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

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Abstract

Contraction and intracellular free Ca^{2+} concentrations $[Ca^{2+}]_i$ in ventricular myocytes from rats receiving sucrose-enriched diets (SED's) were investigated. Groups of Wistar rats received normal rat chow together with either normal drinking water or water containing 500 mM sucrose *ad libitum*. Experiments were performed 12-14 weeks later. Rats receiving SED's responded differently to a standard glucose tolerance test. Fasting blood glucose levels in SED and control rats were 59.4±1.3 and 58.8±1.4 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 SED (256.2 ±32.7 mg/dl) compared to controls (136.6±14.7 mg/dl). At 120 min blood glucose in SED (67.6±7.2) and control (63.0±2.4 mg/dl) rats were not significantly ($P>0.05$) different when compared to initial fasting blood glucose values. Blood plasma insulin concentrations in SED and control rats were 3.01±0.55 (5) and 0.29±0.05 (5) ng/ml, respectively. Amplitude and time to peak (TPK) of ventricular myocyte shortening and Ca^{2+} transient were not altered by SED. Time to half (THALF) relaxation of shortening was significantly prolonged in myocytes from SED (60.6±2.1 ms) compared to control (48.7±2.9 ms) rats. The TPK and THALF relaxation of the Ca^{2+} transient were not altered by SED. However, the amplitude of Ca^{2+} transient was significantly increased in myocytes from SED (0.36±0.02 RU) compared to control (0.31±0.02 RU) rats. Altered myofilament sensitivity to Ca^{2+} may underlie the contractile defects seen in ventricular myocytes from rats fed diets that are supplemented with drinking water containing 500 mM sucrose for 12-14 weeks.

Key words: *Sucrose, ventricular myocytes, contraction, intracellular calcium*

Introduction

The hallmarks of type 2 non-insulin dependent diabetes mellitus (NIDDM) 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 weight gain are all risk factors associated with the etiology of type 2 diabetes. Previous studies have demonstrated that chronic sucrose-feeding in rats leads to a condition which shares some characteristics with type 2 diabetes mellitus including resting hyperglycaemia, high circulating insulin and triglyceride levels.[1,2,3] In this study, we have investigated the effects of sucrose-enriched diets (SED's) on ventricular myocyte contraction and intracellular Ca^{2+} in rats.

Methods

Rats: Adult 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 received normal U/V sterilized tap water (controls) whilst another group received tap water containing 500 mM sucrose (sucrose-enriched diet; SED). Experiments were performed 12-14 weeks after commencement of the SED. Principles of laboratory animal care were followed throughout.

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Measurement of blood glucose and plasma insulin: Glucose tolerance tests were performed during week 12.[4] After an overnight fast rats received an intraperitoneal injection of glucose (2g/kg body weight) and blood glucose was measured (One Touch II glucose meter, Lifescan Inc, USA) at different time points during a period of 2 hrs. Blood glucose was also measured in non-fasting animals immediately prior to experiments. Samples of blood plasma prepared at the time of animal sacrifice were frozen at –70 °C for subsequent analysis using a standard radioimmunoassay kit (Rat insulin RIA kit, Linco Research Inc, USA).

Ventricular myocyte isolation: Single ventricular myocytes were isolated according to previously described techniques with minor modifications.[5,6] 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 reached steady-state conditions recording of contraction was commenced. Unloaded shortening was used as an index of contractility.[7] 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).[5,6]

Measurement of intracellular Ca^{2+}: Myocytes were loaded with the fluorescent indicator fura-2 AM (Molecular Probes, Eugene, OR, USA) as described previously.[8] 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 SED rats: The effects of SED on food and water consumption and weight gain are shown in Table 1. Food consumption was significantly reduced, water consumption was significantly (P<0.01) increased and weight gain was significantly increased in rats receiving SED's. The results of glucose tolerance tests performed at 12 weeks after commencement of FED is shown in Figure

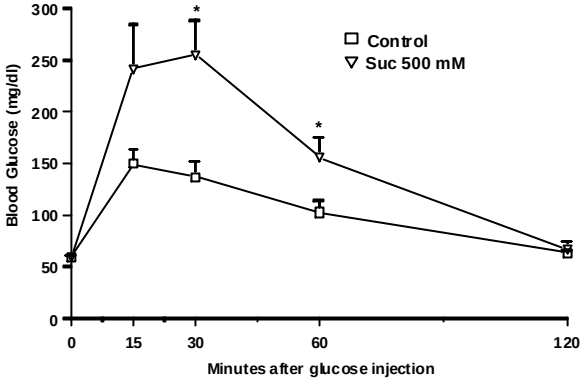

Figure 1: Blood glucose concentrations in response to an intraperitoneal glucose challenge (2g glucose/kg body weight) after an overnight fast at 12 weeks after initiation of SED. Values are mean ± SEM (n=4 animals). * P<0.05 Independent sample t-test.

1. At time zero, after an overnight fast, blood glucose in SED and control rats were 59.4±1.3 (5) and 58.8±1.4 (5) mg/dl, respectively. At 30 min after ip administration of glucose (2 g/ kg bodyweight) blood glucose was significantly higher in SED (256.2±32.7 mg/dl) compared to control (136.6±14.7 mg/dl) rats. At 120 min blood glucose in SED and control rats were 67.6±7.2 and 63.0±2.4 mg/dl, respectively, not significantly higher than blood glucose values at time zero. The general characteristics of the animals immediately prior to experiments are shown in Table 2. Body weight was significantly (P<0.05) increased in SED fed rats compared to controls. Plasma insulin was also significantly (P<0.01) incresaed in SED fed rats compared to controls. Heart weight, non-fasting blood glucose and plasma osmolarity were also marginally, though not significantly (P>0.05) increased in SED rats.

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

	Control	500 mM sucrose
Food consumption during week 10 (g/day/group[1])	112.9±2.5	60.7±2.3 **
Water consumption during week 10 (ml/day/group[1])	223.6±11.4	335.0±11.0 **
Weight at start of experiment (g)	174.80±3.15	171.60±3.49
Weight gain at 12 weeks (g)	316.60±29.16	368.20±16.02 **

Data mean ± SEM (n=5 animals). ** P<0.01 Independent sample t-test. [1]Mean group data.

Table 2: General characteristics of animals immediately prior to experiments

	Control	500 mM sucrose
Body weight (g)	337.80±19.92	397.40±12.73 *
Heart weight (g)	1.22±0.05	1.28±0.02
Non-fasting blood glucose (mg/dl)	69.40±2.62	75.00±3.78
Non-fasting plasma insulin (ng/ml)	0.29±0.05	3.01±0.55 **
Non-fasting plasma osmolarity (mosmol/kg)	300.00±1.05	303.60±1.29

Data mean ± SEM (n=5 animals). ** P<0.01, * P<0.05 Independent sample t-test.

General characteristics of ventricular myocytes from SED rats:

There were no clear visual differences between rod-shaped myocytes from SED and control rats. The viability of myocytes (expressed as percentage rod-shaped vs. round-shaped cells) was not significantly (P>0.05) altered by sucrose feeding. Viability of myocytes from SED rats was 67.4±1.7% (n=5) compared to 68.2±3.0 %(n=5) in controls. Resting cell length (RCL) of myocytes from SED rats (126.7±3.7 μm, n=22) were not significantly different from controls (123.9±4.0 μm, n=26).

Figure 2A is a fast time-base record of shortening from a representative ventricular myocyte isolated from a control rat. Figure 2B-D show mean data describing the amplitude and time course of contractile shortening in cells from SED and control rats. The TPK and amplitude of myocyte shortening was not significantly (P>0.05) altered by SED. However, the time-course of relaxation of shortening was significantly prolonged (P<0.01) in myocytes from SED (60.6±2.1 ms, n=22) compared to control (48.7±2.9 ms, n=26) rats.

Figure 3A is a fast time-base record of a Ca^{2+} transient from a representative ventricular myocyte isolated from a control rat. Resting fura-2 ratio (index of intracellular Ca^{2+} concentration) was not significantly altered in cells from SED (2.10±0.04 RU, n=23) compared to control (2.12±0.03

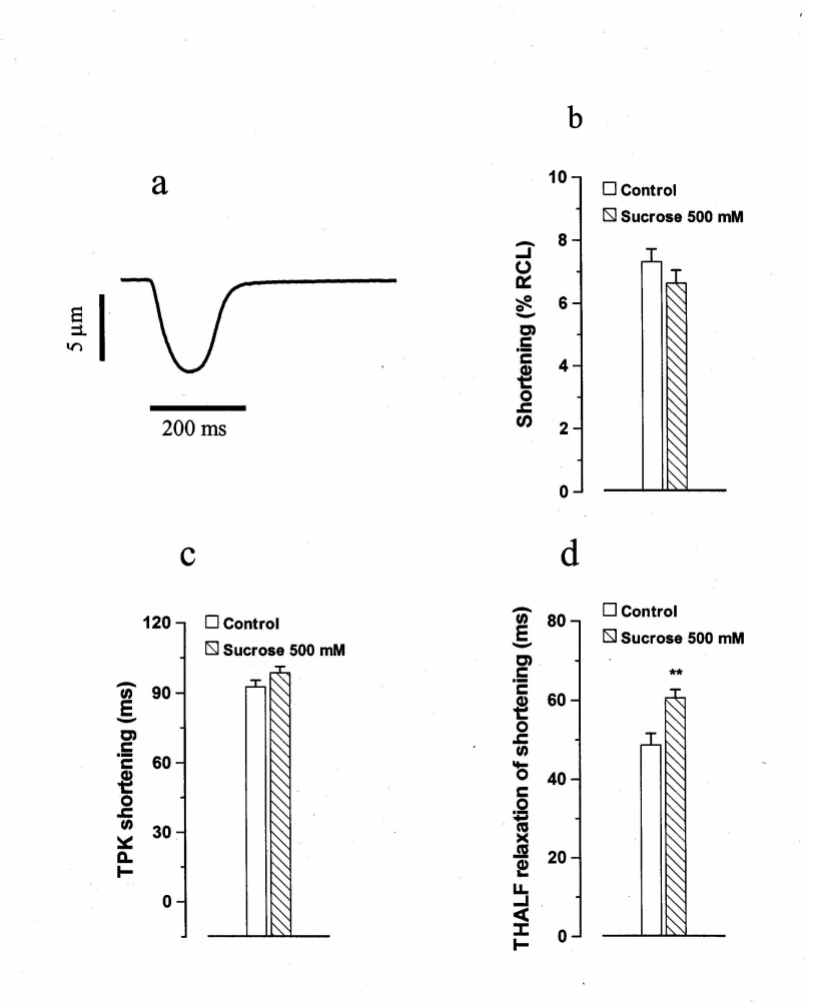

Figure 2: Representative fast time-base recording of unloaded shortening in a control ventricular myocyte (a) Mean data showing amplitude of shortening (b) time to peak shortening (c) and time to half relaxation of shortening (d). Values are mean ± SEM of 22-26 observations taken from 5 different hearts. ** P<0.01 Independent sample t-test.

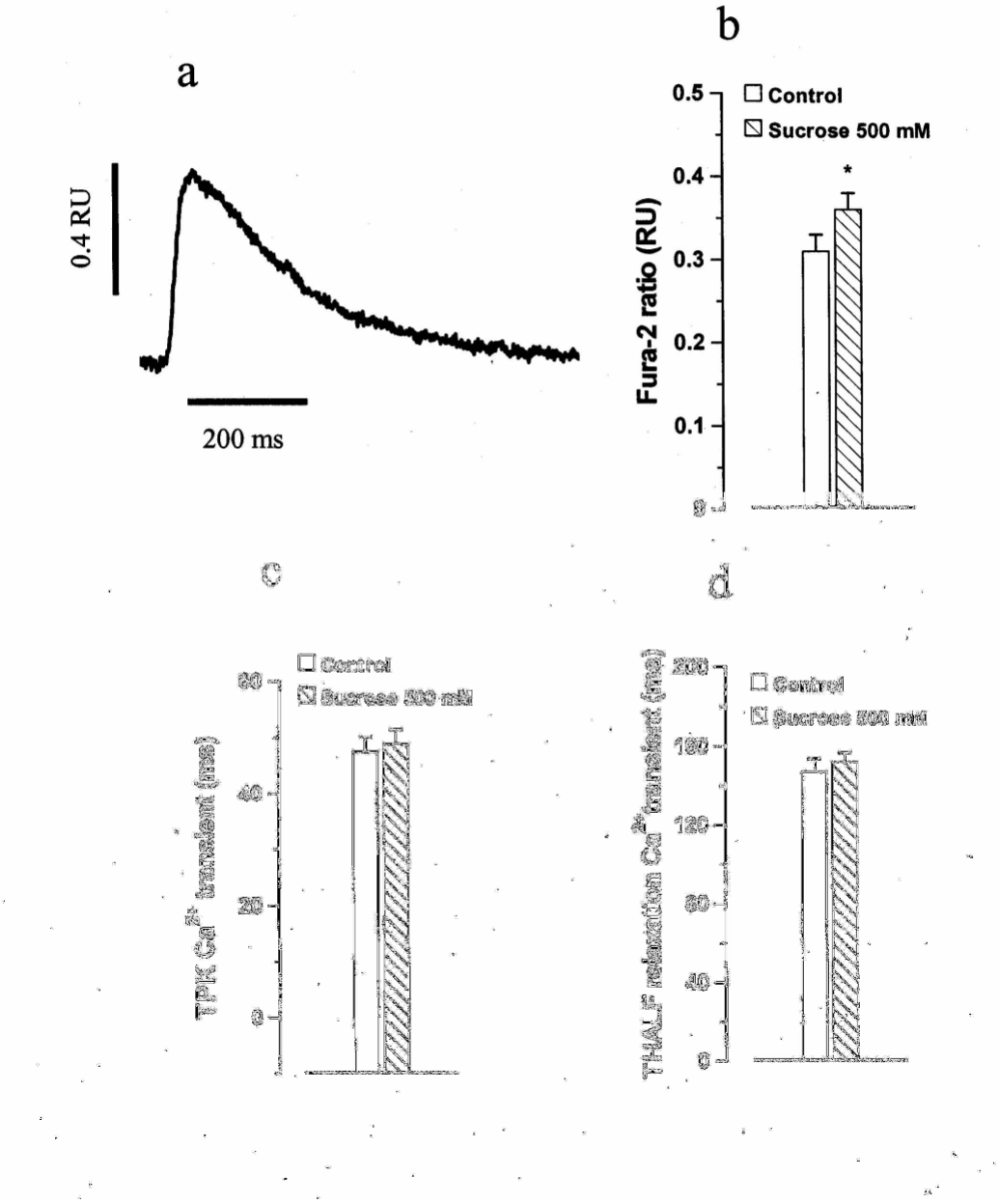

Figure 3: representative fast time-base recording of Ca^{2+} transients in a control ventricular myocyte (a) Mean data showing amplitude of Ca^{2+} transients (b) time to peak Ca^{2+} transients (c) and time to half relaxation of Ca^{2+} transients (d). Values are mean ± SEM of 23-24 observations taken from 5 different hearts. * $P<0.05$ Independent sample t-test.

RU, n=24) rats. Figure 3B-D show mean data describing the amplitude and time course of Ca^{2+} transients in cells from SED and control rats. Whilst the TPK or THALF relaxation of the Ca^{2+} transient were not significantly altered the amplitude of the Ca^{2+} transient was significantly ($P<0.05$) increased in myocytes from SED compared to control rats. The amplitude of the fura-2 ratio, in myocytes from SED and control rats were 0.36±0.02 RU (n=23) and 0.31±0.02 RU (n=24), respectively.

Discussion

Several previous experimental studies have demonstrated that chronic sucrose-feeding in rats leads to a diabetic condition which resembles type 2 diabetes mellitus with characteristics that include resting hyperglycaemia, high circulating insulin and triglyceride levels.[1,2,3,9] In some cases the feeding involved addition of sucrose to drinking water[9] whilst in others sucrose was added to the food.[2,3] Here, sucrose-enrichment was achieved by addition of sucrose (500 mM) to the drinking water. Food consumption was reduced, water consumption was increased in rats receiving SED's. Non-fasting blood glucose was marginally increased and plasma insulin was significantly increased in SED rats. SED rats responded differently to a glucose tolerance test.

At time zero, before administration of glucose, fasting blood glucose was not significantly different in SED compared to control rats. At 30 min after administration of glucose (2g/kg body weight, ip), concentration of blood glucose was significantly higher in SED rats compared to controls. At 120 min, the concentration of blood glucose had fallen to similar levels in SED and control and levels were not significantly different from blood glucose levels at time zero. Impaired early kinetics of insulin release might explain the high concentration of glucose in blood of SED rats at 30 min after ip glucose injection. Features including glucose intolerance and insulin resistance have been previously demonstrated after periods of between 7-10 weeks of sucrose feeding.[2,3]

Although the TPK and amplitude of shortening were not altered the THALF relaxation of myocyte shortening was significantly prolonged at 12-14 weeks after commencement of SED. The findings of this study are partly consistent with previously reported data which demonstrated prolonged time-course of myocyte shortening and relengthening after 7-10 weeks of sucrose feeding.[3] However, another study in myocytes performed after 8 weeks of sucrose feeding demonstrated normal duration of shortening and relengthening and decreased peak shortening compared to myocytes from starch-fed rats.[2] Defective kinetics of sarcoplasmic reticulum Ca^{2+} uptake or plasma membrane efflux of Ca^{2+} via Na^{+}/Ca^{2+} exchange and/or the Ca^{2+} ATPase might underlie prolonged THALF relaxation of shortening. Previously Dutta *et al* have reported prolonged rate of intracellular Ca^{2+} decay in myocytes from sucrose fed rats.[3] However, in the current study the THALF relaxation of the Ca^{2+} transient was only very marginally prolonged in myocytes from SED rats compared to controls suggesting that the relaxation phase of the Ca^{2+} transient was not significantly altered by SED.

In conclusion, defects in the mechanism(s) of Ca^{2+} transport may partly underlie the alterations in the time-course of contraction in ventricular myocytes from rats fed a sucrose-enriched diet.

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