Pelviureteric peristaltic contractions in diabetic rats

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Abstract

The effects of streptozotocin (STZ)-induced diabetes on the spontaneous peristaltic contractions in the upper urinary tract (UUT) of the rat were examined by simultaneously recording the tension in the proximal and distal regions of the renal pelvis and the proximal ureter. All regions of the UUT of diabetic rats contracted at a frequency similar to the contraction frequency of age-matched control rats. In contrast, contraction amplitudes in the proximal and distal renal pelvis and ureter of diabetic rats were 36%, 135% and 121% larger than the equivalent contractions recorded in control rats resulting in a significant increases in the motility index (MI amplitude x frequency) in all 3 regions. Capsaicin (10 µM), substance P (SP 2 µM) and neurokinin A (NKA 2 µM) caused a transient increase in MI in both control and STZ-induced diabetic rats. The rise in basal tension in the proximal and distal renal pelvis evoked by capsaicin, SP or NKA was also significantly greater in the diabetic rats when compared with controls. In contrast, human calcitonin-gene related peptide (hCGRP) produced a relatively small transient inhibition of UUT motility which was little affected by STZ treatment. These results suggest that capsaicin predominantly releases tachykinins from intrinsic sensory nerves in both non-diabetic and STZ-induced diabetic rats. We speculate that the supersensitivity of the diabetic UUT to capsaicin, NKA and SP 8–10 weeks after STZ treatment could be arising from an earlier development of sensory neuropathy.

Key words: pyeloureteric peristalsis, upper urinary tract, diabetes, smooth muscle, contraction

Introduction

Diabetes mellitus (DM) is a metabolic disease arising from defects in insulin secretion or action that result in hyperglycemia. Many diabetic patients display urological symptoms due, in large part, to a dysfunctional bladder arising from a decrease in sensation, compliance, detrusor smooth muscle contractility and responsiveness to both electrical and pharmacological stimulation which results in increased bladder capacity, hypertrophy, hyporeflexia and residual urine retention after voiding. Chronic urine retention and bladder fibrosis can lead to
ureterovesical junction obstruction, hydronephrosis and urinary tract infection which if untreated can lead to acute pyelonephritis and corticomedullary abscesses and other kidney pathologies. Both sensory neuropathy (Kamata et al., 1993; Pinna et al., 1994; Troger et al., 1999) and alterations in the physical properties and pharmacological responsiveness of the remodelled detrusor smooth muscle (Santicioli et al., 1987; Kolta et al., 1985) have been postulated to underlie these alterations in bladder function.

Little is known on the effects of DM on pelviureteric peristalsis. The length and velocity of movement of a bolus of urine in streptozotocin (STZ)-induced diabetic rats is significantly decreased when compared with control or sucrose-diuretic rats (Watanabe and Miyagawa, 2002). Although the spontaneous contractions in the upper urinary tract (UUT) of many mammalian species are thought to myogenic in origin, capsaicin desensitization or inhibition of prostaglandin synthesis profoundly affect pelviureteric peristalsis, changing either the amplitude and/or frequency of contractions in a species dependent manner (Davidson and Lang, 2000; Lang et al., 2002; Weiss et al., 2006). The aim of this study was to further investigate the effects of DM on pelviureteric peristalsis. In particular, we investigated the responsiveness of smooth muscle in the proximal and distal renal pelvis and proximal ureter to capsaicin and the sensory neuropeptides, human calcitonin-gene related peptide (hCGRP), substance P (SP) and neurokinin A (NKA), in control and STZ-treated rats.

**Methods**

*Induction of diabetes*

Male and female Wistar rats were weighed (200–500 g) and then anaesthetized with halothane, a blood glucose reading taken and then animals were injected with streptozotocin (60 mg/kg) in 0.2 M citrate saline (pH=4.5) or vehicle via the tail vein. All procedures were previously approved by the Department of Physiology Animal Ethics Committee.

*UUT preparation*

6–8 weeks after the STZ injection, animals were anaesthetized with chloroform and then killed by decapitation. Blood glucose readings were taken to confirm successful induction of DM and the animal re-weighed. Kidneys were then removed through a midline incision and immediately placed in physiological salt solution (PSS) (see below).

The UUT was dissected free from the surrounding parenchyma and a longitudinal cut was made extending from the proximal renal pelvis to the mid region of the ureter enabling the whole preparation to be laid out flat. Three circumferentially directed cuts, extending to the midline, were then made. The first cut divided the proximal portion of the renal pelvis from the distal renal pelvis, while two smaller cuts were made in the ureter (approximately 1cm from the pelviureteric junction) to create a short circumferential strip (Fig. 1). Threads were then tied around the three separated regions and the preparation was pinned into an organ bath (1.3 ml), perfused with PSS and maintained at a temperature of 30 or 35°C (Fig. 1).

Threads were attached to isometric force transducers, which were connected to a MacLab/4s, analogue-to-digital converter, driven by a Macintosh LC, allowing the simultaneous
recording of tension in the three different regions of the UUT. A tension of approximately 1 mN was placed on each of the regions and the tissue was then left to equilibrate for a period of 30–60 min (Fig. 1) (Teele and Lang, 1998).

After equilibration, the effects of capsaicin, SP, NKA and hCGRP were examined. In general the effects of each treatment on the spontaneous contractility of the UUT were quantified by the use of a motility index (MI), calculated by multiplying the averaged amplitude (mN) of 5 contractions by their frequency, expressed as the number of contractions min⁻¹. Amplitudes, frequencies and MIs were then expressed as a % of their control values. In all experiments, contractions to high K⁺ PSS (20 mM for 2 min) were recorded as a control (Table 2). Contractions in the ureter were often too small to allow a reliable calculation of MIs, particularly in control rats (Fig. 3A). In these cases only the results from the proximal and distal renal pelvis have been presented.

**Statistical analysis**

Results are expressed as means ± standard error of the mean. One-way repeated measures analysis of variance (ANOVA) was conducted when regions of the UUT were the repeated measures. Two-way repeated measures ANOVA were also employed using region and time as within subject factors. Data was tested for homogeneity of variance, and transformed if necessary. Where significant interactions occurred, a Fisher’s least significant difference test was then conducted to determine where the differences occurred; P<0.05 was considered statistically significant.

Time also had a confounding influence on the interpretation of our observations (see Davidson and Lang, 2000). Over a recording period of 240 min, the frequency of contractions in all three UUT regions progressively declined, reaching a significant decrease at time points
≥120 min ($P<0.05$, 1- and 2-way repeated measures ANOVA). In contrast, the force of contractions in the proximal and distal renal pelvis increased over time such that at contraction amplitude at time points ≥120 min were significantly greater than control ($P<0.05$, 2-way repeated measures ANOVA). At 180 min, contraction amplitudes in the proximal and distal renal pelvis were increased by 33% and 17%, respectively (both $P<0.05$). There was no time-dependent change in the contraction amplitudes in the ureter ($P>0.05$, 1-way repeated measures ANOVA). This resulted in no significant changes in the MI of the proximal and distal renal pelvis ($P>0.05$, 2-way repeated measures ANOVA; n=5). In the ureter, MI values were significantly reduced at time points ≥60 min ($P<0.05$, 1-way repeated measures ANOVA; n=5) (Davidson and Lang, 2000).

Drugs

The PSS was of the following composition (in mM): NaCl 120, KCl 5.0, CaCl$_2$ 2.5, MgCl$_2$ 1.0, NaH$_2$PO$_4$ 1.0, NaHCO$_3$ 25 and glucose 11. The pH of this solution was 7.3–7.4, after being bubbled with 95% O$_2$: 5% CO$_2$ gas mixture. The following drugs were used: capsaicin (Sigma, St. Louis, U.S.A), human calcitonin gene-related peptide (hCGRP), neurokinin A (NKA) and substance P (SP) (Auspep). Capsaicin was dissolved in ethanol. hCGRP and SP were both dissolved in filtered distilled water. Drugs were diluted with PSS to their final concentrations as indicated. Before use, solutions were vigorously bubbled with 95% O$_2$: 5% CO$_2$ to restore any changes of pH.

Results

Changes in body weight and serum glucose

Table 1 summarizes the effects of STZ-induced diabetes on the body weight and blood glucose levels over the 6–8 week period of the experiment compared to the control rats which gained weight over this time frame. It was clear that there was a significant difference in the mean body weights and the blood glucose levels in the STZ-induced diabetic rats compared with control.

Spontaneous motility in the UUT

At 35°C, all three regions of the rat upper urinary tract developed spontaneous contractile activity within 5 min of being set up. These contractions were often irregular in shape, with cyclical movements of the baseline frequently being apparent in both control and diabetic rats (Lang et al., 2001). In general, spontaneous contractions were initiated in the proximal renal pelvis, traveled distally to the distal renal pelvis and then into the ureter (Fig. 1C).

<table>
<thead>
<tr>
<th>Table 1. Effects of STZ-induced diabetes on body weights and blood glucose concentrations</th>
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<tbody>
<tr>
<td>Initial Body Weight (g)</td>
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<td>-------------------------</td>
</tr>
<tr>
<td>Control (n=9)</td>
</tr>
<tr>
<td>Diabetic (n=6)</td>
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<td>*$P&lt;0.05$</td>
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amplitude of contractions recorded in the UUT of the rat became more regular and rose from a more stable baseline when the temperature was lowered to 30°C (Fig. 2). The proximal and distal renal pelvis displayed contractions (both at a frequency of 22.3 ± 2.2 min⁻¹) of 0.45 ± 0.09 mN and 0.3 ± 0.05 mN, respectively, at 35°C, compared with 0.55 ± 0.13 and 0.4 ± 0.07 mN (both P<0.05; 2-way repeated measures ANOVA n=8) at a frequency of 13.4 ± 2.4 min⁻¹ (P<0.05; 2-way repeated measures ANOVA) at 30°C. This resulted in a significant decrease in the MIs (amplitude × frequency) in the proximal and mid renal pelvis from 9.8 ± 2.2 and 6.4 ± 1.1, respectively, at 35°C to 6.7 ± 1.8 and 4.9 ± 0.8, respectively, at 30°C (both P<0.05; 2-way repeated measures ANOVA). However, as the measurements of amplitude and frequency at 30°C were far more reliable, all experiments were carried out a 30°C unless otherwise stated.

Effects of diabetes

After 30–60 min equilibration, the amplitude of contractions recorded in all three regions of the UUT of the diabetic rats was significantly greater than that developed at the same time in the corresponding regions of the age-matched control rats. The force of contractions recorded in the proximal and distal renal pelvis, and ureter of the diabetic rats were 0.57 ± 0.08 mN, 0.80 ± 0.12 mN and 0.62 ± 0.1 mN respectively, compared to their respective values of 0.42 ± 0.08 mN (P>0.05), 0.34 ± 0.04 mN (P<0.05) and 0.28 ± 0.06 mN (P<0.05) recorded in control rats (Fig. 2, A, B, Ci) (1-way repeated measures ANOVA; n=10).

In contrast there was little difference between the frequency of contractions recorded in the control and diabetic groups (Fig. 2Cii). In control rats, both the proximal and distal renal pelvis contracted at a rate of 16.6 ± 1.0 min⁻¹ while the ureter was slightly slower, at a rate of 13.9 ± 1.8
min⁻¹. In the proximal and distal renal pelvis and ureter of the diabetic rat, the frequency of contractions were 17.4 ± 1.9 min⁻¹, 18.0 ± 1.7 min⁻¹ and 12.7 ± 2.4 min⁻¹, respectively (all $P>0.05$, 1-way repeated measures ANOVA; n=10) (Fig. 2C ii). However, MIs calculated in all three regions of the UUT of diabetic rats were significantly greater than those calculated in control rats (Fig. 2Ciii) (1-way repeated measures ANOVA; n=10).

Effects of capsaicin

In all regions of the UUT, capsaicin (10 µM for 15 min) rapidly caused an increase in contraction frequency associated with a transient increase in basal tension. In the proximal and distal renal pelvis of the diabetic rat, basal tension increased to 1.49 ± 0.29 mN and 0.86 ± 0.24 mN, respectively, compared to 0.38 ± 0.18 mN and 0.21 ± 0.10 mN in the same regions of control rats (both $P<0.05$, 2-way repeated measures ANOVA; n=6) (Fig. 3A, B). In the proximal renal pelvis, contractions at the peak of the response to capsaicin often fused into a summed response not allowing a measure of the increase of contraction frequency. In the distal renal pelvis, the peak contraction frequency in capsaicin increased to 140.6 ± 6.3% and 133.6 ± 24.4% of control in the non-diabetic and diabetic rats, respectively. As in the guinea pig UUT (Teele and Lang, 1998) this rise in excitability was followed by a short lived decrease in excitability (Fig. 3A, B). However, after 60 min washout in PSS, the amplitude and frequency of contractions recorded in both control and diabetic rats were not different from control values before capsaicin treatment (Fig. 3A, B). MIs calculated as a % of control for the proximal and distal renal pelvis and ureter of diabetic rats after 60 min washout were 105.1 ± 13.8%, 97.1 ± 13.8% and 80.9 ± 13.3%, respectively; compared to the corresponding values in non-diabetic rats of 118.1 ± 14.0%, 105.2 ± 20.8% and 120.2 ± 27.8%, respectively, ($P>0.05$, 1-way repeated measures ANOVA n=6). These results contrast to that seen in the guinea-pig UUT, where capsaicin causes a transient increase
in active and passive tension followed by a sustained and significant decrease in the amplitude of contractions (Teele and Lang, 1998).

**Effects of SP and NKA**

Application of the excitatory tachykinins, SP (2 μM for 2 min) (Fig. 4A, B) or NKA (2 μM for 2 min) (Fig. 4C, D) caused a transient increase in both the amplitude and frequency of contractions of the proximal and distal renal pelvis and ureter in both control and STZ-induced diabetic rats. Table 2 summarizes the effects of these 2 tachykinins on the amplitude, frequency of the contractions in the proximal and distal renal pelvis in control and diabetic rats. It can be seen that there was no difference between the effects of SP or NKA on UUT motility. However, the changes in baseline evoked by SP or NKA were significantly greater in the diabetic animals compared to the age-matched control group ($P<0.05$; 1-way repeated measures ANOVA; n=4). In contrast, contractions to high K PSS were little affected by STZ-treatment ($P>0.05$; 1-way repeated measures ANOVA; n=3) (Table 2).

**Effects of hCGRP**

In comparison to the guinea pig UUT (Teele and Lang, 1998), the effects of 100 nM hCGRP in the rat UUT were small and region specific. Measurements of contractility in the rat UUT were made every 5 min for a period of 20 min, after which time hCGRP was washed out of the bath. In control rats, the amplitude of the contractions in the proximal renal pelvis was significantly increased from 0.63 ± 0.08 to 0.69 ± 0.1 mN after 20 min exposure to 100 nM hCGRP ($P<0.05$; n=5) (Fig. 5i). In the distal renal pelvis, contraction amplitudes were significantly reduced only between 5 and 10 min exposure to hCGRP ($P<0.05$, 2-way repeated measures ANOVA; n=5) (data not shown). Contractions amplitudes returned to control values.
ater 20 min exposure (Fig. 5i). The frequency of contractions was reduced at all time points in both the proximal and distal renal pelvis (Fig. 5ii). After 20 min exposure to hCGRP the frequency of contraction in the proximal and distal renal pelvis was 87.4 ± 4.1% and 90.9 ± 6.0%, respectively, of control (P<0.05, 2-way repeated measures ANOVA; n=5). MIs in the proximal

**Table 2.** Effects of tachykinins on motility in the renal pelvis of control and diabetic rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Proximal Renal Pelvis</th>
<th>Distal Renal Pelvis</th>
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<tr>
<td></td>
<td>Maximum amplitude</td>
<td>Maximum frequency</td>
</tr>
<tr>
<td></td>
<td>(% of control)</td>
<td>(% of control)</td>
</tr>
<tr>
<td>SP Controls</td>
<td>147.7 ± 26.2</td>
<td>132.9 ± 16.0</td>
</tr>
<tr>
<td>Diabetic</td>
<td>140.6 ± 17.3</td>
<td>126.6 ± 4.2</td>
</tr>
<tr>
<td>NKA Controls</td>
<td>130.2 ± 9.1</td>
<td>230.9 ± 47.3</td>
</tr>
<tr>
<td>Diabetic</td>
<td>109.0 ± 3.3</td>
<td>128.3 ± 5.5</td>
</tr>
<tr>
<td>High K PSS  Controls</td>
<td>240.2 ± 17.4</td>
<td>131.3 ± 13.6</td>
</tr>
<tr>
<td>Diabetic</td>
<td>249.7 ± 43.7</td>
<td>112.8 ± 8.3</td>
</tr>
</tbody>
</table>

*P<0.05

**Fig. 5.** Effects of 100 nM hCGRP (for 20 min) on the contractile properties of the proximal and distal renal pelvis of the rat and guinea pig.
renal pelvis of diabetic rats calculated at any time point (5–30 min) after exposure to hCGRP (100 nM) were not significantly different from control values ($P<0.05$, 2-way repeated measures ANOVA; n=5). In contrast, only the MI calculated in the distal renal pelvis after 5 min exposure to 100 nM hCGRP was significantly reduced ($P<0.05$, 2-way repeated measures ANOVA; n=5) (Fig. 5iii). Thus applied hCGRP had a slightly positive inotropic effect (for 5–10 min) on the rat proximal renal pelvis but a negative inotropic effect on the distal renal pelvis.

Also plotted in Fig. 5 are the effects of 100 nM hCGRP (for 20 min) on the contractile properties of the proximal and distal renal pelvis of the guinea pig UUT (n=9) recorded under identical conditions for comparison. It can be seen that a 20 min exposure to hCGRP produced a greater inhibition of both the proximal and distal renal of the guinea pig than in the rat (Teele and Lang, 1998).

Finally, the relative small effects of hCGRP on the rat UUT meant that we could not demonstrate any significant differences between control and diabetic rats in their response to hCGRP, either in terms of UUT region or in any of the parameters measured (all $P>0.05$, 1-way repeated measures ANOVA; n=5).

**Discussion**

The major finding of this study was that the induction of diabetes produced a significant increase in the amplitude but not frequency of the spontaneous contractions in distal renal pelvis and ureter. In addition, a brief application of the sensory neurotoxin, capsaicin, or the sensory neuropeptides, SP and NKA, to the rat UUT caused a transient increase in motility associated with rise in basal tone that was significantly increased after STZ treatment. In contrast, the transient positive chronotropic and inotropic effects of capsaicin, NKA, SP and high K+ PSS on the spontaneously-occurring contractions were not significantly altered in the diabetic animals. The mild positive inotropic and negative chronotropic effects evoked by hCGRP were also not significantly altered by STZ treatment.

The rat UUT contains a dense network of capsaicin-sensitive sensory neurons, which are distributed extensively throughout the muscular, sub-epithelial and epithelial layers of both the renal pelvis and ureter. In the ureter, CGRP-like immuno-reactivity has been also been reported to be 33-fold higher than the SP-like immuno-reactivity present (Amann et al., 1988). Given this relative distribution of the CGRP to SP it was a little surprising that capsaicin did not evoke a marked negative inotropic and chronotropic effect on spontaneous UUT motility, as observed in the guinea pig UUT (Teele and Lang, 1998), even though both capsaicin and CGRP readily inhibit tachykinin-evoked ureteric contractions (Maggi and Giuliani, 1991). However, the small capsaicin-evoked negative inotropic effect on rat UUT motility was consistent with the relatively mild and region-dependent effects of applied hCGRP (Fig. 5). Presumably, capsaicin-evoked SP was functionally antagonizing the negative inotropic and chronotropic effects of any CGRP released. Interestingly, SP and NKA both appeared to produce a negative inotropic effect for several min after their peak excitatory action similar to the effects of capsaicin (Fig. 4). This negative inotropic effect could involve either the presynaptic release of inhibitory substances such as CGRP or, perhaps more likely, the activation of refractory mechanisms within the
smooth muscle cells themselves.

If STZ-induced diabetes was causing a sensory neuropathy it might be expected that the capsaicin–evoked contraction would be diminished while the responses to the applied tachykinins would be enhanced as the smooth muscle layer became super-sensitive upon denervation. The increased change in baseline tension to SP and NKA in the diabetic rats was clearly consistent with such a post-junctional super-sensitivity. The presence of a response to capsaicin could also be arising from a functional antagonism / interaction between neuropathy-evoked reduction of tachykinin release and an increase in smooth muscle sensitivity to these released agents. Alternatively, as has been demonstrated in rat trigeminal ganglia (Troger et al., 1999) and bladder (Santicioli et al., 1987), it seems more likely that the depletion of SP and CGRP in the UUT of STZ-induced diabetic rats was only transient so that sensory nerve function was little altered after 6–8 weeks.

The lack of any significant changes in contraction frequency in any regions of the UUT with diabetes in the present experiments suggests that pacemaker mechanisms are little altered in diabetes. In contrast, the region specific effects of diabetes on contraction amplitude are more difficult to explain. In the bladder, it has been suggested that DM must be inducing some presynaptic alterations such as a decrease in the density of vaniolic (capsaicin) or SP receptors (Kamata et al., 1993) and/or a decrease in the release of SP from nerves which account for the changes in sensitivity seen in the bladder by some researchers. Blockade of PLA₂, cyclooxygenases (Cox) or removal of the urothelium reduces the diabetes-induced increase in detrusor contractility to SP (Pinna et al., 1994). However, contractile responses of detrusor strips to prostaglandins F₂α and E₂ prostaglandins were unaffected, suggesting that prostaglandins may play a role in sensitizing both the sensory nerves in the bladder and the detrusor smooth muscle responses to SP.

In the UUT, it is well established that a tonic production of prostaglandins is critical in the maintenance of UUT contractility (Lang et al., 2002; Weiss et al., 2006). Indeed, we have previously demonstrated that Cox-1 is the primary enzyme involved in synthesizing these prostaglandins in the rat UUT, while Cox-2 is the active enzyme in the guinea pig UUT. Decreases in the MIs of the rat proximal and distal renal pelvis upon Cox-1 inhibition arise from a decrease in contraction frequency. In fact, contraction amplitudes were significantly larger than time matched controls after 60 min exposure to 5 or 50 µM valeryl salicylate (Davidson and Lang, 2000). It seems possible that diabetes is modulating UUT motility via an alteration of prostaglandin synthesis.

In summary, we speculate that, as in the bladder, STZ treatment induces some from of sensitization of the smooth muscle wall of the rat UUT to capsaicin and tachykinins and that it occurs within 6–8 weeks. The underlying mechanisms of this muscle wall sensitization remains unknown but may well arise from a earlier-occurring but transient sensory neuropathy.

References


