Cardiac dysfunction in the Goto-Kakizaki rat
A model of type II diabetes mellitus

Abstract Experimental study of cardiac function in the diabetic heart has focussed mostly on models of Type I diabetes. We studied cardiac function in the Goto-Kakizaki (GK) rat, an inbred model of spontaneous non-obese, Type II diabetes. Methods Both isolated perfused hearts and isolated ventricular myocytes from GK and matched control Wistar rat hearts were studied. Percent myocyte twitch shortening (%TS) and corresponding intracellular calcium transients (indo-1 fluorescence ratio, R) were measured over a range of stimulation frequencies (0.5 – 2.5 Hz; 32 °C, n = 16–24 cells). In isolated Langendorff-perfused hearts, we measured systolic LV pressure (LVPmax), left ventricular end diastolic pressure (LVEDP), maximal rate of LV pressure rise (LV dP/dt max) and fall (LV dP/dt min) and isovolumic LV relaxation (exponential time constant, T) both at baseline and during brief (10 minutes) hypoxia. Results The %TS and corresponding indo-1 R were similar between GK and control myocytes at all stimulation frequencies (e.g. at 2.5 Hz: % TS = 8.6 ± 0.77 and 8.2 ± 0.19; R = 0.19 ± 0.009 and 0.18 ± 0.018, GK and control respectively, P = NS). Similarly, there were no significant differences in baseline LVPmax (129 ± 6.2 and 135 ± 9.6 mmHg; GK and control respectively, P = NS), LV dP/dt max (3169.5 ± 165.80 and 3390.6 ± 232.60 mmHg/s; GK and control respectively, P = NS), LV dP/dt min or T (24 ± 0.7 and 25 ± 0.6 ms, GK and control respectively, P = NS). During 10 min hypoxia, LV dP/dt max decreased significantly more, and LVEDP and T increased significantly more, in GK compared to control hearts (LV dP/dt max: 668.90 ± 32.8 versus 1027.10 ± 84.0 mmHg/s; LVEDP: 21.4 ± 4.3 versus 11.6 ± 0.6 mmHg; T: 102 ± 13.8 versus 56 ± 3.0 ms; GK versus control respectively; all P < 0.05). These abnormalities in GK hearts were reversed with acute addition of insulin (0.01 i.u./ml) to the perfusion buffer. Conclusion The GK model of Type II diabetes displays a mild cardiomyopathy evident as exaggerated diastolic dysfunction during hypoxia. The mechanism is likely to involve substrate deficiency.

Key words Goto-Kakizaki rat – diabetes mellitus – cardiomyopathy – ventricular function – hypoxia
Introduction

Diabetes mellitus is associated with an increased incidence of hyperlipidaemia, hypertension, and ischaemic heart disease, all of which predispose to congestive cardiac failure. However, even in the absence of these risk factors, diabetes mellitus can cause a specific cardiomyopathy characterised by both systolic and diastolic contractile dysfunction [9]. The underlying mechanisms of diabetic heart disease, and the response of the diabetic heart to insults such as ischaemia or hypoxia, are important, clinically relevant issues which are incompletely understood. For instance, it is not clear why myocardial infarction in diabetic patients is associated with a higher mortality despite a similar infarct size to that in their non-diabetic counterparts [38].

A widespread investigative approach to the understanding of the pathophysiology of diabetic heart disease has been to use experimental models of diabetes mellitus, notably with streptozotocin (STZ) or alloxan administration to induce pancreatic islet cell damage and insulin deficiency. Indeed, studies in models such as the STZ-treated rat or the spontaneously diabetic insulin-deficient BB Wistar rat have yielded useful information regarding myocardial dysfunction [9, 40]. In contrast, the effects of Type II diabetes or of insulin resistance on myocardial function have been much less investigated, in large part due to the lack of appropriate rodent or large animal models.

A relatively recently developed model of Type II diabetes is the Goto-Kakizaki (GK) rat. This model was generated by selective inbreeding of non-diabetic Wistar rats with impaired glucose tolerance, over numerous generations [11]. GK rats have a stable, inheritable form of Type II diabetes characterised by mild hyperglycaemia and hyperinsulinaemia, and no obesity, hypertension, or marked hyperlipidaemia. We have established that GK rats from the colony held at our institution display mild hyperglycaemia (1.5-2 mmol/l) versus normal adults) [15, 27]. Despite this mild hyperglycaemia, functional and morphological manifestations of neuropathy and glomerulopathy have been demonstrated in this model [35]. In addition, abnormalities in the hypothalamus [17], retina [1], adipocytes [3], bone [25], foetal development [22], and peripheral nerves [39] have also been described in this model. The GK rat thus offers a convenient model for the study of Type II diabetes, without the confounding effects of obesity or hypertension per se. Cardiac function has not previously been investigated in the GK rat.

The aims of the present study were therefore to: 1) characterise intrinsic myocardial function in the GK rat, using isolated perfused hearts and isolated cardiac myocytes; and 2) study the response of the GK rat heart to brief hypoxia.

Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US Institutes of Health (NIH Publication No. 85–23, revised 1996).

Animals

The GK rat colony at the University of Wales College of Medicine (Cardiff, UK) was established from breeding pairs kindly provided by Professor Y Goto (Tohoku University School of Medicine, Sendai, Japan). Adult male GK rats between 5 and 8 months of age, and matched control male Wistar rats of the same age (Charles River, UK), were studied.

At the time of sacrifice, blood and serum were collected for estimation of glycosylated haemoglobin A1 (HbA1c) and random glucose, respectively.

All animals in this study were maintained under identical conditions, and were allowed free access to tap water and standard laboratory diet.

Heart perfusion

Animals were terminally anaesthetised with intraperitoneal sodium pentobarbitone (90 mg/kg). Hearts were excised into ice-cold HEPES buffer solution and rapidly mounted onto a non-recirculating Langendorff apparatus. The perfusion solution comprised (in mM): NaCl 117; KCl 5.7; NaHCO3 4.4; NaH2PO4 1.2; CaCl2 1.25; MgCl2 1.7; hydroxyethylpiperazine ethanesulfonic acid (HEPES) 20; glucose 10; pH 7.4, 37°C. This was gassed with 100% O2 during normoxic perfusion (pO2 ~700 mmHg) and N2 during hypoxic periods (pO2 ~40 mmHg). Coronary flow rate was adjusted to produce a mean coronary perfusion pressure (CPP) of 80 mmHg, and was then maintained constant. Hearts were paced at 10% above intrinsic rate by a right atrial electrode at 10% above threshold voltage. The pulmonary artery was routinely cut to ensure free coronary drainage.

Left ventricular pressure (LVP) was monitored using an intraventricular latex balloon connected to a Statham (P23XL) pressure transducer. Initial LV end-diastolic pressure (LVEDP) was set at 10 mmHg. Pressure data were sampled via a MacLab data module (AD Instruments, UK), and analysed on a personal computer. The maximal rates of left ventricular pressure rise and fall (LV dP/dtmax and LV dP/dtmin, respectively) were obtained from the first derivative of the LVP signal. The exponential time-constant of isovolumic LV relaxation, T1, was calculated as described previously [43]. The natural frequency of the pressure-recording system was