EFFECTS OF ACETYLCHOLINE ON ISOLATED URINARY BLADDERS OF NORMAL AND STREPTOZOTOCIN-TREATED DIABETIC RATS

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ABSTRACT

This study was prompted by the inconsistent reports and apparent controversies that exist in the biomedical literature on the responses of diabetic bladder strips to cholinergic nerve stimulation and exogenous administration of muscarinic agonists, especially acetylcholine (ACh), *in vitro*. In the present study, acetylcholine-induced contractions of urinary bladders isolated from normoglycaemic (normal) and streptozotocin-treated, diabetic Wistar rats were examined under physiological conditions. Mechanical contractile changes of the isolated urinary bladders of STZ-treated, diabetic rats in response to bath-applied acetylcholine were compared with those obtained from isolated urinary bladders of normal, age-matched, control rats. Results obtained show that urinary bladders from diabetic rats consistently weighed more, and were always more spontaneously active after mounting, than those of the age-matched normal, control rats. Acetylcholine (ACh, 10^{-8}–10^{-4} M) provoked concentration-related, atropine-sensitive contractions of the isolated urinary bladders of both diabetic and age-matched normal, control rats. However, acetylcholine always induced more powerful and greater contractions of the diabetic bladders compared with bladders from the age-matched normal, control rats. The enhanced contractile responses of the diabetic bladder strips to bath-applied ACh were detected soon after induction of diabetes, and the magnitude and/or intensity of the enhanced contractile responses to ACh continued to increase as the diabetic state of the animals progressed. Although this preliminary study could not establish the exact mechanism of the increased contractile responsiveness of the diabetic bladders to the muscarinic agonist (ACh) used, our results tend to suggest that alterations in diabetic urinary bladder synaptosomal, vesicle-bound neurotransmitter (ACh) concentrations and the compensatory increase in the density of muscarinic M_3-receptor population in diabetic bladders are two of the most attractive plausible mechanisms of the increased diabetic bladder responsiveness to bath-applied acetylcholine.

Key Words:

Streptozotocin (STZ); Acetylcholine (ACh); Urinary Bladder Strips; Normal (Non-Diabetic) and Diabetic Rats.

1. INTRODUCTION

The mammalian urinary bladder is innervated and functionally regulated by the autonomic nervous system (ANS). Activation of the parasympathetic motor fibres to the bladder causes an intense stimulation of the muscarinic M_3-receptors in the bladder body, resulting in a strong and efficient bladder contraction, which in turn causes emptying of the bladder. Urinary bladder dysfunction is a common complication of chronic diabetes mellitus. Vesical dysfunction is a well-known manifestation of the peripheral autonomic neuropathy that accompanies diabetes mellitus [1]. It has been suggested that the initial bladder distension seen in diabetic patients is a consequence of impaired sensory transmission, and sensory neuropathy has been shown to correlate with bladder neuropathy [1,2]. Contractile responsiveness of isolated bladder muscles from

streptozotocin (STZ)-treated, diabetic rats to cholinergic nerve stimulation or administration of muscarinic agonist drugs has been studied by various investigators [1,3,4].

Many studies have attempted to clarify the pathogenesis of the urinary bladder dysfunction seen in diabetes mellitus [5]. It has been suggested that urinary bladder dysfunction in chronic diabetes might be due to an altered response of the organ to autonomic stimuli [1]. Segmental demyelination and axonal degeneration of the peripheral autonomic nerves have also been reported to cause dysfunction of lower urinary bladder tract [6]. 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 mammals, contractile responses of the urinary bladder to muscarinic agonists is inconsistent. Some investigators have reported increased responses [1,7,8,9], while others have reported decreased responses [10,11], or no change in contractile responses [4,12] of diabetic urinary bladder smooth muscle strip to muscaric agonists.

The present study was prompted by the existing controversies and inconsistent reports in the biomedical literature on the responses of diabetic urinary bladders to muscarinic agonists. The core aim of this study was, therefore, to investigate the responses of urinary bladders isolated from normal (normoglycaemic) and STZ-treated, diabetic Wistar rats to acetylcholine (ACh), a prototype muscarinic agonist.

2. MATERIALS AND METHODS

The experimental protocol and procedures used in this study were approved by the Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa; and conform with the "*Guide to the Care and Use of Animals in Research and Teaching"* [published by the Ethics Committee of the University of Durban-Westville, Durban 4000, South Africa].

Animals and induction of diabetes

Thirty (30) healthy, young adult, male Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light; and were allowed free access to food and water *ad libitum* one week before the commencement our experiments. The rats were divided into two groups of fifteen normal (non-diabetic) and fifteen STZ-treated, diabetic animals; and were kept in separate cages. Diabetes was induced in the diabetic group of rats by intraperitoneal injections of STZ (75 mg/kg). The STZ-treated rats were kept in their cages for 5–10 days under laboratory conditions, to allow diabetes to develop in the animals. Two to ten week-diabetic rats were used in this study.

Preparation of Bladder Tissues

STZ-treated rats with blood glucose concentrations ≥15 mmol/L were considered to be diabetic and used in this study. The normal (non-diabetic) and STZ-treated diabetic rats were sacrificed by decapitation, and the whole (entire) urinary bladder of each rat was removed, opened up longitudinally to form a semi-rectangular piece of tissue, tied with cotton thread at the upper and lower ends, and suspended in 30-ml 'Ugo Basile Two-Chambered Organ Baths' (model 4050) containing Krebs-Henseleit physiological solution (of composition, in g/litre: NaCl, 6.29; KCl, 0.34; NaH_2PO_4, 0.15; $NaHCO_3$, 2.1; $MgCl_2$, 0.11; $CaCl_2$, 0.26; and glucose, 1.00) maintained at 32±1ºC and continuously aerated with carbogen (i.e., 5% carbon-dioxide + 95% oxygen gas mixture) under an applied resting tension of 1 g. Each bladder muscle strip was allowed to equilibrate for 45–60 minutes, during which time the bathing physiological solution was changed every 15 minutes, before it was challenged with graded concentrations of ACh (and other drugs used).

Acetylcholine (ACh) Treatment

Each isolated urinary bladder muscle strip was challenged with graded concentrations of acetylcholine (ACh, 10^{-8}–10^{-4} M) in the absence, and in the presence, of atropine (ATR, 10^{-7}–10^{-5} M). The tissues were washed out 3–5 times after the maximal contractile response to each ACh concentration was obtained, and thereafter allowed to equilibrate for 5-10 minutes before sequential addition of the next higher concentration of acetylcholine. Isolated urinary bladder strips from diabetic and normal, age-matched control animals were always set-up in parallel under the same experimental conditions, in order to make allowance for adequate comparison of the tissues' contractile responses to acetylcholine. ACh-induced contractile responses of the tissues were recorded isometrically by means of 'Ugo Basile' force displacement transducers and pen-writing 'Gemini' recorders (model 7070).

Data Analysis

Data are presented as means (±SEM) of the contractile responses of the bladder tissues to the various ACh concentrations. Statistical evaluation of the data was done by means of 'Student t-test' for unpaired data. Values of $P \leq 0.05$ were taken to be statistically significant.

3. RESULTS

Changes in blood glucose concentrations, body and bladder weights

One to ten weeks after treatment with STZ, the fasting blood glucose concentrations of the STZ-treated, diabetic rats were significantly elevated ($P<0.05–0.001$), compared with those of the normal (non-diabetic), age-matched, control rats (Table 1). Furthermore, the body weights of the STZ-treated, diabetic rats decreased significantly ($P<0.05–0.01$) after six weeks of STZ treatment, compared with the normal (non-diabetic), age-matched, control rats. The bladders of the STZ-treated, diabetic rats were visibly more distended and larger than those of the normal, age-matched control rats at the time of dissection. Moreover, the wet weights of the bladders from diabetic rats were significantly greater ($P<0.05–0.01$) than those from normal (non-diabetic), age-matched control rats (Table 1).

Acetylcholine (ACh)-induced contractile responses of the isolated bladders

Acetylcholine (ACh, 10^{-8}–10^{-4} M) caused concentration-dependent contractions of bladders isolated from both non-diabetic and STZ-treated, diabetic rats. However, acetylcholine always induced stronger, more powerful and greater contractions of the diabetic bladders compared with bladders from the non-diabetic, age-matched control rats. Figure 1 illustrates typical traces obtained, while Figure 2 summarises the results obtained. The enhanced contractile responses of the bladder strips to bath-applied ACh were detected soon after induction of diabetes, and the magnitude or intensity of the enhanced contractile responses to ACh continued to increase steadily and linearly as the diabetic state of the animals progressed from one week to ten weeks. Atropine (ATR, 10^{-7}–10^{-5} M), a muscarinic antagonist, competitively inhibited the contractile responses of the isolated bladder preparations to bath-applied acetylcholine in a concentration-dependent manner. The mean EC_{50} values for ACh on the isolated whole bladders of diabetic and age-matched, non-diabetic control rats were found to be $0.01\pm0.08 \times 10^{-6}$ M (n=15) and $7.21\pm0.10 \times 10^{-5}$ M (n=15), respectively.

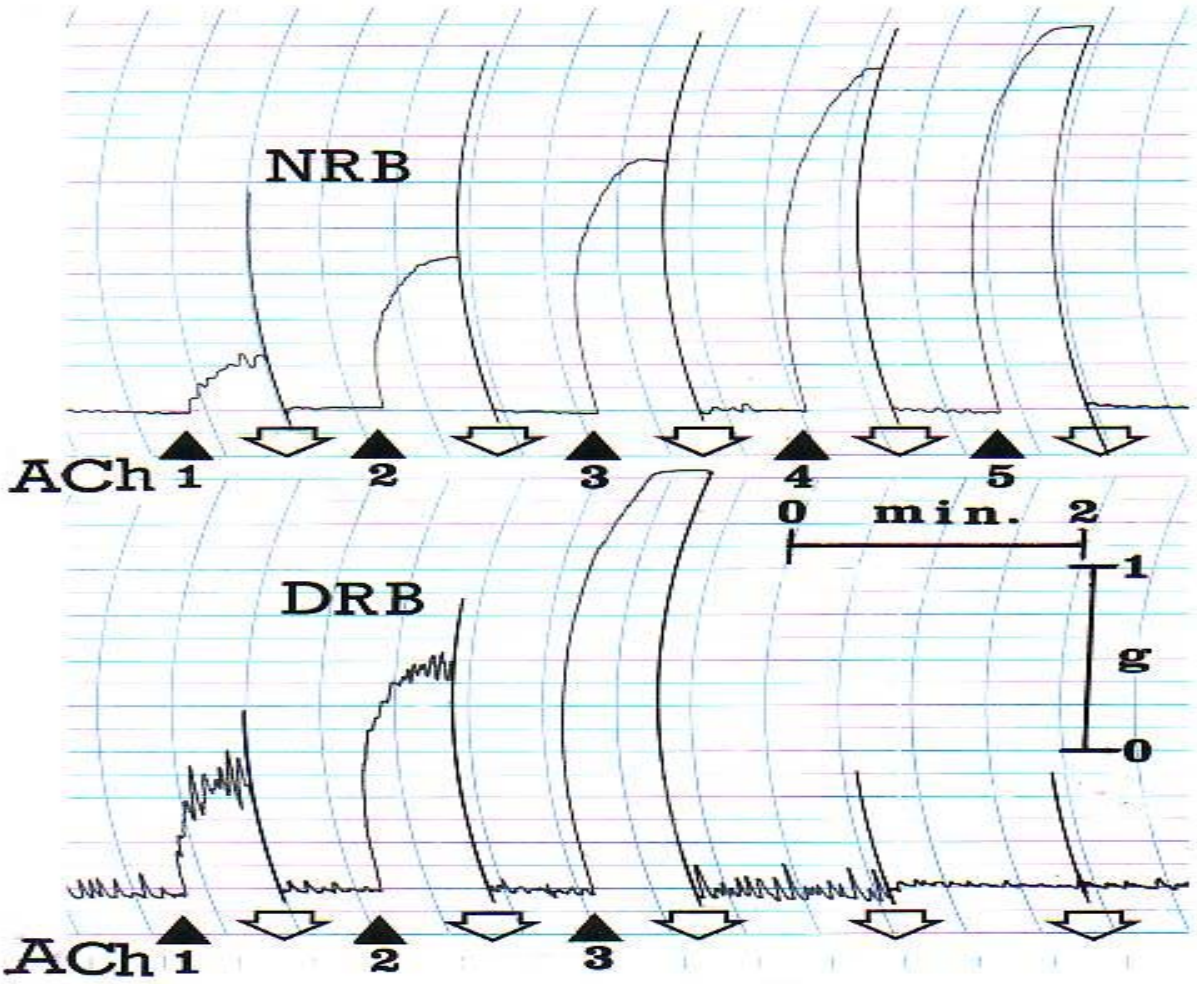

Figure 1.

Effects of graded concentrations of acetylcholine (ACh) on isolated urinary bladder muscle strips from a normal rat bladder (NRB, upper trace) and a diabetic rat bladder (DRB, lower trace) respectively. ACh ▲ 1, 2, 3, 4 and 5 denote acetylcholine, 5.0 x 10^{-8}, 5.0 x 10^{-7}, 5.0 x 10^{-6}, 5.0 x 10^{-5} and 5.0 x 10^{-4} M respectively, sequentially added to the bath-fluid at the solid dots (▲), and washed out 3–5 times at the adjacent, open right-hand-side, downward-pointing arrows.

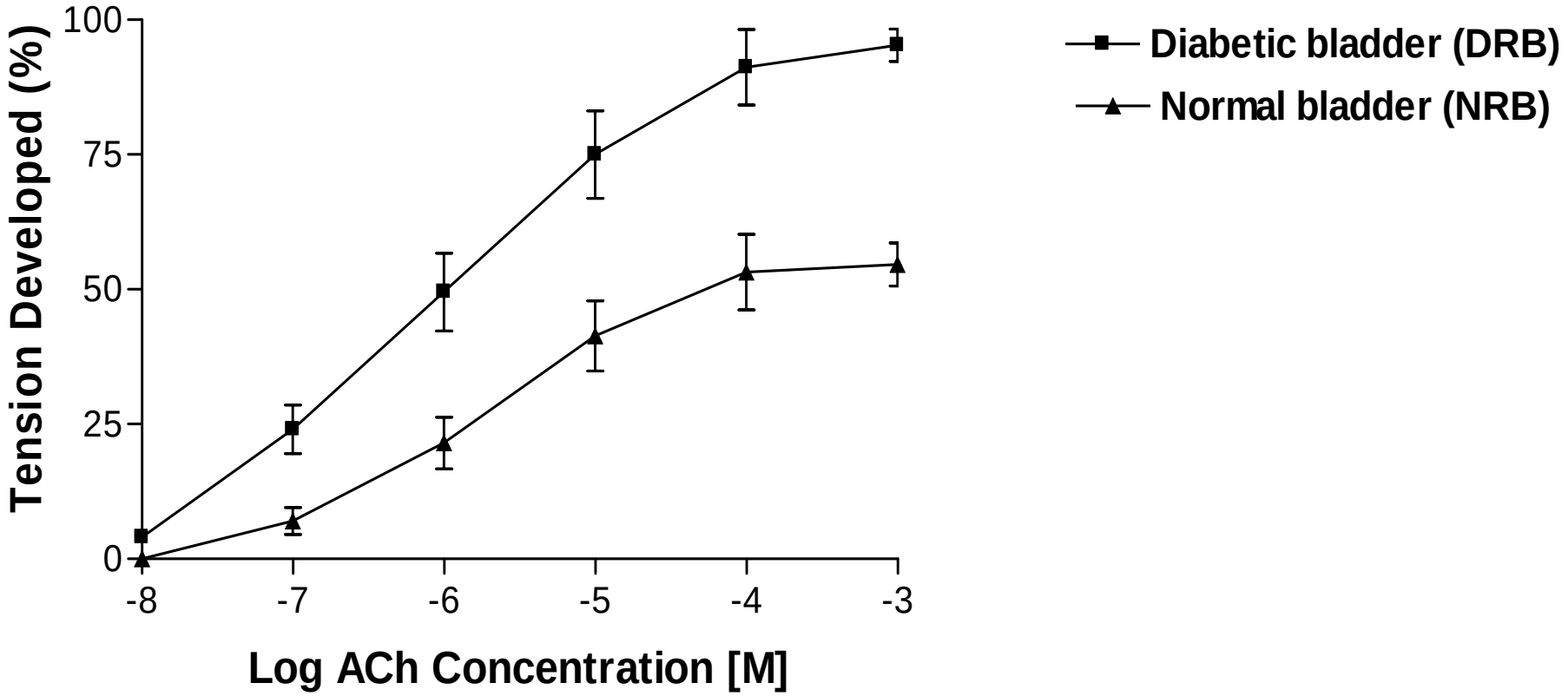

Figure 2.

Comparative effects of graded concentrations of acetylcholine (ACh) on tension developed by isolated urinary bladder tissues from normal (▲, NRB) and diabetic (■, DRB) rats. Each point represents the mean of 15 preparations, while the vertical bars denote standard errors of the means.

Table 1.

Fasting blood glucose (FBG) levels, body weights and wet weights of isolated bladders of normal (non-diabetic) and STZ-treated, diabetic rats.

Parameter	Control, normal rats (n = 15)	Diabetic rats (n =15)
Fasting blood glucose (FBG) levels (in mmol/L)	4.10 (3.8 – 4.6 mmol/L)	15.06 (8.2 – 21.1mmol/L)
Body weights (g)	310±15	203±12
Bladder wet weights (g)	0.63±0.11	0.98±0.14

4. DISCUSSION

The results of the present study show that acetylcnoline (ACh) induced stronger, more powerful and much greater contractions of isolated bladders from STZ-treated, diabetic rats compared with bladders isolated from normal (non-diabetic), age-matched, control rats.

It is now firmly established that the sympathetic and parasympathetic nervous systems of the autonomic nervous system (ANS) regulate the functions of the lower urinary bladder in different ways. During urine storage phase, the roles of the sympathetic nervous system are relaxation of the bladder body, and contraction of the bladder base through α- and β-adrenoceptors, respectively; whereas, the role of the parasympathetic nervous system is a contraction of the detrusor muscle through stimulation of the muscarinic M_3-receptors in the bladder body to expel urine [5,13]. Lincoln *et al*., [12] showed significant increases in the activities of choline acetyltransferase and choline esterase in the bladder after two to eight weeks of diabetes, suggesting that cholinergic nerve activity is increased in the urinary bladder during diabetes. The investigators [12] further observed that norepinephrine content in the bladder showed a tendency to decrease in diabetes.

Previous investigators have shown that in diabetic animals, the contractile response of the urinary bladder to muscarinic agonists is inconsistent. Some investigators have reported increased contractile responses [1,7,8], while others have reported either decreased responses [10], or no change in contractile responses [4,5,12] of the urinary bladder smooth muscle to muscaric agonists. Latifpour *et al*., [8] and Tong and Cheng [9] have also reported an increased number of muscarinic M_3-receptors associated with increased contractile responses to muscarinic agonists in the bladder dome of STZ-induced diabetic rats. On the contrary, Carrier and Aronstam [3] have reported an increased muscarinic responsiveness and a decreased density of muscarinic receptors in ileal smooth muscles from STZ-treated, diabetic rats. Kamata *et al*., [1] have also shown that detrusor strips of urinary bladders from STZ-treated diabetic rats exhibit an enhanced contractile response to acetylcholine, and associated the increased responses to ACh with an increased population of muscarinic receptors in the tissues. However, our results are in agreement with the findings of Kolta *et al*., [7]; Latifpour *et al*., [8]; and Kamata *et al*., [1], who have reported increased contractile responses of diabetic bladders to muscarinic agonists. The discrepancies in the findings of the various earlier investigators who have examined contractile responses of isolated urinary bladders from diabetic mammals to acetylcholine (or parasympathetic nerve stimulation) are unlikely to be due to differences in species, strains and ages of the experimental animals used because our unpublished, preliminary studies have shown that the enhanced, increased contractile responses of isolated bladders from all available diabetic species and strains of mice, rats, guinea-pigs and rabbits of different ages to bath-applied acetylcholine were not modified by the species, strains and ages of the experimental animals. However, the duration of hyperglycaemia, the segment of the bladder used, the breeders of the animals and the experimental conditions employed, might contribute significantly to the differences in the findings of the earlier investigators. Nilvebrant *et al*., [14] and Tong and Cheng [9] noted a "supersensitivity" to muscarinic agonists, and an increased density of muscarinic M_3-receptors. Nilvebrant *et al*., [14] also noted no change in affinity for ^{3}H-QNB with hypertrophied bladders following peripheral parasympathetic denervation of the urinary bladders. Latifpour *et al*., [8] have reported similar alterations in parasympathetic activities in urinary bladders of diabetic rats. Latifpour *et al*., [8] have also shown that STZ-induced diabetic state causes an increased maximum contractile response to muscarinic agonist, an increased density of muscarinic receptors, and no changes in the affinity of the bladder dome smooth muscle for ^{3}H-QNB. However, in their study, Kamata *et al*., [1] found that muscarinic receptors in the detrusor strips from STZ-treated diabetic rats had a lower affinity for ^{3}H-QNB than the detrusor strips from normal, control rats. The latter investigators [1] concluded that although it is unclear why the affinity for ^{3}H-QNB was lower in diabetic state, it is unlikely that the decreased affinity of muscarinic receptors to acetylcholine is related to the increased contractile response to acetylcholine (ACh).

The findings of the present study are in agreement with the works of Kolta *et al*., [15] and Tammela *et al*., [16] who observed enhanced and significantly greater responses of bladder strips from diabetic rats to acetylcholine and carbachol respectively, soon after induction of diabetes with STZ. The investigators [15, 16] further observed that the magnitude and/or intensity of the acetylcholine- and carbachol-induced contractile responses of the diabetic bladder strips continued to increase as the diabetic state of the

animals progressed. The increased diabetic bladder mass observed in the present study is also in consonance with the findings of Tammela *et al*., [16] who found a significantly increased diabetic bladder mass in their study, and concluded that effects of diabetes (and sucrose consumption) on contractile bladder function are related to diuresis-induced increases in bladder mass.

In conclusion, the findings of the present study have demonstrated that urinary bladders from STZ-treated diabetic rats exhibit an increased contractile responsiveness to bath-applied acetylcholine. Although this preliminary study could not establish the exact mechanism of the increased contractile responsiveness of the diabetic bladders to the muscarinic agonist (ACh) used, our results tend to suggest that the alterations in diabetic urinary bladder synaptosomal, vesicle-bound neurotransmitter (ACh) concentrations reported by Tong *et al*., [17], and the compensatory increase in the density of muscarinic M_3-receptor population reported by Latifpour *et al*., [8], would appear to be two of the most attractive, plausible mechanisms of the increased diabetic bladder responsiveness to acetylcholine.

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