Diabetes mellitus- and cooling-induced bladder contraction: an in vitro study

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Abstract

The effects of diabetes mellitus during cooling on ACh- and KCl-induced responses were investigated in rat urinary bladder. Diabetes was induced in the rats by 50 mg/kg streptozotocin via an intraperitoneal injection. Rats¹ body and bladder weights were measured. The isometric tension to ACh (10⁻⁹ – 3 × 10⁻⁴ M) and KCl (5–100 mM) in strips of urinary detrusor muscle of diabetic and non-diabetic rats, in organ baths at 37 and 28ºC were recorded. The body weights were significantly decreased and the bladder weights increased in STZ-induced diabetic group compared to the non-diabetic group. ACh and KCl caused concentration-dependent contractions of urinary bladders from non-diabetic and diabetic preparations. During cooling, the sensitivity and the maximal response were significantly higher than those during 37ºC, both in non-diabetic and diabetic preparations. Cooling of detrusor muscle preparations induces a graded contraction inversely proportional to the temperature in diabetic rats. It may be assumed that the cooling response involves the same mechanisms in the diabetic and non-diabetic animals.

Key words: cooling, carbachol, diabetes mellitus, potassium chloride, rat urinary bladder

Introduction

Diabetes mellitus results in long-term complications of the lower urinary tract, referred to as diabetic cystopathy (Moller, 1976). Many factors probably contribute to the large capacity, atonic bladder that characterizes diabetic cystopathy, including changes in bladder smooth muscle (Michel et al., 2000). Diuresis originating from diabetes mellitus causes, increases in bladder mass and changes in bladder strip contractility (Moller, 1976; Michel et al., 2000). Contractile responses of the bladder are under the control of the parasympathetic nervous system. Cholinergic muscarinic receptors are found throughout the bladder. Activation of the parasympathetic motor fibres to the...
bladder causes an intense stimulation of the muscarinic M₃-receptors in the bladder body, resulting in a strong and efficient bladder contraction, which in turn causes emptying of the bladder (Longhurst et al., 1995; Hegde et al., 1997). Several studies have been undertaken to investigate contractile responses of the bladder to muscarinic drugs in diabetic animals. It has been shown that in diabetic animals, contractile responses of the urinary bladder to muscarinic agonists are inconsistent. Some investigators have reported increased responses (Tong et al., 2002; Nsabimana and Ojewole, 2008), while others have reported decreased responses (Lincoln et al., 1984; Longhurst et al., 1986), or no change in contractile responses (Luheshi and Zar, 1991; Atalık et al., 2000) of diabetic urinary bladder smooth muscle strip to muscarinic agonists.

Cooling has been shown to have a variable influences on smooth muscle sensitivity to various drugs and endogenous substances in different parts of the vascular system of animals (Mustafa and Thulesius, 1999; Atalık et al., 2008). Thus, despite current research to determine the effects of temperature on the vascular reactivity of cutaneous tissues, only very few studies have examined the effect of cooling on the urinary bladder. We (Mustafa and Thulesius, 1999) and the other investigators (Hellstrom et al., 1991; Ismael et al., 2010) showed that cooling-induced bladder contraction in rat. Detrusor contraction in conjunction with bladder cooling is supposed to be initiated by sensory mucosal receptor stimulation followed by detrusor activation through a spinal reflex within the sacral segments. An ice-water test is used clinically in the evaluation of neurogenic disorders of continence as part of a standard urodynamic examination in the differential diagnosis of detrusor overactivity in infants (Geirsson et al., 1999).

The investigators also reported that cooling-induced contraction of isolated rat detrusor muscle preparation is inversely proportional to temperature. Thus, despite current research to determine the effects of cooling on the reactivity of rat urinary bladder, studies with diabetic urinary bladder remain incomplete and to our knowledge, there are no studies that analyze the effects of cooling to ACh and KCl on urinary bladder of streptozotocin (STZ)-induced diabetic rat.

To our knowledge the effects of diabetes on the thermal detrusor smooth muscle responses to carbachol and KCl have not been investigated. It was therefore the objective of this study to clarify the response of smooth muscle cooling in isolated diabetic detrusor muscle preparations.

Materials and Methods

Preparation of bladder strips

Adult female Sprague Dawley rats weighing 250 to 300 g were used in this study. The rats were divided into two groups containing six animals each. They were non-diabetic and streptozotocin (STZ)-induced, diabetic groups. In the diabetic groups, diabetes was induced by a single intraperitoneal injection of 50 mg/kg STZ that was prepared in a 0.1 M citrate buffer solution, pH 4.5. Non-diabetic rats were injected saline only. Body weights of rats were measured in all groups before and after 8 weeks diabetes induction. Plasma glucose levels were determined from tail vein blood samples (Acura Ac 1018) two days after STZ administration. All rats were kept under identical conditions for 8 weeks with free access to food and water. Eight weeks after the initial treatment, blood samples were collected for measurement of plasma glucose levels and rats were sacrificed. STZ-treated rats with blood glucose concentrations ≥250 mg/mL were
considered to be diabetic and used in this study. The lower abdomen was opened and the whole urinary bladder of each rat was removed and rapidly weighed. The bladder was then placed in a Petri dish containing Krebs-Henseleit solution (KHS, mM: NaCl 119, KCl 4.70, MgSO₄ 1.50, KH₂PO₄ 1.20, CaCl₂ 2.50, NaHCO₃ 25, Glucose 11), the connective tissues were cut away. Two strips of detrusor muscle from each bladder were used. Full thickness longitudinal strips (10 × 4 mm) were prepared from the dorsal part of the bladder body.

All experiments were carried out with the approval of local animal use ethical committee of Selçuk University.

**Experimental procedure**

The preparations were mounted 1 g tension in 25 mL organ baths containing KHS maintained at 37ºC and aerated with 95% O₂ and 5% CO₂. Tissues were allowed to equilibrate for 1 h. The responses were recorded isometrically by a force-displacement transducer (Grass FT04, Grass Instruments Co, W. Warwick, RI, USA) connected through amplifiers to a polygraph (Grass 7D, Grass Instrument Co).

Cumulative concentration-response curves were determined in non-diabetic group for ACh (10⁻⁹ – 3 × 10⁻⁴ M) at 37ºC. After the first concentration-response curve was completed, preparations were washed and allowed to reestablish resting tension before being cooled. When preparations stabilized (30 min), bath temperature was decreased to 28ºC. Preparations were allowed to equilibrate at this temperature for 1 h before a second concentration-response curve was determined. Two concentration-response curves were generated in each strip; first at 37ºC and second at 28ºC.

In the other part of the study, cumulative concentration-response curves were determined in non-diabetic group for KCl (5–100 mM) at 37ºC. After the first concentration-response curve was completed, preparations were washed and allowed to reestablish resting tension before being cooled. When preparations stabilized (30 min), bath temperature was decreased to 28ºC. Preparations were allowed to equilibrate at this temperature for 1 h before a second concentration-response curve was determined. Two concentration-response curves were generated in each strip; first at 37ºC and second at 28ºC. The same procedure was repeated with STZ-induced diabetic rat bladders for ACh and KCl. Only one agent was tested in each preparation.

**Drugs**

Acetylcholine and STZ were used. Both drugs were obtained from Sigma, St. Louis, MO, USA.

**Analyses of results**

Concentrations of ACh and KCl causing 50% of the maximal response (EC₅₀) were calculated from each individual concentration-response curve and its 95% confidence interval was obtained for each group of experiments. Maximal responses and EC₅₀ values for curves obtained before (37ºC) and during cooling (28ºC) were compared by using Student’s t-test. Statistical significance was set at $P<0.05$. 
Results

Changes in body weight, bladder weight and plasma glucose levels

Mean body weight of the two groups were similar before inducing diabetes. Eight weeks after the treatment with STZ, the body weights were decreased ($P<0.05$) and the concentration of glucose in plasma levels were elevated significantly ($P<0.05$) in diabetic rats. The weight of the whole urinary bladder in the diabetic group was significantly ($P<0.05$) higher than those in non-diabetics (Table 1).

Table 1. Body weight, bladder weight, and blood glucose levels of non-diabetic and diabetic rats

|                     | Non-diabetic (Control) | Diabetic          |
|---------------------|------------------------|-------------------|
| Initial body weight (g) (n=6) | 272 ± 5.3             | 276 ± 7.2         |
| Final body weight (g) (n=6)    | 315 ± 3.1             | 150 ± 5.9*        |
| Bladder weight (mg) (n=6)      | 110 ± 2.0             | 224 ± 3.1**       |
| Plasma glucose (mg/dl) (n=6)   | 104 ± 2.3             | 435 ± 6.0**       |

Values are mean ± SEM of six animals. *, $P<0.05$ compared to initial weight in the same group. **, $P<0.05$ compared to control group.

Table 2. $EC_{50}$ values and maximum responses ($Emax$) for acetylcholine and potassium chloride of non-diabetic and diabetic rat urinary bladder at both 37 and 28ºC

|                     | $EC_{50}$ (ACh, $\times 10^{-6}$ M) | $Emax$ (g)                       |
|---------------------|-----------------------------------|---------------------------------|
|                     | KCl ($\times 10^{-2}$ M)          | ACh                             | KCl                             |
| Non-diabetic, 37ºC (n=6) | 7.78 ± 0.30                        | 8.47 ± 0.13                     | 1.28 ± 0.20                     | 1.46 ± 0.05 |
| Non-diabetic, 28ºC (n=6)  | 5.13 ± 0.13*                       | 6.47 ± 0.06*                    | 3.23 ± 0.26*                    | 4.87 ± 0.11*|
| Diabetic, 37ºC (n=6)     | 3.43 ± 0.56**                      | 7.93 ± 0.06**                   | 2.16 ± 0.12**                   | 3.50 ± 0.29**|
| Diabetic, 28ºC (n=6)     | 1.80 ± 0.10**,**,**,**,**,**,**,** | 5.13 ± 0.13**,**,**,**,**,**,** | 3.90 ± 0.50,**,**,**,**,**,** | 5.63 ± 0.47** |

Each value is derived from six experiments. Data are means ± SEM. *, **, and #, ##: $P<0.05$ compared to non-diabetic, 37ºC; *** and ###, $P<0.05$ compared to diabetic, 37ºC; ****, $P<0.05$ compared to non-diabetic, 28ºC.

Contractile responses to acetylcholine

Acetylcholine (ACh, $10^{-9} – 3 \times 10^{-4}$ M) caused concentration-dependent contractions of bladder strips isolated from both non-diabetic and STZ-induced, diabetic rats. ACh induced stronger and greater contractions of the diabetic bladders compared with bladders taken from the non-diabetic rats ($P<0.05$).

During cooling, the sensitivity and the maximal response to ACh were significantly higher than those during 37ºC, both in non-diabetic and diabetic preparations. Table 2 compares $EC_{50}$ and $Emax$ values from non-diabetic and diabetic rats at 37 and 28ºC. Figure 1 shows the contractile responses to ACh on urinary bladder strips of non-diabetic and diabetic rats.

Contractile responses to potassium chloride

Figure 2 shows the effects of KCl (5–100 mM) on non-diabetic and diabetic rat urinary bladder at 37 and 28ºC. KCl produced concentration-dependent contractions at both temperatures studied on non-diabetic and diabetic rat urinary bladder strips. KCl induced stronger and greater
Contractions of the diabetic bladders compared with bladders taken from the non-diabetic rats.

During cooling, the sensitivity and the maximal response to KCl were significantly higher than those during 37°C, both in non-diabetic and diabetic preparations. Table 2 compares EC\textsubscript{50} and E\textsubscript{max} (P<0.05) values from non-diabetic and diabetic rats at 37 and 28°C. There was no effect of time on the responses to KCl (P>0.05, data not given).
Discussion

Our results show that cooling of urinary bladder detrusor muscle preparations induces a graded contraction inversely proportional to the temperature in diabetic rats. In our study, increased serum glucose concentrations and bladder weights and decreased body weights in STZ-induced diabetic rats were observed. Our findings are in agreement with those reported by Tammela et al. (1995) and Nsabimana and Ojewole (2008). Itoh et al. (1994) reported that bladder hypertrophy may be due to physiological adaptation to the increased urine volume.

Our results indicate that at 37ºC, both ACh and KCl-induced reproducible contractions in non-diabetic and STZ-induced diabetic urinary bladder strips. As we know while ACh, is the primary neurotransmitter controlling bladder voiding and activates M3 muscarinic receptors. M3 receptors produce direct smooth muscle contraction by a mechanism that relies on entry of extracellular calcium through L-type channels and activation of a rho kinase (Hegde, 2006), potassium chloride can stimulate Ca2+ influx through voltage sensitive Ca2+ channels (Latifpour et al., 1989; Kregel and Gisolfi, 1990). The parasympathetic nervous system plays an important role in the functional regulation of bladder smooth muscle. Histochemical and functional studies have shown that there is a diabetes-induced alteration in the cholinergic innervation and/or changes in the response of bladder smooth muscle to cholinergic agonist in experimental animals (Lincoln et al., 1984; Longhurst and Bellis, 1986; Latifpour et al., 1989; Yenilmez et al., 2006). Nevertheless, results of previous animal studies on cholinergic transmission in diabetic bladders have been controversial. While some investigators reported increased contractile responses (Latifpour et al., 1989; Hegde et al., 1997; Geirsson et al., 1999; Yenilmez et al., 2006) others reported decreased contractile responses (Longhurst and Bellis, 1986; Ichiyanagi et al., 2002), and some other investigators mentioned no change in contractile response (Lincoln et al., 1984; Luheshi and Zar, 1991; Nakamura et al., 1992) of the bladder smooth muscle to muscarinic agonists. The differences in these various reports may be related to differences in methodology, sex differences, ages of the experimental animals and variations in the durations of diabetes. In our study, increased contractile responses after 8 weeks of diabetes were observed. Our results are in agreement with the findings of Latifpour et al. (1989), Kamata et al. (1992), Yenilmez et al. (2006) and Nsabimana and Ojewole (2008) who have reported increased contractile responses of diabetic bladders to muscarinic agonists. Kamata et al. (1992) have also shown that the enhanced contractile response to ACh in detrusor strips of urinary bladders from STZ-induced diabetic rats were associated with an increased population of muscarinic receptors. Furthermore, the overactivity was characterized by an enhanced sensitivity to carbachol, partly due to an increase in receptor density, but without change in receptor/G-protein coupling (Hampel et al., 2003; Mumtaz et al., 2006; Stevens et al., 2006).

As it is known that temperature is one of the factors that induce smooth muscle activity and there is limited information about the effect of temperature in urinary bladder (Atalik et al., 1999; Mustafa and Thulesius, 1999) and furthermore, to our knowledge, there are no studies that analyze the effects of cooling on diabetic urinary bladder to ACh and KCl. The ice-water test is a simple supplementary urodynamic test that increases the precision of the diagnosis of infant detrusor overactivity (Geirsson et al., 1999). The temperature utilized in this study; 28ºC, for cooling was
considered to be “moderate cooling” temperature accordingly to our previous studies (Atalı et al., 2000; Atalı et al., 2001; Atalı et al., 2008). In our study, compared with the control responses at 37°C, cooling increased both the sensitivity and the maximum responses to ACh and KCl in both non-diabetic and STZ-induced diabetic urinary bladder. This suggests that cooling could nonspecifically affect the contractions of urinary bladder when it is activated by different type of contractile agent. In literature, to our knowledge the basic mechanism of the thermal detrusor smooth muscle control has been investigated by Mustafa and Thulesius (1999) and Ismael et al. (2009). These investigators reported that in rat urinary bladder strips cooling-induced contractions were inversely proportional to temperature. Their result is in line with our finding. Mustafa and Thulesius (1999) also observed that cooling affects the processes of translocation of extracellular and intracellular Ca²⁺. Furthermore, cold receptors in the human bladder wall have been reported (Geirsson, 1993).

To our knowledge, our study is the first in the literature to examine the effects of cooling on diabetic-urinary bladder. In the present study, we observed the same results with ACh and KCl during cooling. As we know, while KCl-induced contraction is due to membrane depolarisation leading to an increased Ca²⁺ influx through voltage stimulated Ca²⁺ channels (Goodfraind et al., 1986), ACh can cause contractions both via cellular and extracellular Ca²⁺ (Yang et al., 1993). In a previous study (Atalı et al., 1999), we observed intracellular Ca²⁺ pools may play a functional role in the cooling-induced contractions of rabbit detrusor smooth muscle. Furthermore, in literature sex-related differences in function and distribution of beta-adrenoceptors in rabbit urinary bladder (Morita et al., 1998). Although female rats were used in our experiments, the findings were in line with other reports in male rats (Hellström et al., 1991; Mustafa and Thulesius, 1999), indicating that there are not sex-related differences in the function of rat detrusor during cooling.

In conclusion, cooling of detrusor muscle preparations induces a graded contraction inversely proportional to the temperature in diabetic rats. These findings may indicate that the cooling response involves the same mechanisms in the diabetic and non-diabetic animals.

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