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Effects of ageing and streptozotocin–induced diabetes on connexin43 and P2 purinoceptor expression in the rat corpora cavernosa and urinary bladder

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Abstract

OBJECTIVE—To investigate whether ageing and diabetes alter the expression of the gap junction protein connexin43 (Cx43) and of particular purinoceptor (P2R) subtypes in the corpus cavernosum and urinary bladder, and determine whether changes in expression of these proteins correlate with development of erectile and bladder dysfunction in diabetic and ageing rats.

MATERIALS AND METHODS—Erectile and bladder function of streptozotocin (STZ)-induced diabetic, insulin-treated and age-matched control Fischer-344 rats were evaluated 2, 4 and 8 months after diabetes induction by *in vivo* cystometry and cavernosometry. Corporal and bladder tissue were then isolated at each of these sample times and protein expression levels of Cx43 and of various P2R subtypes were determined by Western blotting.

RESULTS—In the corpora of control rats ageing was accompanied by a significant decrease in Cx43 and P2X₁R, and increase in P2X₇R expression. There was decreased Cx43 and increased P2Y₄R expression in the ageing control rat bladder. There was a significant negative correlation between erectile capacity and P2X₁R expression levels, and a positive correlation between bladder spontaneous activity and P2Y₄R expression levels. There was already development of erectile dysfunction and bladder overactivity at 2 months after inducing diabetes, the earliest sample measured in the study. The development of these urogenital complications was accompanied by significant decreases in Cx43, P2Y₂R, P2X₄R and increase in P2X₁R expression in the corpora, and by a doubling in Cx43 and P2Y₂R, and significant increase in P2Y₄R expression in the bladder. Changes in Cx43 and P2R expression were largely prevented by insulin therapy.

CONCLUSION—Ageing and diabetes mellitus markedly altered the expression of the gap junction protein Cx43 and of particular P2R subtypes in the rat penile corpora and urinary bladder. These changes in Cx43 and P2R expression provide the molecular substrate for altered gap junction and purinergic signalling in these tissues, and thus probably contribute to the early development of erectile dysfunction and higher detrusor activity in ageing and in diabetic rats.

Keywords

connexin43; P2 receptors; ageing; diabetes mellitus; bladder dysfunction; erectile dysfunction

INTRODUCTION

Erectile and urinary bladder dysfunction are common health problems that develop with advancing age and in diabetes mellitus. In people with diabetes the incidence of these urogenital diseases is higher than in the non-diabetic population, and is characterized by an early onset and increased severity. The precise mechanisms whereby ageing and diabetes induce bladder and erectile dysfunction (ED) are still not completely understood. However, it is well known that these urogenital diseases are complex and multifactorial, involving changes in the neural, hormonal, myogenic and urothelial/endothelial components of the bladder and penis. In the present study we investigated whether altered expression of the gap junction protein connexin43 (Cx43) and of specific purinoceptor (P2R) subtypes in the corpus cavernosum and urinary bladder could provide molecular substrates for the development of ED and bladder dysfunction in diabetes and ageing.

Gap junction-mediated intercellular communication is essential for normal erectile function. In the corpora, gap junction channels are formed mainly by Cx43 [1,2] and provide a direct pathway for controlled cytosolic exchange of current-carrying ions and receptor-generated intracellular second messengers (i.e. inositol triphosphate, Ca^{2+}), which ensure the rapid and syncytial corporal smooth responses that are an absolute requirement to achieve proper penile erection and detumescence [3]. The involvement of gap junction channels in mechanisms contributing to diabetic and age-related ED is suggested by recent observations that the permeability of Cx43 channels is increased in cultures of diabetic rat corporal smooth muscle cells [4], and that the expression level of Cx43 mRNA in human corporal tissue is reduced with advancing age [5]. In the urinary bladder the functional importance of Cx43-mediated intercellular signalling is often overlooked. However, there is substantial evidence that increases in Cx43 expression provide a myogenic substrate for detrusor overactivity [6–10], which is one of the most common urodynamic abnormalities in diabetic patients [11].

ATP as a neurotransmitter and extracellular signalling molecule exerts its action through activation of both ionotropic P2X receptors, which are ligand-gated ion channels, and metabotropic P2Y receptors, which are G protein-coupled receptors, that induce Ca^{2+} mobilization from the extracellular and intracellular pools, respectively. Several P2X and P2Y receptor subtypes are expressed in the penis and urinary bladder [12,13]. However, little is still known of the roles played by ATP and of each of its P2R subtypes in the process of penile erection and detumescence. ATP has been proposed to play important modulatory roles that would depend on the basal tone of the corporal smooth muscle, inducing relaxation at high basal tension and contraction at low tension [14]. The relaxation response is believed to be mediated by direct activation of ATP on P2Ys in the cavernosal muscle and indirectly by inducing nitric oxide (NO) release from endothelial cells [15,16]. The contractile action of ATP would involve activation of P2XR, probably P2X₁R and/or P2X₂R in the cavernosal muscle [17]. Although there is still no direct evidence that altered P2R expression could contribute to mechanisms involved in diabetes and age-related ED, their participation is supported by findings that ATP-mediated relaxation is altered in corporal tissue isolated from diabetic men and rats [18], and that ageing alters P2R mRNA expression in vascular smooth muscle [19]. In the urinary bladder ATP participates in both afferent and efferent mechanisms that control bladder function. ATP co-released with acetylcholine from parasympathetic nerves induces contraction by activating detrusor smooth muscle P2XRs, mainly P2X₁Rs, while ATP released from urothelial cells and activation of P2X₃R in afferent pathways communicate bladder fullness and contribute to the initiation of the micturition reflex [12,13]. In healthy human bladders the relative contribution of the purinergic component to parasympathetic neuromuscular transmission is minor. Interestingly, there is a significant positive correlation between age and the contribution of

the purinergic component in neurogenic contractions of human bladder detrusor muscle, as well as increased ATP-mediated detrusor excitation in pathological conditions such as interstitial cystitis, idiopathic detrusor instability and outlet obstruction [12,13]. The mechanisms underlying this enhanced ATP signalling in ageing and diseased bladders are still not completely understood, and have been proposed to involve augmented ATP release, reduced ecto-ATPase activity and/or altered P2R expression.

In the present study we used the Fischer-344 rat model of streptozotocin (STZ)-induced diabetes, which recapitulates important aspects of urogenital diseases in diabetic people. We analysed a combination of *in vivo* measurements of penile and urinary bladder function, with biochemical analysis of isolated corporal and bladder tissue from age-matched control and STZ-treated rats at 2, 4 and 8 months after inducing diabetes. Findings from this longitudinal study showed that the most significant effects of diabetes on Cx43 and P2R expression occur early, at 2 months after inducing diabetes, and that advancing age markedly decreases Cx43 expression and alters the expression profile of the P2R subtypes in the corpora and bladder. Thus, altered expression of Cx43 and of particular P2R subtypes contribute to the early onset of urogenital complications in diabetes and to development of these complications with advancing age.

MATERIALS AND METHODS

All animal handling and experimental protocols were approved by the Animal Institute and Committee of the Albert Einstein College of Medicine. Male F-344 rats (200–240 g; Taconic Farms, NY, USA) were used in this longitudinal study. One group of rats was made diabetic by a single i.p. injection with 35 mg/Kg STZ dissolved in citrate buffer (60 mL of 0.1 M citric acid and 40 mL of 0.2 M Na₂HPO₄, pH 4.6). The control group received an i.p. injection with vehicle alone. Blood glucose levels of STZ-treated rats were measured for 3 days after STZ administration and the induction of diabetes was confirmed in rats showing consistent blood glucose levels of >250 mg/dL. Half of the STZ-diabetic rats were then treated daily by an i.p. injection with humulin NPH insulin (insulin-treated group) and the other half remained untreated (diabetic group). Finally, the rats were separated into three groups and used at 2, 4 and 8 months after diabetes induction to evaluate the effects of ageing, diabetes progression and insulin-replacement therapy (IRT) on erectile and bladder function, and on the expression of the proteins of interest in the corpora and bladder tissue.

The body weight, blood glucose level and variables for both erectile and bladder function were measured from each rat in the diabetic, IRT and age-matched control groups following the protocol and surgical techniques previously described by us [20]. Erectile function was evaluated by determining the ratio between the intracavernous pressure (ICP) generated in response to cavernous nerve stimulation (20 Hz, pulse width 0.22 ms, 1 min duration, 6 mA) and blood pressure (ICP/BP); bladder activity and contractility were evaluated by measuring *in vivo* the spontaneous bladder pressure oscillations between micturitions (spontaneous bladder activity, SA), calculated by subtracting the basal bladder pressure from the intermicturition pressure. At completion of the functional evaluation the rats were killed humanely in a CO₂ asphyxiation chamber, and the urinary bladders and corpora cavernosa were rapidly harvested and stored at –80 °C.

Frozen bladder and corporal tissue samples were thoroughly minced using a mortar and pestle frozen in liquid N₂, and then sonicated in cell lysis buffer (1 mM NaHCO₃, 2 mM phenylmethylsulphonyl fluoride, 1 mM Na₃VO₄, 5 mM EDTA, and 1 × complete protease inhibitor cocktail, Roche Laboratories, Basle, Switzerland). Total protein content in each sample was measured using the BCA protein assay kits (Pierce, Rockford, IL, USA).

Protein extracted from bladder and corporal tissue was electrophoresed on 10% SDS-PAGE and transferred onto nitrocellulose membranes (Whatman, Dassel, Germany). After 30 min incubation with blocking buffer containing 0.5% Tween-20 (TBS-T) and 5% nonfat dry milk, at room temperature, the membranes were incubated overnight at 4 °C with polyclonal anti-Cx43 (kindly provided by Dr Elliot Hertzberg, Albert Einstein College of Medicine) or anti-P2R (P2Y₁R, P2Y₂R, P2Y₄R, P2X₁R, P2X₃R, P2X₄R or P2X₇R, 1 : 1000, Alomone, Jerusalem, Israel) or monoclonal anti-glyceraldehyde phosphate dehydrogenase (GAPDH) antibody (1 : 20 000; Research Diagnostics, Concord, MA, USA). After several washes in TBS-T the membranes were then incubated with horseradish peroxidase-conjugated antirabbit or antimouse IgG (1 : 10 000, Santa Cruz Biochem, Santa Cruz, CA, USA) for 1 h at room temperature. Bands were detected on X-ray film after incubation of the membranes with the enhanced chemiluminescence reagents (Amersham, Piscataway, NJ, USA).

Data were analysed statistically, with all data presented as the mean (SEM) and by a one-way ANOVA, followed by Tukey's multiple comparison test for statistical comparisons, with all differences considered significant at $P < 0.05$.

RESULTS

The mean values of body weight, blood glucose level and variables describing erectile and bladder function that characterize the rats used in this longitudinal study are shown in Table 1. Consistent with previous reports, 2 months after inducing diabetes by STZ treatment the diabetic rats had a significantly lower mean body weight and higher mean glucose levels than the age-matched controls [20]. With ageing there was a significant increase in body weight in control rats, while in diabetic rats, regardless of age, the body weight was maintained at similar low values, of 200–210g (Table 1). IRT prevented this chronic diabetes-associated weight loss and also reinstated the glycaemia to levels that were not significantly different from those in age-matched control rats (Table 1). In addition, there were significant differences in the body weight of IRT-treated STZ-rats at the 2 and 8 month sample times, indicating that IRT also restored the age-related increase in body weight seen in healthy rats (Table 1). Bladder function, as determined from *in vivo* cystometry, was also altered with ageing. At the 8-month sample, control rats had significantly higher bladder capacity, micturition volumes and spontaneous bladder activity (SA) than younger rats in the 2- and 4-month groups (Table 1). There was a significant positive correlation between ageing and increase in bladder activity, as evidenced by the higher SA of bladders in the 4- and 8-month compared with the 2-month control rat group ($P < 0.001$; $r = 0.36$). Bladder dysfunction, as evidenced by an increase in SA, developed early in diabetic rats, and the values for these cystometric variables were already significantly higher at 2 months after STZ treatment (Table 1). There was also an early onset of ED in STZ-diabetic rats; at 2 months after STZ treatment, the development of ED was shown by the significantly lower mean ICP/BP values than in age-matched control rats, which persisted with ageing and progression of the diabetic state. Typically, penile rigidity sufficient for coitus is achieved at ICP/BP ratio values of >0.6 . As shown in Table 1, diabetic rats had a mean ICP/BP value that was characteristically below the threshold of 0.6. The effects of diabetes on bladder and penile function were ameliorated by IRT (Table 1), and both bladder SA and erectile capacity were normalized in the STZ-rats on IRT. However, bladder capacity and micturition volume remained elevated in the IRT group, as the long-acting insulin did not prevent glucosuria over the entire 24 h after therapy.

Ageing markedly altered the expression of Cx43 and of particular P2R subtypes in rat corporal and bladder tissue. In the corpora, there was a significant negative correlation between age and Cx43 expression ($P < 0.05$; $r = -0.74$). As shown in Fig. 1, the expression levels of Cx43 in the rat corpora decreased progressively with ageing, reaching a significant

60% reduction in the corpora of rats in the 8-month compared to those in the 2-month group (Fig. 1A). In the bladder, Cx43 expression was also lower in older rats. Interestingly, in contrast to what was observed for the corpora, such age-related decrease in Cx43 expression was not progressive (Fig. 1B). There was half the Cx43 expression in the bladder from the younger 2-month than in the 4-month group and it was maintained at these lower levels in the older 8-month group (Fig. 1B).

Regarding the expression of P2Rs, various subtypes have been shown to be present in the penis and urinary bladder [12,13]. In this study we focused on the metabotropic P2Y₁R, P2Y₂R, P2Y₄R and ionotropic P2X₁R, P2X₃R, P2X₄R and P2X₇R subtypes. Except for the P2Y₁R subtype, all other P2R subtypes were detected by Western blotting at variable expression levels in both corpora and bladder. Figure 2 compares the expression levels of the different P2R subtypes detected in the corpora (Fig. 2A) and bladder (Fig. 2B) of healthy rats in the 2-, 4- and 8-month groups. In the rat corpora, P2Y₄R was the most abundantly expressed P2R subtype, followed by P2Y₂R, P2X₁R, P2X₄R and P2X₇R. There was a clear tendency towards a decrease in expression levels of P2Y₄R, P2X₁R and P2X₃R in the ageing corpora. However, the reduction in protein levels was only statistically significant for P2X₁R, and there was a significant negative correlation between age and P2X₁R expression in the rat corpora ($P < 0.01$; $r = -0.86$). In relation to the other ionotropic P2X₄R and P2X₇R subtypes, there was a different age-related expression pattern, characterized by increased expression from younger to more mature rats, followed by a decrease with further ageing. Also notable was the change in P2R expression profile in the ageing corpora (Fig. 2A), with a clear shift in the relative expression and possible contribution of the P2X₁R and P2X₇R to penile function in older rats.

In the bladder, the P2X₁R was the most abundantly expressed P2R subtype, consistent with previous reports of the importance of this receptor for normal bladder function [12,13]. Interestingly, in contrast to what was seen in the corpora, the mean expression level of P2X₁R was largely maintained in the ageing bladder. The behaviour was similar in relation to the P2Y₂R and P2X₄R subtypes. The P2X₃R was detected at a very low level only in the bladder of rats in the 2-month group. In this aspect, the pattern of P2X₃R expression in the bladder could be compared to that in the corpora, where the expression of this receptor decreases with age. Also in a similar manner to that in the ageing corpora, the expression of P2X₇R increased from the younger to more mature rats, and then decreased with further ageing. However, perhaps the most interesting age-related change in P2R expression in the bladder was that of the P2Y₄R. While in the corpora the P2Y₄R is the most abundant P2R subtype expressed at all ages investigated in this study (Fig. 2A), in the bladder the expression levels of this receptor significantly correlated with rat age ($P < 0.01$; $r = 0.78$), increasing in the bladders from the 2-month to 8-month rats, ultimately reaching expression levels that were comparable with those of the predominant P2X₁R subtype.

The effects of diabetes progression and IRT on P2R and Cx43 protein expression in the rat corpora and bladder at 2, 4 and 8 months after STZ treatment are shown in Figs 3 and 4, respectively. The overall analysis of these longitudinal data indicated that the most significant diabetes-related changes in P2Rs and Cx43 expression occurred early, at the first sample measured, 2 months after inducing diabetes. At this time the expression profile of P2Rs in the STZ-rat corpora was significantly altered, showing increased P2X₁R ($P < 0.01$) and decreased P2Y₂R ($P < 0.05$), P2X₃R, P2X₄R ($P < 0.05$) and P2X₇R expression (Fig. 3A). Interestingly, in contrast to expression patterns in the diabetic corpora, the expression of P2Y₂R, P2Y₄R ($P < 0.05$) and P2X₄R was markedly increased in the bladders 2 months after STZ-treatment, an effect that was nonetheless prevented by IRT (Fig. 3D).

There were opposite effects of diabetes on the bladder and corpora in relation to Cx43 expression at 2 months after inducing diabetes. At this time point, Cx43 expression in diabetic corporal tissue was decreased by 80% ($P < 0.05$; Fig. 4A), while in the diabetic bladders it was almost double that in age-matched controls (Fig. 4B). There were significant negative and positive correlations between blood glucose levels of STZ-rats after diabetes induction and Cx43 expression levels in the corpora ($P < 0.01$; $r = -0.91$) and bladder ($P < 0.05$; $r = 0.67$), respectively. Another difference between the effects of diabetes on these tissues was in the level of Cx43 phosphorylation. In the 2-month STZ-diabetic bladders the non-phosphorylated (P0) and phosphorylated (P1 + P2) forms of Cx43 reached levels that were two and five times higher than those in age-matched control bladders, respectively (Fig. 4C). Interestingly, with the progression of the diabetic state, the expression of Cx43 in the diabetic bladders remained almost double that in age-matched controls and still with higher phosphorylation levels than those in control bladders (Fig. 4C). In the corpora, at 4 months after inducing diabetes, Cx43 expression and phosphorylation were slightly higher than those in control corpora, resembling the Cx43 expression pattern seen in the diabetic bladders. However, at 8 months after inducing diabetes Cx43 expression and phosphorylation levels in the STZ-diabetic rat corpora were no different from those in the 8-month control corpora (Fig. 4A,C).

DISCUSSION

In this longitudinal study we found that the development of bladder and erectile dysfunction in either ageing or diabetic rats was accompanied by significant changes in the expression levels of particular P2R subtypes and of the gap junction protein Cx43. Increased detrusor activity is one of the common urodynamic abnormalities that develop in ageing and in diabetic bladders. Evidence that enhanced purinergic signalling is probably a contributing factor to development of this age- and diabetes-related bladder dysfunction has been provided by results in human, rabbit and rat bladders showing that the purinergic component of the neurogenic detrusor contraction [21,22], as well as the sensitivity of the detrusor muscle to ATP stimulation [23–25], are significantly increased both in ageing and in diabetes. Recent studies investigating whether increased expression of P2Rs might provide the basis for this augmented purinergic signalling in ageing bladders have logically focused on the P2X₁R subtype, the main subtype mediating the contractile response of the detrusor muscle to ATP. However, contrary to expectations, the P2X₁R mRNA levels in ageing human bladders were shown to be either no different [26] or reduced [27] when compared with those in younger bladders. In the present study there was also no significant changes in P2X₁R expression in ageing rat bladders or in STZ-diabetic rat bladders. This latter finding is in agreement with the previous report that the higher atropine-resistant neurogenic-mediated contraction of diabetic detrusor muscle was also P2X₁R antagonist-resistant, thereby excluding the contribution of P2X₁Rs in this phenomenon [28]. Extending our investigation to other P2R subtypes, we found that ageing was accompanied by a significant increase in expression levels of the P2Y₄R subtype, and that changes in bladder activity significantly correlated with changes in P2Y₄R expression levels. These findings suggest that enhanced P2Y₄R signalling in ageing bladders probably contributes to heighten the detrusor response to purinergic stimulation. Support for this proposed key participation of P2Y₄Rs in the modulation of detrusor activity is further provided by our findings showing that the early development of bladder overactivity in STZ-diabetic rats was also accompanied by significantly higher levels of P2Y₄R expression, and that both the increase in P2Y₄R and bladder activity was prevented by IRT despite an increase in micturition volume in those rats. However, interestingly, with progression of the diabetic state the higher expression levels of not only P2Y₄R but also of the P2Y₂R and P2X₄R returned to levels that were not significantly different from those in age-matched control bladders. Although puzzling, this observation is consistent with recent findings by Daneshgari *et al.*

[24] that the response of STZ-diabetic rat bladder strips to ATP increased progressively to peak 9 weeks after inducing diabetes and then decayed to reach values that were similar to controls. The mechanisms involved in this time-dependent modulation of P2R expression in the bladder during the progression of the diabetic state are still unknown. However, it is clear that changes in expression of these receptors, especially the P2Y₄R subtype, would probably contribute to increase the sensitivity of the diabetic detrusor to ATP and thus provide molecular substrates for the development of bladder overactivity in early stages of diabetes. In addition to enhanced purinergic signalling, our findings of higher Cx43 expression in diabetic bladders suggest that intercellular coupling and gap junction-mediated signalling in the detrusor muscle are also enhanced in diabetic bladders. Interestingly, we also found that Cx43 phosphorylation was markedly higher in diabetic than in control bladders. Post-translational phosphorylation of connexins has been associated with changes in gap-junction formation and channel properties. In the case of Cx43, the translated 42 kDa protein can be converted to two main phosphorylated forms, the Cx43-P1 (~44 kDa) and then to Cx43-P2 (~46 kDa). In gap-junction plaques Cx43 is primarily phosphorylated to the P2 form and Cx43-P2 occurs only in communication-competent cells [29]. This finding, taken together with our observations of higher quantity of both Cx43-P1 and P2 in diabetic bladders (Fig. 4C), further suggests that gap junctional communication is increased in diabetic bladders. Moreover, higher detrusor activity in neurogenic overactive bladders [8], urgency [9] and in animal models of bladder obstruction [6,7] has already been associated with increased Cx43 expression and gap-junctional coupling. Therefore, it is likely that Cx43 also provides a molecular substrate for development of detrusor overactivity in diabetic bladders.

ED, defined as the inability to develop and maintain an erection for satisfactory sexual intercourse, is another common urogenital complication that develops with ageing and in diabetes [30]. As shown in this study, ED developed as early as 2 months after inducing diabetes and was accompanied by a significant increase in P2X₁R and decrease in P2Y₂R, P2X₄R and P2X₇R expression in the STZ-diabetic rat corpora. Similar to what we observed in diabetic bladders, with progression of the diabetic state the expression of these receptors returned to levels that were no different from those in age-matched control corpora, suggesting that similar time-dependent compensatory mechanisms might operate in both bladder and penile corporal tissue. Little is known of the roles played by each P2R subtype in erectile function. It is currently believed that P2YR subtypes participate in penile erection by directly evoking relaxation of the cavernous muscle and indirectly inducing NO release from endothelial cells [15,16], and that P2XR subtypes, probably the P2X₁R and/or the P2X₂R, contribute to penile detumescence by directly evoking contraction of the cavernous muscle [17]. Previous studies have shown that at early stages of diabetes ATP-induced relaxation of phenylephrine-pre-contracted diabetic corpora is significantly lower than that in control rat corpora [18]. Down-regulation of P2Y₂R in diabetic corpora probably account for this decrease in ATP-induced relaxation of the diabetic corpora, and consequently contributes to impairment of the erectile process. In healthy control corpora, the levels of P2X₇R expression were significantly higher in mature adult rats than in younger and older rats. A similar pattern of expression was also followed by the P2X₄R subtype. In the early stages of diabetes the development of ED was accompanied by down-regulation of these receptors and, most importantly, both ED and decrease in P2X₇R expression were prevented by IRT. Combined, these findings indicate that these P2R subtypes not only are likely to play key roles in mature penile function but also contribute substrates to the development of ED in diabetes.

We previously showed in Sprague-Dawley rats that ageing is accompanied by a significant decrease in erectile capacity, as evidenced by the ICP/BP ratios of >0.6 in 4-month-old vs <0.4 in 9-month-old rats [31]. In the present study, using Fischer-344 rats within the same

age range, there were no significant age-related differences in erectile capacity. Interestingly, there was a significant negative correlation between ICP/BP values and P2X₁R expression levels in the corporal tissue of these rats ($P < 0.05$; $r = -0.78$). This finding, taken together with the observation that early diabetes-induced reduction in the erectile capacity of STZ-rats was accompanied by a significant doubling in corporal P2X₁R expression (Fig. 3A), suggest that this P2R subtype plays a key role in the early development of ED in diabetes. Interestingly, at this same stage diabetes significantly reduced Cx43 expression levels in the corpora of young STZ-rats, which reached mean values similar to those in the corpora of rats in the older 8-month control group (compare Figs 1A,3A). We have shown that Cx43 expression levels in the rat (Fig. 1A) and in human corporal tissue [5] significantly decrease with ageing. In the corpora of the penis, gap junction channels formed mainly by Cx43 provide the anatomical substrate for the rapid and synchronised response of myocytes to autonomic stimulation that is required for proper erectile function. It is thus likely that premature reduction of Cx43 expression and consequent decrease in coupling and coordination within the corporal smooth muscle also contribute to the early development of ED in STZ-diabetic rats. That IRT prevented this 'ageing' effect of diabetes on Cx43 expression (Fig. 4A), and that the reduction of erectile capacity of insulin-treated STZ-rats, which was no different from that of age-matched control rats, further supports a role for gap junctions in the early development of ED in diabetes.

The aetiology of urogenital complications in ageing and in diabetes is well known to be multifactorial. In the present study we provided evidence that changes in Cx43 and P2R expression would contribute to alter gap junction and purinergic signalling in these tissues, and thus provide molecular substrates for the development of ED and higher detrusor activity in ageing and diabetes.

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Abbreviations

SA	spontaneous activity
ICP/BP	intracavernous pressure/blood pressure
STZ	streptozotocin
IRT	insulin-replacement therapy
ED	erectile dysfunction
Cx43	connexin43
P2R	P2 purinoceptor
NO	nitric oxide
TBS-T	blocking buffer + Tween-20
GAPDH	glyceraldehyde phosphate dehydrogenase.

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FIG. 1.

The effect of ageing on Cx43 expression in healthy rat penile corporal and urinary bladder tissues. Expression levels of Cx43 in the corporal (**A**) and bladder (**B**) tissue of 2-, 4- and 8-month control rat groups were determined by Western blot and normalized by GAPDH. Note the significant decrease in Cx43 expression as the corpora ages and the earlier halving in Cx43 expression in bladders from younger (2-month group) to more mature rats (4-month group), which persisted with further ageing (8-month group). Values are the mean ($_{SEM}$) of tissues from at least three rats in each group. * $P < 0.05$ vs the 2-month group.

FIG. 2.

The effect ageing on P2 receptor expression in healthy rat penile corporal and urinary bladder tissues. Expression levels of metabotropic P2Y₂R and P2Y₄R, and ionotropic P2X₁R, P2X₃R, P2X₄R and P2X₇R subtypes in the corporal (**A**) and bladder (**B**) tissue of 2-, 4- and 8-month control rat groups were determined by Western blot and normalized by GAPDH. Note the significant change in P2R expression profile in the corpora, characterized by a decrease in P2X₁R as the corpora ages and significant increase in P2X₇R in younger 4-month adult rats that is followed by a significant decrease in older rats. Ageing did not significantly alter P2R expression profile in the bladder but expression of P2Y₄Rs markedly increased in ageing bladders. Values are the mean (SEM) of tissues from at least 3 rats in each group. Significant differences between the 2-, 4- and 8-month groups for each P2R subtype were determined by one-way ANOVA followed by Tukey's multiple comparison test.

FIG. 4.

The effect of diabetes on Cx43 expression in rat penile corporal and urinary bladder tissues. Expression levels of Cx43 in 2-, 4- and 8-month STZ-diabetic, IRT-treated and age-matched control corporal (**A**) and bladder (**B**) tissue were determined by (**C**) Western blot and normalized by GAPDH. Note the significant decrease in Cx43 expression as the corpora ages and the 'ageing effect' of diabetes in the corpora of 2-month STZ-rats. In diabetic bladders Cx43 was markedly up-regulated and in older diabetic bladders remained almost double that in age-matched controls. Note also the changes in Cx43 phosphorylation, with a marked increase in the slower migrating P1 and P2 phosphorylated forms of Cx43 in diabetic bladders. Values are the mean (SEM) of tissues from at least three rats in each group. * $P < 0.05$ vs age-matched control; @ $P < 0.05$ vs 2-month control corpora. Significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test.

TABLE 1

The general characteristics of the rats and the values of some variables derived from cystometric and cavernosometric studies that characterize bladder and erectile function as a function of time after STZ treatment

Time and group (n rats) [*]	Mean (SEM) variable				
	Body weight, g	Blood glucose, mg/dL	Micturition, mL	Bladder capacity, mL	ICP/BP SA, cmH ₂ O
2 months					
Control (17–23)	326.5 (7.1) ^J	104 (6)	1.0 (0.1) ^J	1.1 (0.1) ^J	5.2 (0.7) ^J 0.65 (0.04)
Diabetic (65–58)	202.1 (3.7) ^A	410 (9) ^A	2.9 (0.1) ^A	3.0 (0.1) ^A	10.1 (0.8) ^C 0.54 (0.03) ^D
IRT (23–15)	257.6 (6.9) ^E	125 (15)	2.1 (0.2) ^E	2.2 (0.2) ^E	6.4 (0.9) 0.63 (0.05)
4 months					
Control (31–13)	374.5 (3.4) ^J	97 (5)	1.2 (0.1)	1.3 (0.1)	6.6 (0.6) 0.74 (0.02)
Diabetic (45–35)	202.7 (5.1) ^A	425 (12) ^A	2.9 (0.1) ^G	2.9 (0.1) ^A	11.0 (1.1) ^C 0.56 (0.03) ^B
IRT (15–12)	278.4 (11.1) ^E	99 (9)	2.4 (0.3) ^G	2.5 (0.3) ^F	5.7 (1.3) ^I 0.72 (0.04)
8 months					
Control (28–13)	401 (6.2)	115 (8)	1.4 (0.1)	1.4 (0.1)	7.6 (0.7) 0.75 (0.05)
Diabetic (31–20)	209.6 (6.7) ^A	423 (9) ^A	3.1 (0.1) ^{GH}	3.2 (0.1) ^{GH}	12.2 (1.6) ^C 0.55 (0.05) ^D
IRT (25–11)	302.0 (11.7) ^{EK}	108 (18)	2.6 (0.2) ^G	2.7 (0.2) ^G	7.4 (0.9) 0.66 (0.04)

^{*} for cystometric-cavernosometric studies. P values from one-way ANOVA followed by Tukey's multiple comparison test:

^A <0.001;

^B <0.01;

^C <0.05 vs control and IRT;

^G <0.001;

^F <0.01,

^D <0.05 vs control;

^H <0.05 vs IRT;

^E <0.001 vs control and diabetic, and

$J < 0.001$,
 $J < 0.05$ vs 8-month control;
 $K < 0.05$ vs 2-month IRT.