TRPV1 and TRPA1 channel function following streptozotocin-induced diabetes in rats

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ABSTRACT: The aim of this study is to study the change in TRPV1 and TRPA1 channel responses with time following the treatment with streptozotocin. The results showed that as early as 36 hours after induction of diabetes by STZ, the contractile responses to capsaicin were significantly reduced in comparison to those of the controls and this reduction persisted until the eight weeks time point. In contrast, responses to the TRPA1 agonist allyl isothiocyanate were not affected at early time points but were reduced one week after STZ treatment. This detailed time course analysis suggests that there are novel mechanisms of modulation of the TRPV1 channels in this STZ model. In conclusion, in the rat urinary bladder or colon preparations, diabetes mellitus using STZ animal model caused the impairment caused by STZ-induced diabetes occurred very early (within 36 hours after diabetes induction) in TRPV1 channel but not TRPA1 channel. There are specific early effects of STZ treatment on TRPV1 channel function at a time when other afferent nerve terminal channels (TRPA1) are functioning normally, suggesting that early onset of dysfunction in TRPV1 signalling may not merely be the consequence of nerve damage. The mechanism of this impairment may not be the effect of neuropathy on neurotransmitter release or nerve damage. Improving the responsiveness of nerves of bladder in diabetic patients might be of therapeutic benefit.

Introduction

Diabetic bladder dysfunction is characterized by a triad of decreased sensation, increased capacity and poor emptying with a prevalence estimated to be between 32% and 45% (Hunter and Moore, 2003). The study in animal models of diabetes especially in streptozotocin induced-diabetes in rats indicated that diabetes both decreased (Longhurst and Belis, 1986) and increased detrusor contractility (Warning and Wrendt, 2000). Abnormalities of bladder function such as reduced contractile responses to nerve stimulation and applied acetylcholine have been reported (Longhurst and Belis, 1986). In addition, there are abnormalities in afferent nerve signaling in bladder from streptozotocin-induced diabetic rats (Steer et al., 1994). Transient Receptor Potential (TRP) channels are a recently identified large group of calcium permeable ion channels that allow calcium entry without requiring cell depolarization. TRPV1 is the most investigated channel compared to other TRP subfamilies. TRPV1 channel is activated by capsaicin, which has been shown to cause contraction of the rat bladder (Saitoh et al., 2007) and has been used in the treatment of neurogenic bladder dysfunction (Fowler et al., 1992). Pinna et al. (1994) have shown decreased capsaicin responses in STZ-diabetic rat bladder. TRPV1 levels are reduced in skin biopsies from patients with diabetic neuropathy (Facer et al., 2007) and insulin has been shown to cause sensitization and translocation of TRPV1 receptors (Van Buren

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et al., 2005). Therefore we hypothesize that TRPV1 function will be reduced in the diabetic bladder. Another TRP channel, TRPA1 is also emerging as playing a significant role in bladder function. TRPV1 is co-expressed with TRPA1 and the later is reported to be expressed on capsaicin-sensitive primary sensory neurons. Peripheral neuropathy, one of the consequences of diabetes, is reproduced in the streptozotocin (STZ) model of diabetes in rats (Bestetti et al., 1981; Filho and Fazan, 2006). The study in animal models of diabetes especially in streptozotocin induced-diabetes in rats indicated that diabetes both decreased (Longhurst and Belis, 1986) and increased detrusor contractility (Warning and Wrendt, 2000). Abnormalities of bladder function such as reduced contractile responses to nerve stimulation and applied acetylcholine have been reported (Longhurst and Belis, 1986). In addition, there are abnormalities in afferent nerve signaling in bladder from streptozotocin-induced diabetic rats (Steer et al., 1994). A decrease in nerve growth factor in bladder from rats with 12 weeks streptozotocin-induced diabetes has also been reported (Sasaki et al., 2002). In preliminary observations we confirmed the reduction in contractile responses to the TRPV1 agonist capsaicin in diabetic rat bladder eight weeks after STZ treatment. It was interesting to examine the time course of onset of this dysfunction. The aim of these experiments was to investigate the time cause of onset of TRPV1 channel dysfunction in STZ model of diabetes in rat bladder.

Materials and Methods

Animals

Male wistar rats with body weight of 300-400 g were used. These animals were supplied by the Biological Science Unit (BSU), School of Life Sciences, Faculty of Health and Human Science, University of Hertfordshire. Animals were divided into two groups: control and diabetic. All rats were kept in separate cages. One control and one diabetic rat were kept in the same cage. They were provided with feed and water daily for up to 8 weeks until used in the study. All groups were kept in a temperature-controlled room (22 ± 2 °C), artificially lit from 6.00 to 18.00 hours daily. The initial weights and blood glucose levels of the rats were recorded and again at sacrifice.

Induction of diabetes mellitus

The initial blood glucose level of rats was measured using an Accu-Check active testing kit. The blood was taken from the tail vein. The initial body weight was also measured to quantify the change in body weight over the eight weeks. In order to induce diabetes, streptozotocin (STZ) at dose of 65 mg/kg bodyweight was injected intraperitoneally to the rats with a single injection. Streptozotocin was freshly dissolved in 20 mM citrate buffer at pH 4.5. The control rats were injected with 20 mM citrate buffer (pH 4.5) at an equal volume to the diabetic group. To avoid the initial hypoglycemia, 2% sucrose was added to drinking water for the streptozotocin-induced diabetic rats for 48 hours. The weight of the rats and their blood glucose levels were measured
immediately after sacrifice. The blood glucose level in control rats should be approximately 100 mg/dl. For diabetic groups, the blood glucose level of 400 mg/dl or more is confirmed as diabetic.

**Tissue preparations**

The bladder was immediately removed by opening the lower abdomen. The bladder was cut at the bladder neck to obtain the whole bladder. Then the removed bladder was kept in Krebs solution of the following composition (in mM): NaCl 118.3, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, D-glucose 11.1, CaCl₂ 2.5 and gassed with 95% O₂ and 5% CO₂.

The bladder was cut longitudinally along the neck to the body. Then four strips were taken from the whole bladder. The strips were approximately 1 cm long and 0.5 cm wide. The strip was tied with tread at one end to be attached to the force transducer. The other end was tied to a hook to be attached to organ bath. The bladder strip preparations were mounted in organ bath contained a Krebs solution and gassed with 95% O₂ and 5% CO₂.

Capsaicin at concentrations of 10⁻¹⁰ M to 10⁻⁶ M was added to the organ bath in cumulative manner. The comparisons of contractile responses to capsaicin of the carbachol contracted tissues of control and STZ-treated rat bladders were measured.

To study the effect of time frame of STZ-diabetes induction on another TRP ion channel, TRPA1, which is reported to be expressed on the sensory nerve terminal similar to the TRPV1 channel, the contractile responses to the TRPA1 agonist allyl isothiocyanate at concentrations of 10⁻⁵ M to 10⁻⁴ M were measured. Allyl isothiocyanate was added to the organ bath in a non-cumulative manner. The dose response studies to allyl isothiocyanate were performed using the tissues from the rat treated with STZ or citrate buffer (control) for 8, 2, 1 week, and 36 hours to see the changes in the responses according to the time courses of STZ treatments.

**Results**

**The effect of STZ-induced diabetes on blood glucose level and body weight**

8 weeks after induction of diabetes by STZ in rats, the blood glucose was elevated four-folds compared to the controls (treated with citrate buffer) confirming a diabetic state (Figure 1A).

In contrast, body weight of rats treated with STZ was stable while it was increased in the controls which were treated with citrate buffer. These suggest that in diabetes, there is no weight gain while the control gained weight over time (Figure 1B).
The effect of time frame of STZ-induced diabetes on TRPV1 channel function

To emphasize the changes in blood glucose and TRPV1 induced contractions over time the data for 10^{-8} M capsaicin is summarised in Figures 3 and 4 respectively. It is clear that in the STZ model of diabetes in rat, blood glucose levels were markedly increased from 24 hours after induction with STZ and constant for up to 8 weeks thereafter (Figure 3A).

In contrast, changes in capsaicin-induced contractility became evident 36 hours after induction of diabetes by STZ and remained depressed over the 8 weeks period. Surprisingly, contractile responses in controls declined at 2 weeks but appear to partially recover at 8 weeks (Figure 3B). The reasons for these unexpected changes in controls are unclear.

The effect of time frame of STZ-induced diabetes on TRPA1 channel function

In contrast to TRPV1, responses to the TRPA1 agonist allyl isothiocyanate were not affected at 36 hours but were reduced one week after STZ treatment (Figure 4).

Figure 1 Blood glucose level (A) and body weight (B) of age-matched controls and STZ-induced diabetic rats, 8 weeks after the administration of STZ. Values represent the mean ± S.E.M for 6 animals. *P<0.05 is significantly different from age-matched controls (Student’s t test for unpaired observations).

Figure 3 Blood glucose levels (A) and contractile responses of bladder (B) to TRPV1 agonist capsaicin at the concentration of 10^{-8} M from age-matched controls and STZ-induced diabetic rats, 8, 2, 1 week, and 36 and 24 hours after the administration of STZ. Values represent the mean±S.E.M for 6 animals. Means are different between age-matched controls and STZ-induced diabetic rats (P<0.05, two-way ANOVA).
The results indicated that as early as 36 hours after induction of diabetes by STZ, the contractile responses to capsaicin were significantly reduced in comparison to those of the controls and this reduction persisted until the eight weeks time point. In contrast, responses to the TRPA1 agonist allyl isothiocyanate were not affected by early time points but were reduced eight weeks after STZ treatment. The contractile responses of bladder strips to TRPV1 agonist capsaicin were not affected by exposure to elevated glucose. There are specific early effects of STZ treatment on TRPV1 channel function at a time when other afferent nerve terminal channels (TRPA1) are functioning normally, suggesting that early onset of dysfunction in TRPV1 signalling may not merely be the consequence of nerve damage.

Although there is no direct study on the time course of STZ-induced diabetes on the TRP channel function in rat urinary bladder, it was previously reported that 8-week streptozotocin-induced diabetes clearly leads to a number of significant alterations in the functional responses of the rat ileum (Talubmook et al., 2003). There is the presence of neuropathy in Schwann cell 4 months after induction of diabetes by STZ (Bestetti et al., 1981a) and there are neuropathy and myopathy in the diaphragm of rat after 12 months of STZ-induced diabetes (Bestetti et al., 1981b).

In addition, STZ-induced diabetes provokes impairment of capsaicin-sensitive sensory fibers but not of the cholinergic system even at early stage (4 week) of the disease in rat urinary bladder and the bladder response to capsaicin gradually decreased with the progression of diabetes (Pinna et al., 1994).

From the result obtained in the present study, it suggests that at the early time point of the onset, diabetes mellitus affected TRPV1 but not TRPA1 which are reported to be expressed in the same nerve terminals and that the early impairment of TRPV1 channel function may not due to the diabetic neuropathy.
References


