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Contraction and intracellular Ca^{2+} in ventricular myocytes from rats receiving fructose-enriched diets

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Abstract

Contraction and intracellular Ca^{2+} in ventricular myocytes from rats receiving fructose-enriched diets (FED) were investigated. Groups of male Wistar rats received normal rat chow together with either normal drinking water or water containing 500 mM fructose *ad libitum*. Experiments were performed 12-14 weeks later. Rats receiving FED responded differently to a standard glucose tolerance test. Fasting blood glucose levels in FED and control rats were 48.5±2.3 (4) and 47.0±1.9 (4) mg/dl, respectively. At 30 min after intraperitoneal administration of glucose (2g/kg body weight), concentrations of blood glucose were significantly ($p<0.05$) higher in rats receiving FED (206.3±19.5 mg/dl) compared to controls (150.3±8.5 mg/dl). At 120 min, blood glucose in FED (87.3±4.2) and control (80.3±3.4 mg/dl) rats were still significantly ($p<0.01$) higher than initial values. Amplitude and time to half (THALF) relaxation of ventricular myocyte shortening and Ca^{2+} transient were not altered by FED. Time to peak (TPK) shortening was unaltered, however, the TPK Ca^{2+} transient was significantly prolonged in FED rats (65.1±3.4 ms) compared to controls (56.4±2.0 ms). In conclusion, diets supplemented with drinking water containing 500 mM fructose for a period of 12-14 weeks did not have significant effects on ventricular myocyte shortening but did prolong the time course of the Ca^{2+} transient suggesting that defects in Ca^{2+} transport may precede defects in myocyte contractility in this experimental model.

Key words: *Fructose, ventricular myocytes, contraction, intracellular calcium.*

Introduction

The hallmarks of type 2 diabetes are impaired insulin action in peripheral tissues and decreased pancreatic beta-cell function. Genetic and lifestyle factors including consumption of high-calorie diets, lack of exercise and consequent obesity are all risk factors associated with the etiology of type 2 diabetes. Previous experimental studies have demonstrated that chronic fructose-feeding may lead to hyperglycaemia and glucose intolerance which is associated with hyperinsulinaemia and loss of normal insulin sensitivity. [1-3] The insulin resistance is due, at least in part, to diminished ability of insulin to suppress hepatic glucose output.[4] Other characteristics reported in fructose fed rats include hypertriglyceridaemia and increased systolic blood pressure.[3] In this study we have investigated the effects of fructose-enriched diets (FED) on ventricular myocyte contraction and intracellular Ca^{2+} in rats.

Methods

Rats: Male Wistar rats, weighing between 160-180 g, were maintained on a 12-hr dark-light cycle. All rats received normal rat chow and water *ad libitum*. One group of rats (n=4) received normal U/V sterilized tap water (controls) whilst another group (n=4) received tap water containing 500 mM fructose (fructose-enriched diet; FED). Experiments were performed 12-14 weeks after commencement of the FED. Principles of laboratory animal care were followed throughout.

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Measurement of blood glucose and plasma insulin

Glucose tolerance tests were performed at week 12 according to established methods.[5] Briefly, after an overnight fast rats received an intraperitoneal (ip) injection of glucose (2g/kg body weight) and blood glucose was measured (One Touch II glucose meter, Lifescan Inc, USA) during a period of 2 hr. Blood glucose was also measured in non-fasting animals immediately prior to experiments. Insulin was measured in samples of blood plasma stored at –80 °C using a standard radioimmunoassay (Rat insulin RIA Kit, Linco Research Inc, USA).

Ventricular myocyte isolation

Single ventricular myocytes were isolated according to previously described techniques with minor modifications.[6-7] In brief, rats were killed humanely by decapitation with a guillotine. Hearts were then removed rapidly, mounted on a Langendorff perfusion apparatus and perfused retrogradely at a constant flow of 8 ml/g heart/min with a HEPES-based salt solution (isolation solution - see below) containing 0.75 mM Ca^{2+}. When the coronary circulation had cleared of blood, perfusion was continued for 4 min with Ca^{2+} - free isolation solution containing 0.1 mM EGTA, and then for 6 min with solution containing 0.05 mM Ca^{2+}, 0.75 mg/ml collagenase (type 1; Worthington, N.J., USA) and 0.075 mg/ml protease (type X1V; Sigma). After this time, the ventricles were excised from the heart, minced and gently shaken in collagenase-containing isolation solution supplemented with 1 % BSA. Cells were filtered from this solution at 4 min intervals and resuspended in isolation solution containing 0.75 mM Ca^{2+}.

Measurement of shortening

Ventricular myocytes were allowed to settle on the glass

bottom of a Perspex chamber mounted on the stage of an inverted microscope (Axiovert 35, Zeiss, Germany). Electrically stimulated (1 Hz) myocytes maintained at 35-36 °C were superfused (3-5 ml/min) with HEPES-based normal Tyrode solution (see below) pH adjusted to 7.4. Once contractility had reached steady-state recording of contraction was commenced. Unloaded shortening was used as an index of contractility.[8] The shortening of myocytes was followed using a video edge detection system (VED-114, Crystal Biotech, USA). Amplitude of shortening (expressed as a % of resting cell length; RCL), time to peak (TPK) shortening and time from peak to half (THALF) relaxation were measured (Signal Averager v 6.37 software, Cambridge Electronic Design Ltd, UK).[6-7]

Measurement of intracellular Ca^{2+}

Myocytes were loaded with the fluorescent indicator fura-2 AM (Molecular Probes, Eugene, OR, USA) as described previously.[9] In brief, 6.25 µl of a 1.0 mM stock solution of fura-2 AM (dissolved in dimethylsulphoxide, DMSO) was added to 2.5 ml of cells to give a final fura-2 concentration of 2.5 µM. Myocytes were shaken gently for 10 min at 24 °C (room temperature). After loading, myocytes were centrifuged, washed with normal Tyrode to remove extracellular fura-2 and then left for 30 min to ensure complete hydrolysis of the intracellular ester.

To measure intracellular Ca^{2+} concentration $[Ca^{2+}]_i$, myocytes were alternately illuminated by 340 nm and 380 nm light using a monochromator (Cairn Research, England) which changed the excitation light every 2 ms. The resultant fluorescent emission at 510 nm was recorded by a photomultiplier tube and the ratio of the emitted fluorescence at the two excitation wavelengths (340/380 ratio) was calculated to provide an index of $[Ca^{2+}]_i$.

Solutions

The cell isolation solution contained (in mM) 130.0 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.4 NaH_2PO_4, 5 HEPES, 10 glucose, 20 taurine and 10 creatine set to pH 7.3 with NaOH. Normal Tyrode solution contained (in mM): NaCl 140; KCl 5; $MgCl_2$ 1; glucose 10; HEPES 5; $CaCl_2$ 1 set to pH 7.4 with NaOH.

Statistical analysis

Results are expressed as the mean ± S.E.M of *n* observations. Statistical comparisons were performed using Independent Samples t-test (SPSS, v 11.0). *p* values less than 0.05 were considered significant.

Results

General characteristics of FED rats

The effects of FED on food and water consumption and weight gain are shown in Table 1. Food consumption was reduced, water consumption was significantly ($p<0.05$) increased and weight gain was marginally increased in rats receiving FED. The results of glucose tolerance tests performed at 12 weeks after commencement of FED is shown in Figure 1. At time zero, after an overnight fast, blood glucose in FED and control rats were 48.5±2.3 (n=4) and 47.0±1.9 (n=4) mg/dl, respectively. At 30 min after ip administration of glucose (2 g/ kg bodyweight) blood glucose was significantly higher in FED (206.3±19.5 mg/dl) compared to control (150.3±8.5 mg/dl) rats. At 120 min blood glucose in FED and control rats were 87.3±4.2 and 80.3±3.4 mg/dl, respectively, still significantly ($p<0.01$) higher than the time zero values. The general characteristics of the animals immediately prior to experiments are shown in Table 2. Body and heart weight, non-fasting blood glucose and plasma insulin were marginally increased in FED rats compared to controls. Blood plasma osmolarity was significantly ($p<0.05$) increased in FED rats compared to controls.

General characteristics of ventricular myocytes from FED rats

There were no clear visual differences between rod-shaped myocytes from FED and control rats. The viability of myocytes (expressed as percentage rod-shaped vs. round-shaped cells) was not significantly ($p>0.05$) altered by fructose feeding. Viability of myocytes from FED rats was 67.8±1.2 (n=4) compared to 66.1±0.5 (n=4) in controls. Resting cell length (RCL) of myocytes from FED rats (137.8±4.5 µm, n=22) were not significantly different from controls (126.2±4.5 µm, n=23).

Figure 2a is a fast time-base record of shortening from a representative ventricular myocyte isolated from a control rat. Figure 1a-d show mean data describing the amplitude and time course of contractile shortening in cells from FED and control rats. FED did not significantly ($p>0.05$) alter the amplitude of shortening or either the TPK shortening or the THALF relaxation.

Figure 3a is a fast time-base record of a Ca^{2+} transient from a representative ventricular myocyte isolated from a control rat. Figure 3b-d show mean data describing the amplitude and time course of Ca^{2+} transients in cells from FED and control rats. Whilst FED did not significantly alter either the amplitude or the THALF relaxation of the Ca^{2+} transient, the TPK Ca^{2+} transient was significantly ($p<0.05$) prolonged in myocytes from FED rats compared to controls. The TPK in FED and control myocytes were 65.1±3.4 ms compared to 56.4±2.0 ms, respectively.

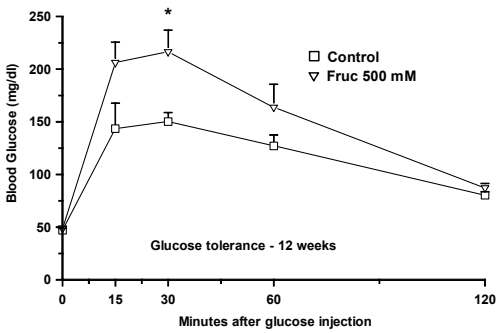

Figure 1: Blood glucose concentrations in response to an intraperitoneal glucose challenge (2g glucose/kg body weight) after an overnight fast at 12 weeks after initiation of FED. Values are mean ± SEM (n=4). * $p<0.05$ Independent sample t-test.

Table 1: Effects of fructose-enriched diets on food and water consumption at 10 weeks and weight gain at 12 weeks after commencement of FED.

	Control	500 mM fructose
Food consumption during week 10 (g/day/group[1])	90.1±6.2	74.2±7.7
Water consumption during week 10 (ml/day/group[1])	154.3±22.0	286.6±37.7*
Weight gain after 12 wks (%)	178.1±5.9	185.1±9.8

Data mean ± SEM (n=4). * $p<0.05$ Independent sample t-test. [1]Mean group data.

Table 2: General characteristics of animals immediately prior to experiments

	Control	500 mM fructose
Body weight (g)	311.50±10.94	338.75±18.91
Heart weight (g)	1.10±0.04	1.23±0.08
Non-fasting blood glucose (mg/dl)	79.25±9.14	92.00±2.94
Plasma insulin (ng/ml)	1.62±0.35	2.80±0.37
Plasma osmolarity (mosmol/kg)	294.75±3.07	307.25±2.17*

Data mean ± SEM (n=4). * $p<0.05$ Independent sample t-test.

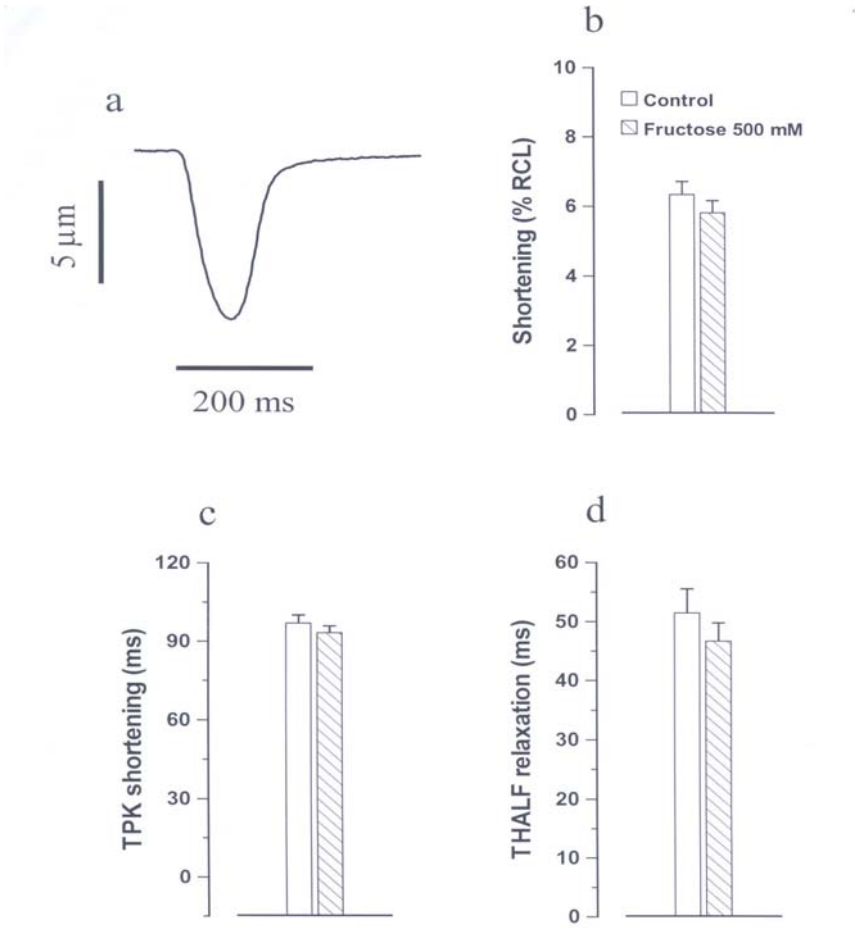

Figure 2: Representative fast time-base recording of unloaded shortening in a control ventricular myocyte (a). Mean data showing amplitude of shortening (b) time to peak shortening (c) and time to half relaxation of shortening (d). Values are mean ± SEM of 22-23 observations.

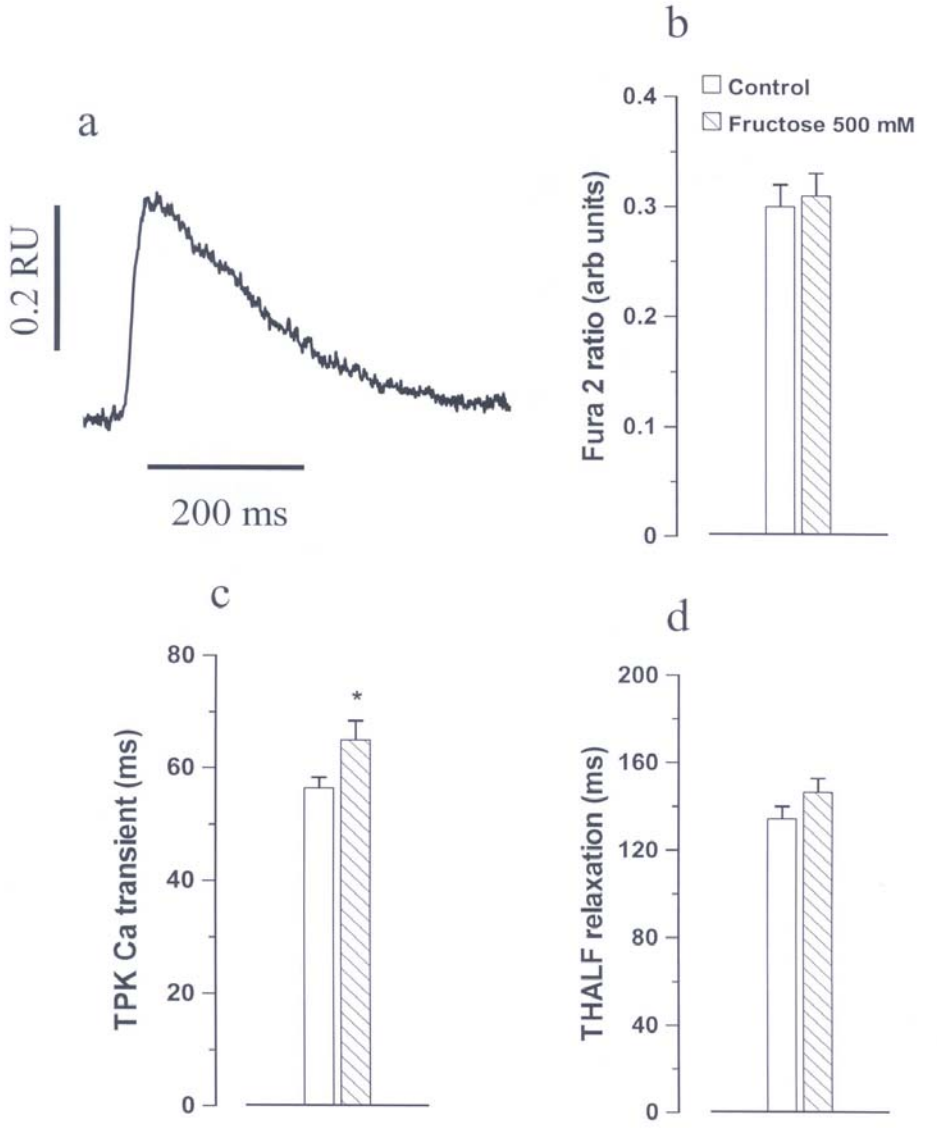

Figure 3: Representative fast time-base recording of Ca^{2+} transients in a control ventricular myocyte (a). Mean data showing amplitude of Ca^{2+} transients (b) time to peak Ca^{2+} transients (c) and time to half relaxation of Ca^{2+} transients (d). Values are mean ± SEM of 27-32 observations. * $p<0.05$ Independent sample t-test.

Discussion

Several previous studies have shown that chronic feeding with fructose-enriched diets produces hyperinsulinaemia and insulin resistance.[2-4] Other characteristics reported in fructose-fed rats included hypertriglyceridaemia and hypertension.[3] In these studies fructose-enrichment was achieved by supplementation of the food with fructose for example a diet containing 66% fructose, 12% fat and 22% protein.[3] In our study fructose-enrichment was achieved by addition of fructose (500 mM) to the drinking water. Food consumption was reduced, water consumption was significantly ($p<0.05$) increased and weight gain was increased in rats receiving FED. Non-fasting blood glucose and plasma insulin were marginally increased in FED rats. FED rats responded differently to a glucose tolerance test. At 30 min after administration of glucose (2g/kg body weight, ip) concentration of blood glucose was significantly higher in FED rats compared to controls. At 120 min the concentration of blood glucose had dropped to similar levels in FED and control though both were still significantly above basal glucose levels. Impaired early kinetics of insulin release might explain the high concentration of glucose in blood of FED rats at 30 min after ip glucose injection.

The amplitude and time course of shortening were not altered in myocytes at 12-14 weeks after commencement of FED. Although the amplitude of the Ca^{2+} transient was not altered the TPK Ca^{2+} transient was prolonged in myocytes from FED rats. This may suggest that defects in Ca^{2+} transport may precede defects in contractility at least after this period of dietary intervention.

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