997; Ren et al, 1997), in which they observed early alterations in Ca^{2+} transient characterized by slowed intracellular Ca^{2+} clearing and mechanical relaxation. These reports strongly support the hypothesis that hyperglycemia is the principal causal factor of diastolic dysfunction in diabetic cardiomyopathy.

Pathways by which high extracellular glucose alters SR Ca^{2+} handling appear to involve changes in intracellular glucose metabolism (Ren et al, 1997). However, the detailed mechanism of glucose-induced alterations in the SR Ca^{2+} uptake function is left mostly unknown. It is quite possible that an increased glucose uptake results in increased metabolic flow into glycolysis, pentose phosphate shunt, and/or other minor metabolic pathways such as glucosamine pathway (Nishio et al, 1988; Marshall et al, 1991) or polyol pathway (Kashiwagi et al, 1997; Mezzetti et al, 1997). Alterations in the SR Ca^{2+} uptake function might be ultimately correlated with one of those intracellular metabolic changes.

However, it can also be hypothesized that changes in mitochondrial glucose metabolism, rather than the alterations in glucose metabolism in cytosolic compartment, might play a critical role in the development of diabetic cardiomyopathy. Increased glucose uptake and glycolytic flow results in increased pyruvate entry into mitochondria, and increased pyruvate oxidation in mitochondria may induce major alterations in cellular physiology. Therefore, it would be beneficial to clarify whether the effect of high glucose on Ca^{2+} dysregulation is mediated by altered metabolic flow in cytosolic compartment or in mitochondrial compartment. In the present study, we sought to reveal the effect of experimental manipulation to enhance mitochondrial pyruvate utilization on the SR Ca^{2+} uptake function of cultured cardiomyocytes. Elevated pyruvate concentration in culture medium resulted in suppression of Ca^{2+} uptake, which was comparable to the effect of high glucose medium. These effects were accompanied by decreased glucose uptake, indicating decreased glycolytic flow in cytosolic compartment. However, it could not be excluded that increased pyruvate concentration in cytosolic compartment might cause accumulation of certain intermediate metabolite of cytosolic glucose metabolism, without increasing glucose entry into glycolytic pathway. Therefore, in another series of experiments, we treated cells with dichloroacetate (DCA), which is known to stimulate pyruvate entry into mitochondria by enhancing the activity of pyruvate dehydrogenase complex (PDH) (Abdel-aleem et al, 1996). Similar to pyruvate, DCA also induced suppression of SR Ca^{2+} uptake and decreased glucose uptake.

The second aim of the present study was to reveal the effects of fatty acid and explore the possible relationship with glucose metabolism. Fatty acid is one of the major energy substrates in myocardial cells, and its serum concentration is markedly increased in diabetic state. Moreover, fatty acid and glucose are closely linked in their metabolism. Glucose utilization in skeletal and cardiac muscle is inhibited by increasing the rate of fatty acid oxidation, which is presumed to be mediated through the inhibition of pyruvate dehydrogenase complex and phosphofructokinase by end products of fatty acid oxidation (Randle et al, 1963). Likewise, the reverse process, such as the decrease of fatty acid oxidation by increase of glucose utilization, has also been reported, and the role of malonyl-CoA as the mediator has been suggested (Saddik et al, 1993). Therefore, it is reasonable to hypothesize that glucose and fatty acid may affect each other in the development of SR dysfunction.

METHODS

Cell culture

We established an *in vitro* model of diabetic cardiomyopathy using H9c2 cardiac myocyte cell line (ATCC, cardiac myoblasts from rat). This commercially available cell line has extensively been characterized and confirmed to be a useful surrogate for researches on cardiomyocyte physiology (Hescheler et al, 1991; Sipido & Marban, 1991), and the confluent H9c2 cells have been proved to possess SR Ca^{2+} regulatory function which was comparable to that of primary cell culture from isolated cardiac myocytes (Szalai et al, 2000). Cells were cultured as monolayers in Dulbecco's modified Eagle's medium (GIBCO BRL) supplemented with 10% fetal bovine serum (GIBCO BRL). The medium contained glucose 1,000 mg/L, sodium pyruvate 110 mg/L, and glutamine 584 mg/L, along with other common components. Cells were grown under an atmosphere of 10% CO_2 in humidified air at 37°C. The medium was replaced every 2 or 3 days. A stock of cells was grown in a culture flask and split before confluence at a subcultivation ratio of 1 : 6 or 1 : 7. Cells used in experiments were cultured in flasks for 2~3 weeks, and then harvested by trypsinization and subjected to calcium uptake measurement.

H9c2 cells reached confluence in about 1 week, and further cultivation resulted in partial multinucleation, presumably indicating differentiation into myocytes. In preliminary experiments of fluo-3 imaging, we observed that subconfluent cells did not respond to caffeine, while confluent cells showed an increased cytosolic Ca^{2+} concentration in response to caffeine (data not shown).

Determination of Ca^{2+} uptake

We developed a method to measure SR Ca^{2+} uptake in permeabilized H9c2 cells, which was modified from the method established by Wimsatt et al. (Wimsatt et al, 1990). After trypsinization and washing with phosphate-buffered saline, approximately 3×10^6 cells were suspended in 6 ml of assay buffer containing (mM) histidine 40 (pH 7.0), KCl 100, $MgCl_2$ 5, EGTA 0.5, potassium oxalate 10, sodium azide 5, rotenone 0.016, and oligomycin 0.01. Digitonin was added to this suspension to the final concentration of 40 μg/ml. Aliquots of the suspension were preincubated in a water bath at 37°C with stirring, and ruthenium red was added to the final concentration of 0.03 mM. Various concentrations of $CaCl_2$ solution, containing $^{45}CaCl_2$, were added to yield the final pCa of 7.6, 7.0, 6.6 and 6.0 according to the method of Robertson and Potter (Robertson & Potter, 1984). After equilibration for 3 minutes, ATP was added to the final concentration of 5 mM to initiate ATP-dependant calcium uptake. The amount of calcium transported into the SR was quantitated using the Millipore filtration technique described by Martonosi and Feretos (Martonosi & Feretos, 1964). A 250-μl aliquot of permeabilized, calcium-challenged cell suspension was filtered

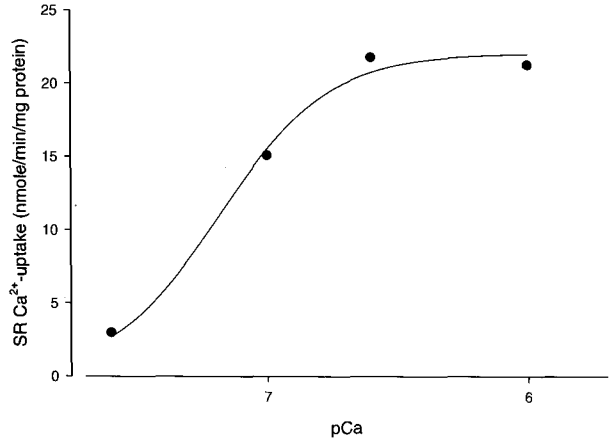

Fig. 1. SR Ca^{2+}-uptake of permeabilized H9c2 cardiomyocytes. Ca^{2+}-uptake, expressed as nmoles/min/mg protein, was measured in pCa 7.6, 7.0, 6.6 and 6.0 conditions, and the plots were fitted to Hill's equation. See Methods for details.

through Milipore filter before the initiation of ATP-dependant calcium uptake for estimation of nonspecific binding, and 500-μl aliquots were filtered at 2 minutes after the addition of ATP for estimation of calcium uptake rate (nmole/min/mg protein). The filters were immediately washed with 5 ml of wash buffer (Tris-Cl 20 mM, KCl 100 mM, EGTA 0.5 mM, pH 7.0), dried, and then counted for radioactivity. Specific radioactivity was calculated by counting the aliquots of the suspension. Protein concentration of the suspension was measured with BCA reagent (Sigma) as described by the manufacturer.

The residual Ca^{2+} uptake activity in the presence of mitochondrial inhibitors and ruthenium red was completely blocked by a SR Ca^{2+}-ATPase inhibitor, thapsigargin (data not shown), which confirmed that the Ca^{2+}-uptake measured with this method represents the SR-specific Ca^{2+} uptake.

Calculation of maximal Ca^{2+} uptake value

Fig. 1 illustrates the results of a representative experiment, showing SR Ca^{2+}-uptake value (calculated as nmole Ca^{2+}/min/mg protein) plotted against the initial pCa value. These plots were, using commercially available curve fitting program (Sigmaplot), fitted to Hill's equation:

$$Ca^{2+}\ \text{uptake} = \frac{M \cdot [Ca^{2+}]^{\alpha}}{K^{\alpha} + [Ca^{2+}]^{\alpha}}$$

where M is the maximal Ca^{2+}-uptake, K the Ca^{2+} concentration exerting half maximal Ca^{2+}-uptake, and a the Hill's coefficient. The calculated maximal SR Ca^{2+}- uptake value, presented as the percent of the corresponding control cells, was used as the index of SR Ca^{2+}-uptake in each experiment.

Measurement of glucose, pyruvate and lactate in culture medium

Media glucose concentrations were measured with a glucose analyzer (YSI, USA). Concentrations of pyruvate and lactate were assayed using conventional enzymatic analysis (Bergmeyer, 1983) after acid treatment. Briefly, medium samples were treated with 100% trichloroacetic acid (final concentration 10%), centrifuged, and neutralized with 3 M KOH. For pyruvate assay, 40 μl sample was added to 955 μl of reaction buffer (100 mM potassium phosphate, pH 7.4, 0.1 mM NADH), and the reaction was initiated by adding 5 μl of 5 units/ml lactate dehydrogenase. Changes in OD at 340 nm were measured after incubation for 10 minutes at room temperature. For lactate assay, 100 μl sample was added to 876 μl of reaction buffer (116 mM sodium glutamate, pH 8.9, 0.93 mM NAD), and the reaction was initiated by adding 17 μl of 80 units/ml alanine aminotransferase and 7 μl of 4 units/ml lactate dehydrogenase. Changes in OD at 340 nm were measured after incubation for 60 minutes at room temperature. Standard solutions of pyruvate and lactate were used for calculation of concentrations.

Fatty acid treatment

Stock solution of oleic acid-bovine serum albumin (BSA) complexes were prepared according to published protocols (Zahabi & Deschepper, 2001), in which 20 mg of oleic acid (free acid, Sigma) was added to 2 ml of water, and dissolved by adding 30 μl of 1 N NaOH and heating briefly at 70°C with agitation. Eight milliliters of BSA solution (250 mg/ml) was then added dropwise to the dissolved oleic acid to yield stock solutions containing 8 mM oleic acid complexed in an approximate 3:1 ratio with BSA. The stock solution was added to culture media to the final concentrations of 0.2, 0.4, or 0.8 mM oleic acid. Control cells were treated with oleic acid-free BSA solution.

Statistical analysis

Values in each experiment were expressed as mean ± SEM. Statistical significance between two groups was assessed by unpaired Student's t-test. A p-value less than 0.05 was defined as statistically significant.

RESULTS

Effects of glucose and pyruvate on SR Ca^{2+} uptake

After seeding in culture flasks, cells were grown for about 2 weeks before experimental manipulation. In this period, a group of cells were cultured in high glucose (25 mM) medium instead of the standard DMEM medium containing 5.5 mM glucose. Compared to control cells, the cells cultured in high glucose medium did not show any differences in morphology and proliferation rate. As reported in previous studies (Davidoff & Ren, 1997; Ren et al, 1997), exposure to high glucose medium resulted in a significant suppression of SR Ca^{2+}-uptake function (Fig. 2).

In each of control and high glucose groups, effects of pyruvate were tested by changing the culture medium with DMEM containing 5 mM sodium pyruvate and further incubation for 2 days before Ca^{2+}-uptake measurements. In low glucose-cultured pyruvate-treated cells, SR Ca^{2+}-uptake was suppressed, which was comparable to the results in high glucose-cultured cells. However, in high glucose-cultured cells, pyruvate treatment exerted no further suppression of SR Ca^{2+}-uptake (Fig. 2), implying

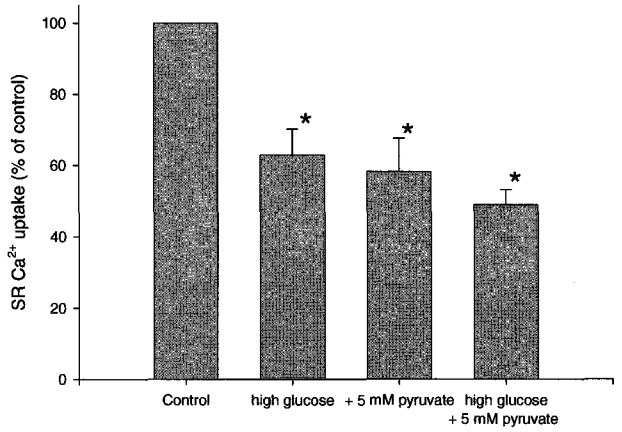

Fig. 2. Suppression SR Ca^{2+}-uptake by glucose and pyruvate. High glucose group was cultured in 25 mM glucose for 2 weeks. Pyruvate was added to culture medium 2 days before the Ca^{2+}-uptake measurements. Calculated maximal SR Ca^{2+}-uptake value was presented as the percent of the parallel control cells. *: $p<0.05$.

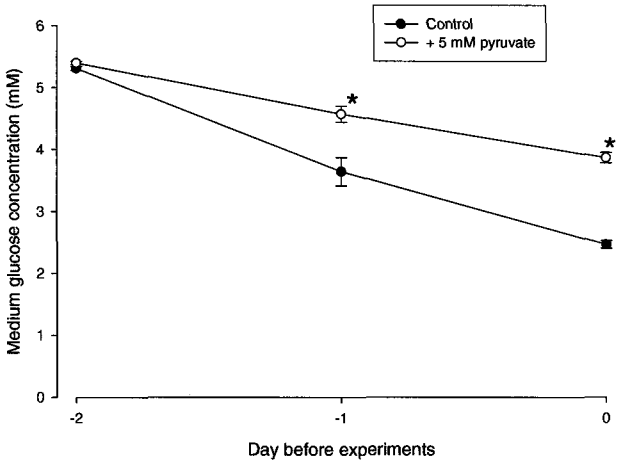

Fig. 3. Decrease of medium glucose concentrations in the presence or absence of pyruvate. Culture medium was changed with fresh DMEM with or without 5 mM pyruvate, and the glucose concentration was measured for 2 days before Ca^{2+}-uptake experiments. *: $p<0.05$.

a common mechanism involved in effects of glucose and pyruvate on SR Ca^{2+}-uptake.

Cultured cells are known to convert a large amount of glucose to lactate through anaerobic glycolysis, with relatively little pyruvate entering the mitochondria in basal state (Neermann & Wagner, 1996). It was presumed that supraphysiological concentration of pyruvate in culture medium would enhance the mitochondrial pyruvate uptake and oxidation, with decreased demand for glucose. For 2 days after changing the culture medium with fresh DMEM containing 5 mM glucose, medium glucose concentration was decreased by a rate of approximately 1.4 mM/day. As expected, 5 mM pyruvate in culture medium inhibited glucose uptake (Fig. 3). Therefore, it was shown that, unlike in the high glucose-cultured cell group where glucose uptake and lactate production was increased (data not shown), the suppression of SR Ca^{2+}-uptake in pyruvate-

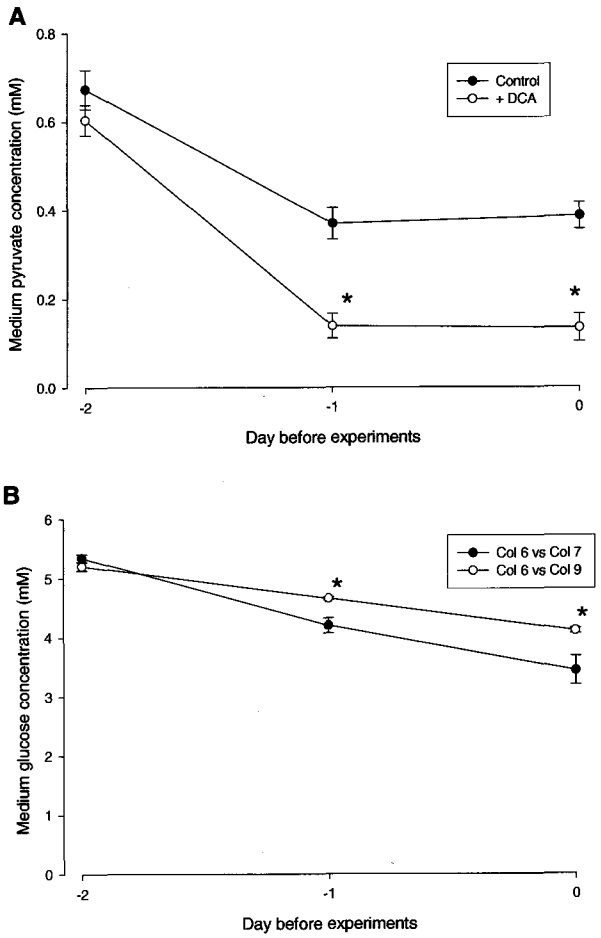

Fig. 4. Changes of medium glucose and pyruvate concentrations in the presence or absence of DCA. Culture medium was changed with fresh DMEM with or without 5 mM DCA, and pyruvate (A) or glucose (B) concentrations were measured in aliquots of culture medium for 2 days before Ca^{2+}-uptake experiments. *: $p<0.05$.

treated cell group was accompanied by a decreased glucose uptake.

Effects of DCA on SR Ca^{2+} uptake

The results mentioned above supported the hypothesis that suppressed SR Ca^{2+}-uptake observed in high glucose-cultured cells correlated with increased pyruvate uptake by mitochondria, rather than increased glucose uptake. To confirm this hypothesis, we tested the effects of DCA, a known stimulator of PDH complex.

Cells were treated with 5 mM DCA and incubated for 2 days before SR Ca^{2+}-uptake measurements. In this period, the pyruvate concentration in culture medium was significantly lower that that of control cells (Fig. 4A), indicating low intracellular pyruvate concentration. Concurretly, the glucose uptake was lowered by DCA treatment (Fig. 4B), although the cells were not supplied with alternative energy source. These results indicated that the cellular energy metabolism was shifted toward higher mitochondrial pyruvate oxidation with concomitant suppression of anaerobic glycolyis. In these DCA-treated cells,

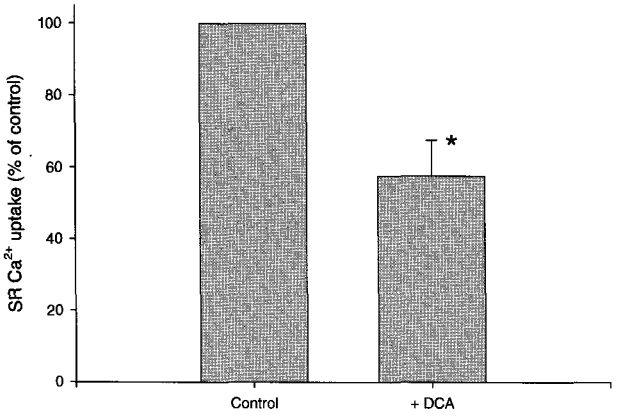

Fig. 5. Suppression of SR Ca^{2+}-uptake by DCA. Cells were incubated with 5 mM DCA for 2 days before the Ca^{2+}-uptake measurements. Calculated maximal SR Ca^{2+}-uptake value was presented as the percent of the parallel control cells. *: $p<0.05$.

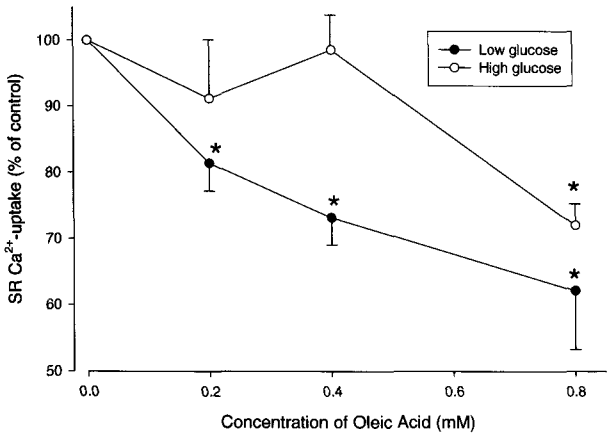

Fig. 6. Suppression SR Ca^{2+}-uptake by oleic acid in low glucose- or high glucose-cultured cells. Various concentrations of oleic acid complexed to BSA were added to culture medium 2 days before Ca^{2+}-uptake experiments. Calculated maximal SR Ca^{2+}-uptake value was presented as the percent of the parallel control cells, to which fatty acid-free BSA of equimolar concentration was added. *: $p<0.05$.

SR Ca^{2+}-uptake was suppressed in a degree comparable to those in high glucose-cultured cells or pyruvate-treated cells (Fig. 5).

Concluding from the effects of high glucose concentration, pyruvate treatment and DCA treatment, the suppression of SR Ca^{2+}-uptake by altered glucose metabolism was not due to either increased glucose uptake or increased cytosolic pyruvate concentration, however correlated with enhanced pyruvate oxidation in mitochondria.

Effects of fatty acid on SR Ca^{2+} uptake

As a substrate for energy production, fatty acid is an alternative source of intramitochondrial acetyl-CoA, which enters Krebs cycle. Access of fatty acid-derived acetyl-CoA to Krebs cycle influences the intramitochondrial metabolism of pyruvate and vice versa. In an attempt to elucidate

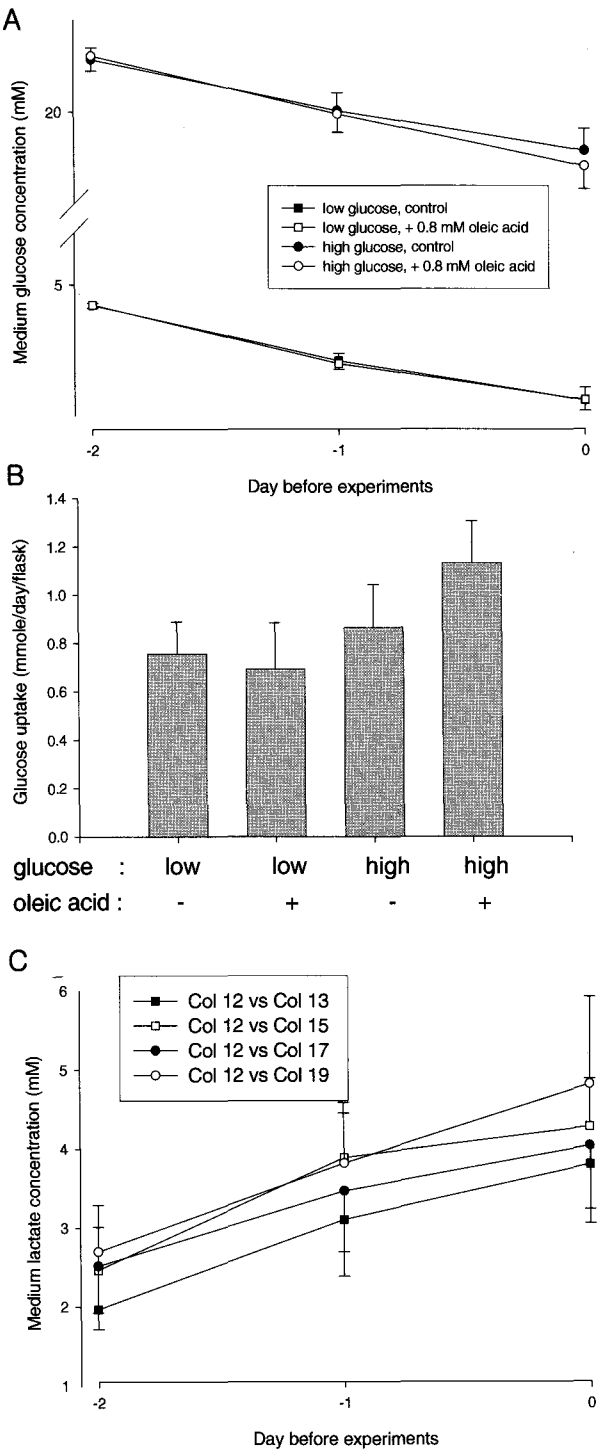

Fig. 7. Glucose uptake and lactate production in cells cultured with oleic acid supplementation. (A) Medium glucose concentration was monitored for 2 days of oleic acid (0.8 mM) supplementation in low glucose- or high glucose-cultured cells. (B) Averaged glucose uptake rate was calculated in each group. (C) Accumulation of lactate in culture medium was measured for 2 days of oleic acid (0.8 mM) supplementation in low glucose- or high glucose-cultured cells.

the effects of fatty acid on SR Ca^{2+}-uptake, we added various concentrations of oleic acid, the most abundant extracellular fatty acid, to cultured cardiomyocytes and compared the SR Ca^{2+}-uptake rate to those of corresponding control cells treated with the vehicle. As illustrated in fig. 6, 0.2 to 0.8 mM oleic acid concentrations dose-dependently suppressed SR Ca^{2+}-uptake.

In another series of experiments, effects of the same concentrations of fatty acid were examined in high glucose-cultured cells to clarify the relationship between glucose- and fatty acid-induced SR dysfunction. In cells cultured for 2 weeks in 25 mM glucose, low concentration (0.2 and 0.4 mM) of oleic acid failed to induce the suppression of SR Ca^{2+}-uptake, while 0.8 mM oleic acid induced a significant suppression (Fig. 6).

In either low glucose or high glucose culture conditions, the glucose uptake was not altered by the addition of oleic acid to the culture medium (Fig. 7A and B). On the other hand, the accumulation of lactate, the end product of anaerobic glycolysis, was slightly enhanced by oleic acid treatment both in low glucose- and high glucose-cultured cells, indicating lower pyruvate oxidation (Fig. 7C).

DISCUSSION

Our results show that the dysfunctional SR Ca^{2+}-uptake induced by altered glucose metabolism is mimicked by increased pyruvate supply. Moreover, SR dysfunction induced by pyruvate disappeared in high glucose-cultured condition, indicating a shared mechanism in the effects of glucose and pyruvate. As glucose uptake was decreased in pyruvate-treated cells, it is clear that increased glucose uptake is not necessarily involved in hyperglycemia-induced SR dysfunction. To elucidate the detailed mechanism of pyruvate-induced SR dysfunction, we attempted to find out the altered metabolic step which is causally related with suppressed SR Ca^{2+}-uptake. Therefore, PDH activation by DCA treatment was adopted as an experimental tool to enhance the pyruvate oxidation by mitochondria. This manipulation lowered cellular glucose uptake and medium pyruvate concentration. In preliminary experiments, when the medium was manipulated to various initial concentrations of pyruvate (0, 0.3, or 1 mM) and applied to cells, the concentration of pyruvate was adjusted to approximately 0.3 mM within 24 hours and maintained for several days (data not shown), indicating that the extracellular pyruvate concentration was regulated by cellular components. Therefore, lower extracellular pyruvate concentration in DCA-treated cells most likely reflect the lower cytosolic pyruvate concentration, resulting from the increased flow of pyruvate into PDH pathway. Taken together, these results indicated that increased mitochondrial pyruvate uptake, rather than enhancement of certain metabolic pathway in the cytosolic compartment, is required for the altered SR Ca^{2+}-uptake induced by glucose or glucose-related metabolites.

While the defects of SR calcium transport in diabetic cardiomyopathy have been implicated in numerous studies (Ganguly et al, 1983; Bouchard & Bose, 1991; Lagadic-Gossman et al, 1996), the mechanism of hyperglycemia-induced SR dysfunction has rarely been studied. However, Ren et al. (Ren et al, 1997) have proposed that an increased flow into glucosamine pathway, branching from fructose-6-phosphate in glycolytic cascade (Nishio et al, 1988; Marshall et al, 1991), is responsible for dysfunctional Ca^{2+} regulation induced by hyperglycemic conditions. Our results, which excludes cytosolic glucose metabolism as a causal mechanism of SR dysfunction, contradicts the glucosamine hypothesis.

On the other hand, several researchers raised a question as to whether the diabetic cardiomyopathy is related to hyperglycemia itself or there are factors in addition to hyperglycemia which contribute to the alteration of cardiac function seen during diabetes. In this regard, it has been reported that, in animal and clinical studies of diabetes, diminished myocardial compliance was either unaffected or only partially reversed by rigid control of hyperglycemia (Kannel & McGee, 1974; Regan et al, 1981). These data support the assumption that metabolic derangement other than hyperglycemia might play an important role in the pathogenesis of diabetic cardiomyopathy. In this regard, it was proposed that altered fatty acid metabolism could be candidates for the causal factor of the development of diabetic cardiomyopathy (Rodrigues & McNeill, 1992). It is widely known that diabetes is associated with abnormalities in plasma lipid level, which may persist in spite of hypoglycemic therapy.

In the present study, we showed that physiologic concentrations of oleic acid induce dose-dependent suppression of SR Ca^{2+}-uptake, supporting the role of elevated fatty acid in the development of diabetic cardiomyopathy. However, the effects of oleic acid were markedly attenuated in high glucose-cultured cells. These results implied that a common mediator is involved in the SR dysfunction induced by glucose or by fatty acid. Presumably, in diabetic conditions where both glucose and fatty acid levels are elevated in cellular environment, these two factors might synergistically affect the SR function. Moreover, normalization of one of these two factors may not be sufficient to remedy the SR dysfunction.

Exploration of the detailed mechanism of suppressed SR Ca^{2+}-uptake by pyruvate or fatty acid requires further researches. In this regard, several studies have indicated that fatty acids at high concentrations might, directly or indirectly through potentially toxic metabolites, affect intracellular Ca^{2+} homeostasis (Adams et al, 1979; Lopaschuk et al, 1983). Adams et al. showed that palmitylcarnitine, an endogenous fatty acyl ester, was incorporated into isolated SR membrane and concomitantly inhibited Ca^{2+}-ATPase activity (Adams et al, 1979). In a more recent study, Lopaschuk et al. reported increased levels of long chain acylcarnitines associated with SR preparation of drug-induced diabetic rat heart (Lopaschuk et al, 1983). On the basis of observation that palmitylcarnitine potently inhibited SR Ca^{2+}- uptake, they argued that high endogenous long chain acylcarnitines in diabetic rat heart caused the suppression of SR Ca^{2+}-uptake. We speculate that high extracellular fatty acid concentration in cultured cells might exert effects on SR Ca^{2+}-uptake through a similar mechanism.

On the other hand, increased fatty acid metabolites might also be involved in the suppression of SR Ca^{2+}-uptake by increased pyruvate oxidation in mitochondria. It was reported that acetyl-CoA produced from pyruvate oxidation inhibits the β-oxidation of fatty acid in mitochondira (Abdel-aleem et al, 1996). Although the standard cell culture condition in our experiments does not include additional fatty acid supplementation, the high lipid content of serum included in culture media is likely enough

to maintain the contribution of fatty acids in mitochondrial energy metabolism. Increased pyruvate oxidation by high glucose, pyruvate or DCA would inevitably suppress the entry of acylcarnitine into mitochondria, with resultant accumulation of this metabolite.

In conclusion, we demonstrated that: (1) increased pyruvate oxidation is the key mechanism in the SR dysfunction observed in high glucose-cultured cardiomyocytes; (2) exogenous fatty acid also induces the suppression of SR Ca^{2+}-uptake, presumably through a mechanism shared by glucose.

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