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Stress–strain analysis of jejunal contractility in response to flow and ramp distension in type 2 diabetic GK rats: Effect of carbachol stimulation

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ABSTRACT

Investigation of intestinal motility in a genetic model of GK rats abandons the possible neurotoxic effect of streptozotocin in streptozotocin-induced diabetic model. Seven GK male rats (GK group) and nine normal Wistar rats (Normal group) were used in the study. The motility experiments were carried out in an organ bath containing physiological Krebs solution. Before and after 10^{-5} M carbachol application, the pressure and diameter changes of jejunum were obtained in relation to (1) basic contraction, (2) flow-induced contraction with different outlet resistance pressures and (3) contractions induced by ramp distension. The frequency and amplitude of contractions were analyzed from pressure–diameter curves. Distension-induced contraction thresholds and maximum contraction amplitude of basic and flow-induced contractions were calculated in terms of stress and strain. (1) The contraction amplitude increased to the peak value in less than 10 s after adding carbachol. More than two peaks were observed in the GK group. (2) Carbachol decreased the pressure and stress threshold and Young's modulus in the GK group ($P<0.01$). (3) Carbachol increased the maximum pressure and stress of flow-induced contractions at most outlet pressure levels in both two groups ($P<0.001$). Furthermore, the flow-induced contractions were significantly bigger at low outlet pressure levels in GK group ($P<0.05$ and $P<0.01$). (4) The contraction frequency, the strain threshold and the maximum contraction strain did not differ between the two groups ($P>0.05$) and between before and after carbachol application ($P>0.05$). In GK diabetic rats, the jejunal contractility was hypersensitive to flow and distension stimulation after carbachol application.

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1. Introduction

Gastrointestinal (GI) disorders are common in patients suffering from diabetes mellitus (Horowitz and Samsom, 2004). Diabetes can affect the entire GI tract including the small intestine. However, the pathogenesis of GI disorders in diabetes mellitus is complex in nature, multi-factorial (motor dysfunction, autonomic neuropathy, glycemic control, psychological factors, etc.) and is not well understood (Horowitz and Samsom, 2004). Our previous studies have shown that the morphological and biomechanical properties of the GI tract are changed in type-1 diabetic patients (Frokjaer et al., 2007) and animals (Yang et al., 2004; Zhao et al., 2003; Zhao et al., 2006). Remodeling was also demonstrated in the esophagus (Zhao et al., 2007), stomach (Liao et al., 2006) and intestine (Zhao et al., 2013) from type-2 diabetic animals. The morphometric and biomechanical remodeling in diabetes may affect intestinal motility (Zhao et al., 2006).

Intestinal motility disorders have been found in diabetic patients (Horowitz and Samsom, 2004) and animals (Yamamoto et al., 2008). Either delayed or rapid transit in the small intestine was observed in diabetic animal models (Chang et al., 1995; Kumar and Prashanth, 2004; el Salhy, 2001, 2002). Small intestinal transit disorders in diabetes patients have also been documented (de Boer et al., 1993; Iida et al., 2000; Kawagishi et al., 1992; Folwaczny et al., 1995; Keshavarzian et al., 1986; Nguyen et al., 1997). It is well known that the contraction threshold during stimulation is a proxy of intestinal sensitivity whereas the contraction amplitude reflects the contractile strength. However, to the best of our knowledge, contraction thresholds and contraction amplitude,

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especially in terms of mechanical stress and strain parameters, have not been reported for the diabetic intestine.

Type 2 diabetes is the most common form which accounts in adults for about 90–95% of all diagnosed cases of diabetes (Stone et al., 2010). In the present study, we used the Goto–Kakizaki (GK) rat which is a model of type 2 diabetes (Goto and Kakizaki, 1981). It exhibits similar metabolic and hormonal disorders as human diabetes (Goto et al., 1988; Miyamoto et al., 1996). Intestinal motility disorders have been reported in streptozotocin-induced type-1 diabetes in rats. However, it cannot be excluded that the disorders are due to neurotoxicity of streptozotocin. The GK rat is a genetic model of non-insulin-dependent diabetes. Investigating intestinal motility in GK rats avoid the neurotoxic effect.

Carbachol is a parasympathomimetic drug that directly stimulates cholinergic receptors (van Zwieten and Doods, 1995). It may also act indirectly by promoting release of acetylcholine and by a weak anticholinesterase action (Marshall, 1971). Treatment of rats with streptozotocin induces a diabetic state in which the bladder muscle in streptozotocin-induced diabetes is hyperactive and hypersensitive to muscarinic agonists such as carbachol (Stevens et al., 2006). Gastric antral smooth muscle cells from streptozotocin-induced rats and db/db spontaneously diabetic mice have impaired contractile response to carbachol (Soulié et al., 1992). Therefore, carbachol stimulation was used in the present study to evaluate the effect on flow and distension stimulated contractility.

The hypotheses of the present study were the contraction threshold, the frequency and the amplitude of basic, flow-induced and distension-induced contractions change due to the remodeling induced by diabetes. These motility changes are associated with altered stress–strain properties. The aims of this study were to test the hypotheses and to compare the frequency and amplitude of basic, flow-induced and distension-induced contractions in terms of mechanical stress and strain in the normal and GK diabetic rats with and without carbachol application.

2. Materials and methods

2.1. Animals and groups

Seven inherited type 2 diabetic rats (Goto–Kakizaki rats, GK group), 12 weeks old and weighing 330 g, were purchased from Taconic Europe (DK-8680 Ry, Denmark). Nine age-matched normal rats (same strain as the GK rats) served as controls (Normal group). During the breeding, the body weight was measured every week from 12 weeks. The rats drank tap water and ate food without restrictions. The fasting glucose level of the rats was measured once every second week starting from 16 weeks and also at the last day of the experiment. The rats survived for 32 weeks and fasted overnight before the experiments. Approval of the protocol was obtained from the Danish Committee for Animal Experimentation.

2.2. In vitro intestinal preparation

When the scheduled time had arrived, the rats were anesthetized with Hypnorm 0.5 mg and Dormicum 0.25 mg per 100 g body weight. The abdominal cavity was opened with careful dissection of the intestine. A 10-cm-long jejunal segment was harvested from 5 cm distal to the ligament of Treitz. The residual contents in the lumen were gently cleared using physiological saline. A 2-cm-long tissue was cut from the proximal end of the segments and fixed in 10% formalin for histological examination. From another short segment, two tissue rings were cut and used for no-load state and zero-stress state analysis. The segment was immersed into the organ bath containing Krebs solution of the following composition (mmol/l): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; NaH_2PO_4, 1.0; MgCl, 1.2; $CaCl_2$–H_2O, 2.5; Glucose, 11; and ascorbic acid, 0.11 as soon as possible. The Krebs solution was 37 °C and aerated with a gas mixture (95% O_2 and 5% CO_2, pH 7.4). Thirty minutes equilibrating time was needed for recovery of the motility before the experiments started.

2.3. Flow and ramp distension experiment

The proximal and distal ends of each jejunal segment were tied with silk threads on to two cannulas fixed to opposite walls of the organ bath. The length of the segment was adjusted between two cannulas without overstretching the segment. The proximal cannula was connected via a tube to a fluid container, containing the same Krebs solution as mention above for applying luminal flow (rate 1 ml/min) and for ramp distension using a pump (Genie Programmable Syringe Pump, World Precision Instrument, Stevenage, UK). The distal end of the intestinal segment was connected to a reservoir (Fig. 1A). The outlet pressure was modulated by the fluid level in the reservoir. The outlet resistance was varied at 0, 2.5, 5.0, 7.5, and 10 cm water column. Ramp distension (0–10 cm H_2O) was done with closed outlet (Fig. 1B). The intestinal diameters were videotaped by a CCD camera (Sony, Japan) through a stereomicroscope. The frequency of pressure and diameter data collection was 10 frames per second.

Three minutes after finishing the experiment above, 10^{-5} M carbachol was applied to the fluid in the organ bath. The same experimental protocol was repeated. The Krebs solution was replaced by calcium-free Krebs solution with 0.4% EGTA and 2 mg papaverine in order to abolish smooth muscle contractility. The same protocol was repeated again. Papaverine inhibits enzyme phosphodiesterase causing elevation of cyclic AMP levels, altering mitochondrial respiration, and inhibition of calcium influx.

2.4. General analysis of contractions from pressure–diameter curves

Pressure changes during flow and ramp distension tests were recorded and digitized. Corresponding diameter changes were measured by analyzing the video clip. The resolution of the images is 0.05×0.07 mm/pixel. In order to analyze the videos automatically, several imaging techniques were employed such as simple color thresholding for tissue area segmentation, morphological operation for denoising and third order polynomial curve fitting of the tissue shape for determining the circumferential direction. The analysis software was developed in the C++ language with Microsoft Direct Show SDK library for movie handling. The contraction frequency and maximum amplitude were analyzed in relation to basic (without flow and without distension, outlet open), flow-induced contraction (outlet open) and distension induced contractions (outlet close) with and without carbachol stimulation.

It is well known that the mechano-sensory receptors located in the intestinal wall sense mechanical wall stress and strain rather than pressure and volume changes. Therefore, the distension-induced contraction thresholds and maximum contractions of basic and flow-induced contractions were calculated in term of stress and strain with reference to the zero-stress state.

2.5. Zero-stress state of the intestinal segment

Methods for the determination of zero-stress state have been previously described (Gregersen, 2000, 2002). The 1–2 mm wide rings were transferred to a calcium-free Krebs solution with EGTA. This represented the no-load state which was photographed. Each ring-shaped segment was cut radially under the microscope and opened up into a sector. Photographs were taken ~60 min after the radial cutting to allow viscoelastic creep to take place. This represented the zero-stress state that was also photographed.

2.6. Stress and strain calculation

Morphometric data like circumferential length (C), the wall thickness (h), and the wall area (A) at no-load and zero-stress state were measured from digitized images of the segments in the zero-stress and no-load states by using image analysis software (Sigmascan Pro 4.0). Furthermore, the outer diameter (D) was measured from the images of the pressurized segments by using a custom-made software subroutine. The intestinal segment in the pressurized state is assumed circular cylindrical and the wall can be regarded as a membrane. Hence, the mean circumferential stress $\sigma_\theta = \Delta \mathrm{Pr}_{i-p}/h_p$ can be assumed uniformly distributed and the circumferential stress was then derived as

$$S_\theta = \frac{\sigma_\theta}{\lambda_\theta^2} = \frac{\partial(\rho_0 W)}{\partial E_\theta} = \frac{\Delta \mathrm{Pr}_{i-p}}{h_p \lambda_\theta^2} \quad (1)$$

and the circumferential strain was computed from the circumferential stretch ratio λ_θ as

$$E_\theta = 1/2(\lambda_\theta^2 - 1) \quad (2)$$

r_{i-p}, h_p, λ_θ, ΔP, ρ_0 and W are the luminal radius, the wall thickness, the circumferential stretch ratio, the recorded pressure, wall density and strain-energy function. Calculations of r_{i-p}, h_p, h_p and λ_θ have been described in detail by Gregersen (2002) and Zhao et al. (2003). S_θ is circumferential 2nd Piola-Kirchhoff's stress and E_θ is circumferential Green strain. These mechanical parameters were selected because intestinal tissue exhibits large deformation.

The stress and strain immediately before the distension-induced contraction (stress and strain threshold) and at the maximum basic and flow-induced contractions point were used as endpoints.

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