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The direct effects of streptozotocin and alloxan on contractile function in rat heart

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ABSTRACT

Streptozotocin (STZ) and alloxan (ALX) are widely used to induce diabetes mellitus in experimental animals. The direct effects of STZ and ALX on the amplitude and time course of ventricular myocyte shortening and on cardiac action potentials were investigated. STZ and ALX (10^{-5} M) were dissolved in normal Tyrode (NT), maintained at pH 7.4 and 37 °C and stored for either 15 or 60–120 min. Both compounds reduced the amplitude of myocyte shortening. Compared to NT the amplitude of shortening was 34.7 ± 5.0% and 35.2 ± 6.8% with STZ and 41.0 ± 5.5% and 37.3 ± 5.7% with ALX stored for 15 and 60–120 min, respectively. During a 10 min NT washout STZ myocytes recovered to 56.2 ± 8.3% and 60.5 ± 8.2% and ALX myocytes recovered to 88.9 ± 10.0% and 83.7 ± 9.9% after storage of compounds for 15 and 60–120 min, respectively. Perfusion of the whole heart with ALX induced bradycardia but had no effects on the duration of action potential repolarization at 50% and 70% from peak action potential. The negative inotropic effects of STZ and ALX were not altered by storage. The results suggest that some of the effects on heart reported in STZ- and ALX-induced diabetes may be partly attributed to direct action of these compounds.

1. Introduction

Streptozotocin (STZ, 2-deoxy-2-3(3-(methyl-3-nitrosoureid)-D-glucopyranose) and alloxan (ALX, 2,4,5,6-tetraoxypyrimidine; 5-6-dioxyuracil) have been widely used to induce diabetes mellitus (DM) in experimental animals in order to study the disease itself and complications of the disease which includes diabetic cardiomyopathy [1–9]. The cytotoxic action of these diabetogenic agents in pancreatic β-cells has been well described. Within days of administration of STZ and ALX animals develop hypoinsulinemia and hyperglycaemia and a variety of other metabolic disturbances [10]. Functionally, STZ- and ALX-induced diabetic heart is in a compromised condition according to several indices of cardiac performance. In the STZ-induced diabetic heart, the heart rate and development (+dP/dt) and decline (−dP/dt) of ventricular pressure are reduced, time to peak pressure, time to half-relaxation from peak pressure, rate of ventricular myocyte contraction and relaxation are prolonged while amplitude of contraction is often reduced [1–3,8,9]. Similar defects have also been demonstrated in ALX-induced diabetic heart [4–7].

Assessment of the effects of STZ- and ALX-induced experimental DM on heart function has generally been conducted on the assumption that these agents have no direct action on heart muscle and its function. However, recent studies in Langendorff perfused rat heart have demonstrated that STZ reduces spontaneous heart rate [11]. Moreover, experiments in rat ventricular myocytes have provided convincing evidence that STZ induce a concentration-dependent depression of peak shortening, prolonged time to peak shortening and time to 90% relengthening and reduce maximal velocities of shortening and relengthening [12]. Recent *in vivo* biotelemetry studies have demonstrated a rapid decline in heart rate after administration of STZ suggesting that STZ may have direct actions on the heart [13,14]. While the direct effects of STZ have been reported [12] no study has yet investigated the direct effects of ALX on ventricular myocytes. The aim of the present study was to examine direct effects of ALX and compare them with STZ on the amplitude and kinetics of rat ventricular myocyte contraction. The direct effects of ALX on action potentials in the isolated heart were also investigated.

2. Methods

2.1. Preparation of STZ and ALX

STZ (Sigma, S-0130) and ALX (Sigma, A-7413) were prepared as a 10^{-3} M stock solution in citrate buffer (sodium citrate/citric acid) at pH 4.4. The stock solution was stored in a refrigerator at 4 °C and

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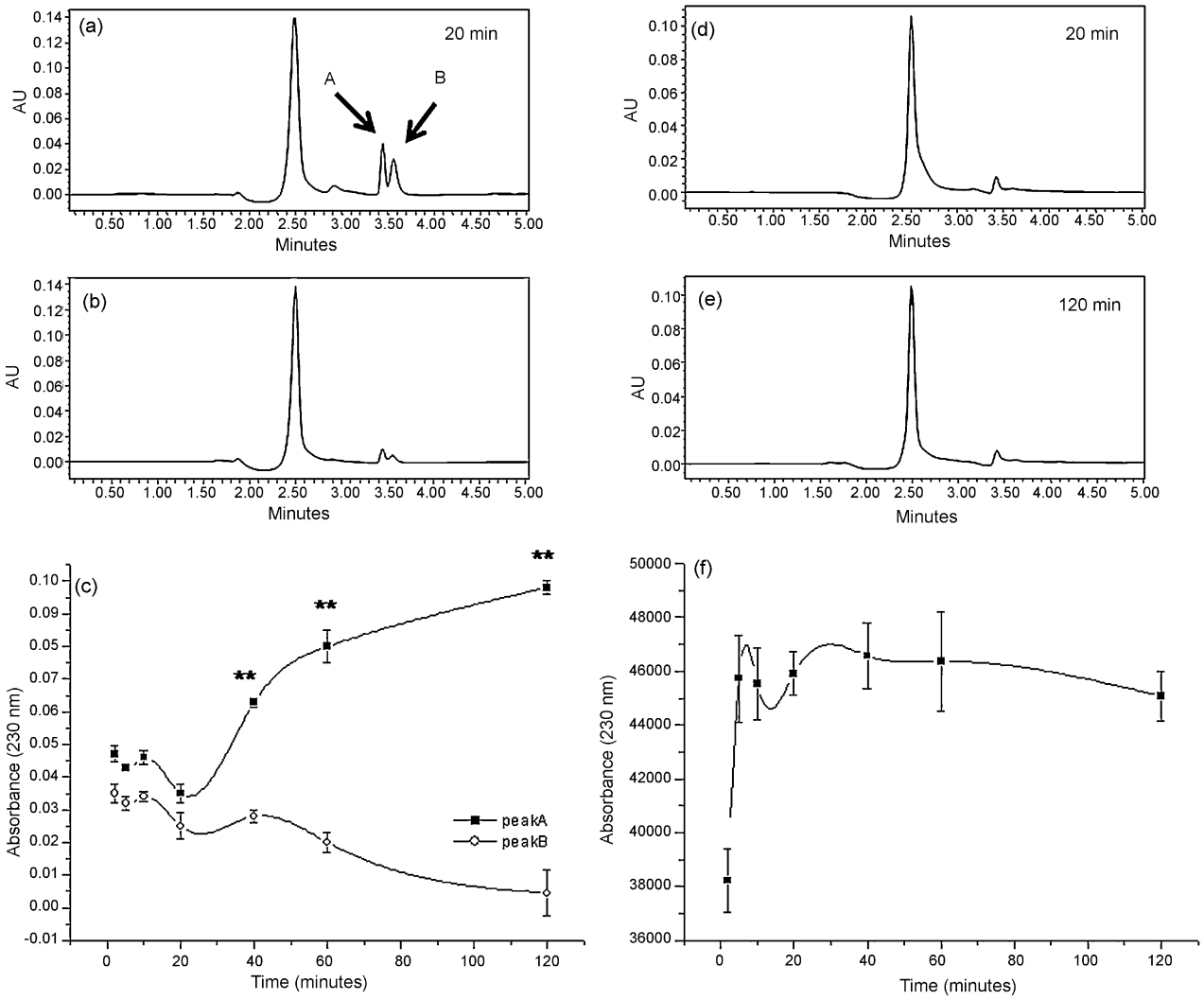

Fig. 1. Chromatograms and the time course of compound stability for STZ (left side panels) and ALX (right side panels). Chromatograms at different sampling times after storing STZ/NT for 20 min (a) and 120 min (b) and the time course of STZ/NT stability recorded at 230 nm (c). Chromatograms at different sampling times after storing ALX/NT for 20 min (d) and 120 min (e) and the time course of ALX/NT stability recorded at 230 nm (f). Data are mean ± S.E.M., $n = 4$. AU = absorbance units.

prepared fresh on a daily basis. Experimental test solutions were prepared by addition of STZ or ALX stock to normal Tyrode (NT; see below).

2.2. High performance liquid chromatography analysis of STZ and ALX

STZ and ALX 10^{-5} M test solutions were prepared by addition of 10^{-3} M stock solutions to NT containing 1.8 mM Ca^{2+} (see below). Test solutions were maintained at 37 °C in a water bath until use. Samples were analyzed by reverse phase high performance liquid chromatography (HPLC) with UV detection (RP HPLC-UV) at λ 230 nm. The system comprised a Waters 486 absorbance detector, a W717 autosampler and a W515 HPLC pump (Waters-Milford, MA, USA). HPLC analysis was performed in STZ and ALX test solutions stored for different times in the range 0–120 min. Samples (10 μL) were injected and separation was carried out by means of a Beckman ODS column, particle size 5 μM, 4.6 mm × 250 mm (Beckman Instr., San Ramon, CA, USA). The mobile phase consisted of 90% water (Milli-Q, 18 MΩ cm^{-1} Millipore, Milford, MA, USA) and 10% acetonitrile (Sigma–Aldrich, St Louis, MO, USA). The flow rate was 1 ml/min. Chromatograms were stored and processed by means of Millennium 32 software (Waters-Milford, MA, USA). For STZ, due to incomplete separation of A and B peaks, calculation was based on peak height values.

2.3. Ventricular myocyte isolation

Forty male Wistar rats were used in the study. All animals were maintained on the same diet and water *ad libitum*. The experiments were approved by the local Animal Ethics Committee, FMHS UAE University. Ventricular myocytes were isolated according to previously described techniques with minor modifications [1]. Initially hearts were perfused with cell isolation solution containing 1.8 mM Ca^{2+}. After stabilization hearts were then perfused for 4 min with a cell isolation solution containing 0.1 mM EGTA (see below), and then for 6 min with cell isolation solution containing 0.05 mM Ca^{2+}, 0.75 mg/ml collagenase (Type 2; Worthington, NJ, 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 0.75 mM Ca^{2+}-containing isolation solution.