Reactivity of Diabetic Urinary Bladder to the Cholinesterase Inhibitor Neostigmine

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OBJECTIVE To examine the effects of neostigmine, an acetylcholinesterase inhibitor that has been used to treat impaired bladder emptying on diabetic rat urinary bladder smooth muscle.

METHODS Rat urinary detrusor muscle strips were suspended in organ baths containing Krebs' solution for isometric tension recording. Streptozotocin-diabetic (12 weeks) bladder tissue activity compared with control was assessed by using electrical field stimulation (EFS) in the presence and absence of the cholinesterase inhibitor, neostigmine.

RESULTS EFS-induced contractions; a major part of it is cholinergic in origin. Neostigmine significantly enhanced EFS-induced contractions in diabetic strips than control at all frequencies. Neostigmine caused concentration-dependent contractions of control and diabetic bladder tissues, which were completely abolished by atropine. Carbachol-induced bladder contraction was significantly reduced in diabetes.

CONCLUSION In diabetes mellitus, cholinesterase modulation (increase) may play a role in the development of inadequate bladder contraction, seen in later stage diabetic bladder dysfunction. UROLOGY ■: ■—■, 2014. © 2014 Elsevier Inc.

Impaired bladder contraction is a common problem among diabetic patients. Excitatory cholinergic and purinergic systems are involved in the maintenance of bladder continence and micturition. Acetylcholine and adenosine triphosphate (ATP) were found to provide all excitatory input in the rat bladder.[1] It was found that electrical nerve stimulation caused co-release of acetylcholine and ATP from the same source in the rat bladder.[1] It is known that reflex activation of cholinergic nerve is responsible for bladder emptying.[2] Voiding of the bladder is the result of mainly muscarinic receptor activation.[3] Acetylcholinesterase (AChE) is an enzyme that specially cleaves acetylcholine to acetate and choline and terminates its actions. Inhibitors of AChE indirectly provide a cholinergic action by prolonging the lifetime of the acetylcholine produced. Neostigmine combines with the enzyme making it very slow to be hydrolyzed, taking minutes rather than microseconds. The anticholinesterase drug is hydrolyzed at a negligible rate compared with acetylcholine. Pharmacotherapy using muscarinic agonists and AChE inhibitors such as neostigmine has been used to treat impaired bladder emptying.[4,5] Neuropathies of the autonomic nervous system as a complication of diabetes mellitus have been well known.[6,7] The most common complication of diabetes is diabetic cystopathy and diabetic bladder dysfunction.[8-10]

The aim of the study was to examine the effects of AChE inhibitor, neostigmine, on diabetic rat urinary bladder smooth muscle.

METHODS

Animals

Forty-eight adult male Sprague-Dawley rats weighing approximately 200 g were housed individually on a 12-hour light-dark cycle (lights on from 6 AM to 6 PM). The ambient temperature was kept at 21°C, and the rats had free access to standard laboratory food and tap water.

Induction of Diabetes

Diabetes was induced in 24 rats by intravenous injection of streptozotocin (55 mg/kg body weight) dissolved in 0.05-M sodium citrate, pH 4.5; control rats received buffer alone by the same route. The rats were kept for 12 weeks, and induction of diabetes was ascertained by the determination of blood glucose concentrations.

Preparation of Bladder Strips

Rats were fasted for 6 hours before sacrificing by decapitation. Blood was collected into heparinized tubes, centrifuged at 3000× g for 15 minutes, and the plasma used for the measurement of glucose concentration at death. The urinary bladder was removed and placed in Krebs' solution of the following composition (mM): NaCl, 118; KCl, 5.9; $MgSO_4$, 1.2; $CaCl_2$, 2; KH_2PO_4, 1.2; $NaHCO_3$, 26; and glucose, 11.1 at pH 7.4. The bladder was cut longitudinally into equal strips 10 × 5 mm from control rats and diabetic rats, which were suspended in10-mL organ baths

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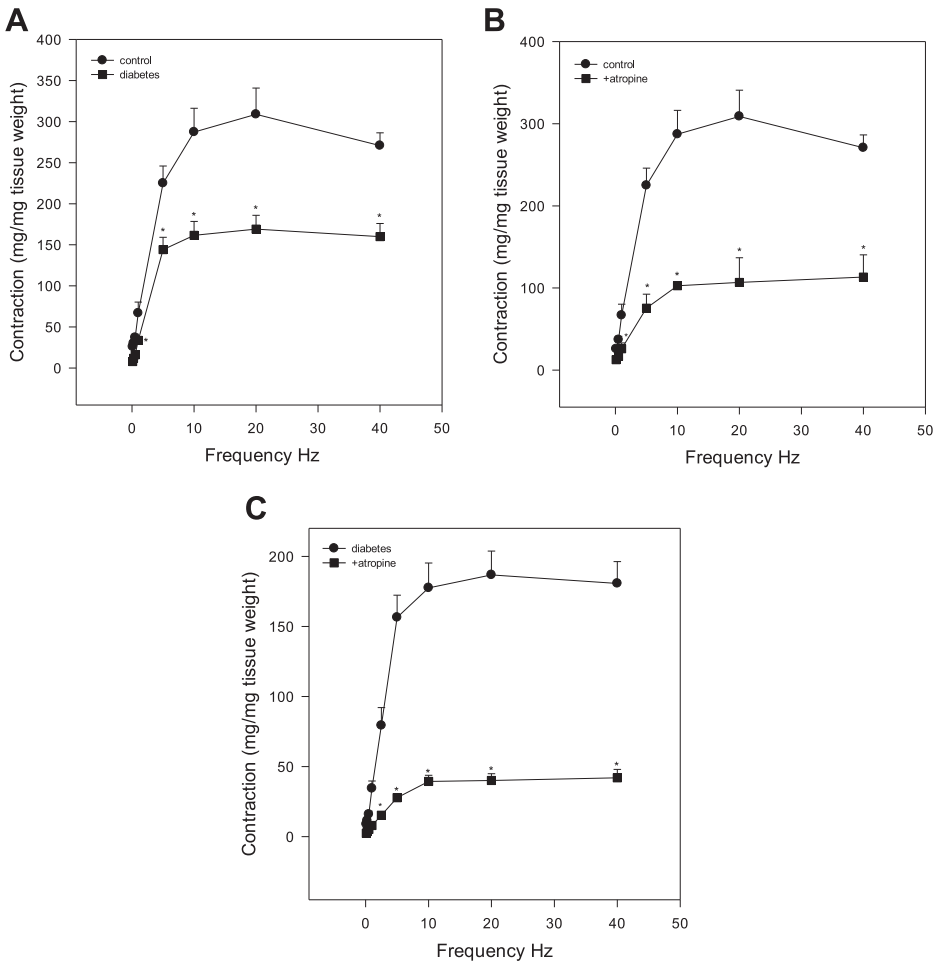

Figure 1. **(A)** Effect of diabetes on frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of bladder strips from control (●) and diabetic rats for 12 weeks (■). Results are means ± standard error of the mean (SEM) of 6 experiments for each. Significant difference between normal and diabetic groups (*P <.05). **(B)** Effect of atropine (1 μM) on frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of bladder strips from control (●) and after atropine (■). Results are means ± SEM of 4 experiments for each. Significant difference between the groups (*P <.05). **(C)** Effect of atropine (1 μM) on frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of bladder strips from diabetic rats for 12 weeks (●) and after atropine (■). Results are means ± SEM of 4 experiments for each. Significant difference between the groups (*P <.05).

containing Krebs' solution, maintained at 37°C, and gassed with 95% O_2 and 5% CO_2. Tension was continuously recorded using a computerized automated isometric transducer system (Schuler organ bath type 809; Hugo Sachs Elektronik) connected to a Gould recorder (Gould Inc). The strips were initially loaded to a tension of 1 g and allowed to equilibrate for 60 minutes during which time they were washed twice. At the end of each experiment, the muscle was dried with filter paper and weighed, and then responses were calculated as mg × mg^{-1} tissue weight. This method was used in our previous researches.[11-13]

Electrical Field Stimulation Studies

For electrical field stimulation (EFS), bladder strips were passed through a pair of platinum ring electrodes. The electrodes were connected to a Grass S8800 stimulator (Astro-Med), delivering square wave pulses. Optimum electrical stimulations parameters, previously determined (50 V, 0.5 ms for 10 s using frequency ranging from 0.1-40 Hz), were used in that study.

Frequency-response curves were elicited using the previous parameters every 3 minutes.

Drugs. Acetylcholine hydrochloride, neostigmine bromide, carbamylcholine chloride (carbachol), and atropine sulfate were obtained from Sigma Chemicals, St. Louis, MO. All drugs were dissolved in distilled water.

Calculation. Data are presented as mean ± standard error of the mean of (n) experiments. Where necessary, differences between 2 mean values were compared using the Student t test, paired or unpaired as appropriate. Where multiple comparisons were necessary, 1-way analysis of variance was used followed by the Student-Newman-Keuls test. The difference was assumed to be significant at P <.05.

RESULTS

In our study, diabetic bladders weighed more than the normal rat bladders. The average bladder weights of control and diabetic for 12 weeks were 0.1020 ± 0.009 and 0.2414 ± 0.03 g, respectively (n = 12; ***P >.001).

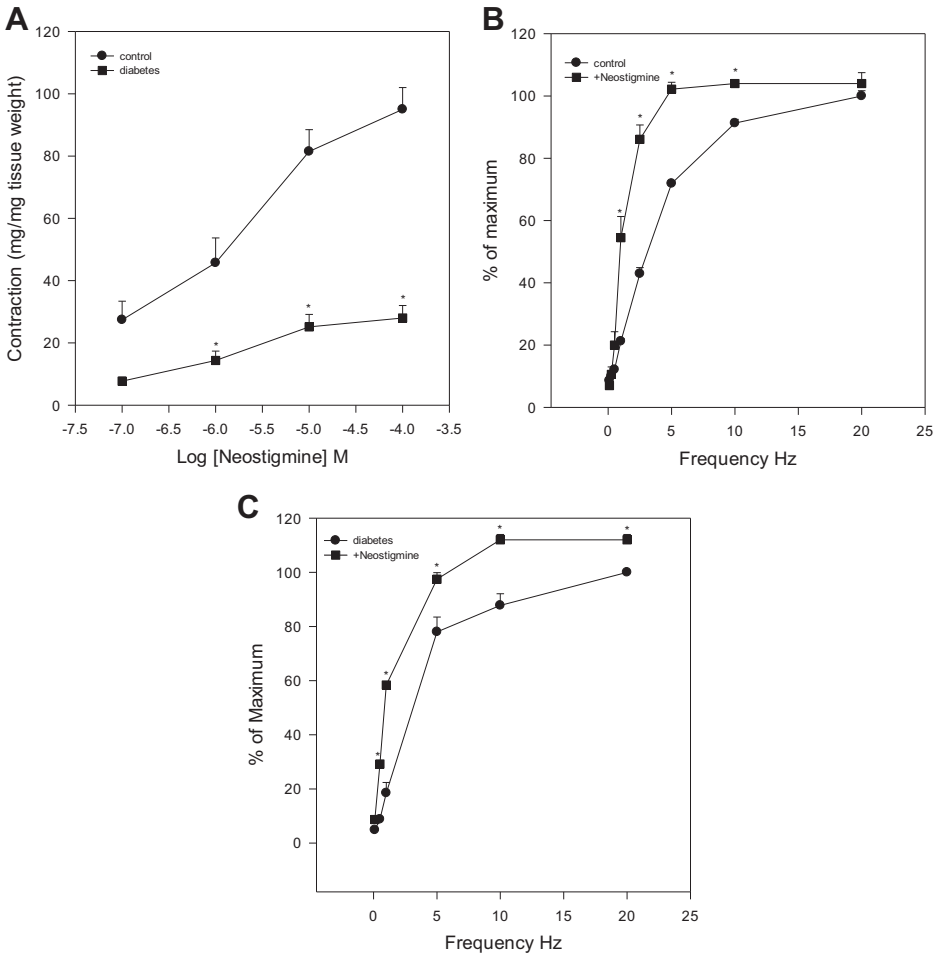

Figure 2. (A) Effect of diabetes on neostigmine concentration-contraction curves of bladder strips from control (•) and diabetic rats for 12 weeks (■). Results are means ± standard error of the mean (SEM) of 4 experiments for each. Significant difference between normal and diabetic groups (*P <.05). **(B)** Effect of neostigmine (10 μM) on frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of bladder strips from control (•) and after neostigmine (■). Results are means ± SEM of 4 experiments for each. Significant difference between the groups (*P <.05). **(C)** Effect of neostigmine (10 μM) on frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) of bladder strips from diabetic rats for 12 weeks (•) and after neostigmine (■). Results are means ± SEM of 4 experiments. Significant difference between the groups (*P <.05).

EFS-induced Contraction

Preparations Under Resting Conditions. EFS (0.1-40 Hz) of the control and diabetic bladder preparations elicited frequency-dependent contractions as shown in Figure 1A. The contractions were rapid at onset and stopped immediately when stimulation ceased. These contractions were abolished by tetrodotoxin (1 μM; n = 8; data not shown) confirming that they were neurogenically mediated. EFS-induced contractions were also inhibited by atropine (1 μM) in the 2 preparations, also confirming that the major part of excitatory innervation in rat bladder smooth muscle is cholinergic in origin as shown in Figure 1B,C.

EFS-induced contractions last 10 seconds, whereas in the presence of atropine, they last 4 seconds. The peak of contraction reached after 10 seconds, and the contraction only stopped when the stimulation was ceased. No relaxant responses to EFS were observed in the presence of atropine.

Effect of Neostigmine Under Resting Conditions. Neostigmine (0.1-100 μM), an AChE inhibitor caused concentration-dependent contractions of bladder strips from normal and diabetic rats. Neostigmine-induced bladder contraction was significantly reduced in diabetic strips (Fig. 2A). The increase of the basal tone by neostigmine was completely abolished by atropine, indicating that the effect is cholinergic.

Effect of Neostigmine EFS-induced Contraction. Neostigmine 10 μM produced leftward shifts of the EFS-induced contraction in urinary bladder strips from normal and diabetic rats. Pretreatment of the preparations with neostigmine (10 μM) markedly augmented the EFS-induced contractions as shown in Figure 2B,C.

Neostigmine significantly affected both urinary bladder strips from normal and diabetic rats; however, the enhancement of neostigmine on EFS in diabetic strips were higher than in control strips for all the frequencies. The percentage of frequency-induced contraction to EFS enhancement of bladder strips from control and diabetic rats due to neostigmine is shown in Figure 3. These results confirm that AChE is more effective in diabetic bladder than control bladder.

Carbachol-induced Contraction. Carbachol 10 nM-to-100 μM—induced concentration-dependent contractions of bladder strips are shown in Figure 4. Dose-response curves for carbachol were obtained in control and diabetic rats. Carbachol-induced bladder contraction was significantly reduced after the induction of diabetes.

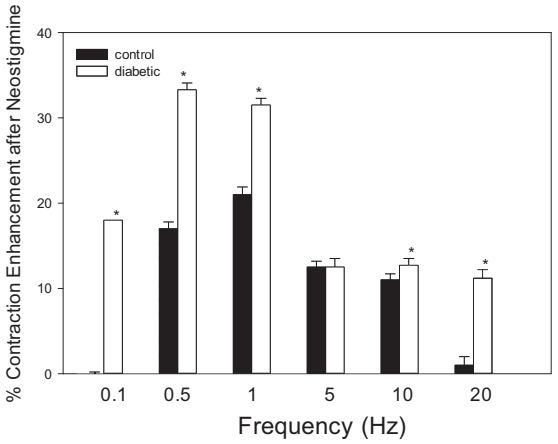

Figure 3. Percentage of frequency-induced contraction to electrical field stimulation (50 V, 0.5 ms, 10 s) enhancement of bladder strips from control and diabetic rats in the presence of neostigmine (10 μM). Results are mean ± standard error of the mean of 4 experiments (*P <.05).

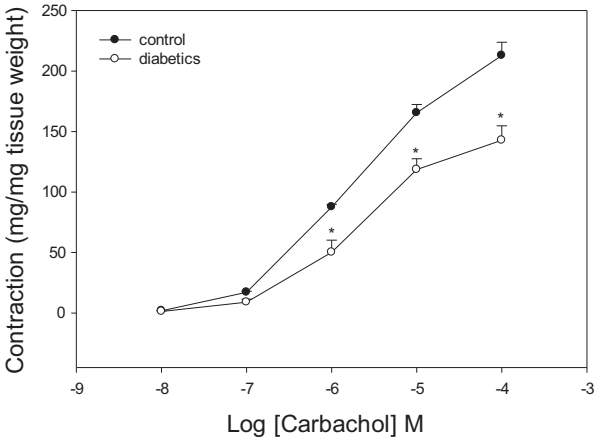

Figure 4. Effect of diabetes on carbachol concentration-contraction curves of bladder strips from control (●) and diabetic rats for 12 weeks (■). Results are means ± standard error of the mean of 4 experiments. Significant difference between normal and diabetic groups (*P <.05).

COMMENT

Our results demonstrated that the major part of the endogenous agonist released by EFS is cholinergic in origin in the control and diabetic bladders. Previous studies proved that in rat bladder, EFS caused co-release of acetylcholine and ATP from the same source, and acetylcholine and ATP were provided all excitatory input.[1,2,14] In addition, our study showed that cholinergic component of the nerve-mediated detrusor contraction decreased in the diabetic rat bladder than control bladder. These results are supported by previous studies.[7,15,16] Lincoln et al[17] and Wahba et al[18] showed significant increases in the activities of AChE in the bladder after 2 weeks of diabetes. They suggested that cholinergic nerve activity was increased in the urinary bladder during diabetes by using histochemistry and biochemical assays.

Because muscarinic receptors are physiologically most important for the mechanism to elicit contraction of the urinary bladder, pharmacotherapy using cholinergic agonists and AChE inhibitor neostigmine is used to treat diabetes complications in bladder. Our study showed that the enhancement of neostigmine on EFS in diabetic strips was higher than in control strips indicating that AChE enzyme is more active in diabetic bladder than in control. This result also reveals that although neostigmine enhanced endogenous acetylcholine-induced contractions through inhibition of AChE enzyme, its effect on basal tone was clearly different. Neostigmine-induced contraction, which was completely abolished with atropine, indicates that neostigmine may act directly on the muscarinic receptors.[19-20] The direct effect of neostigmine was shown in different body tissues such as trachealis muscle,[21] sympathetic neurons,[22] Aplysia neurons,[23] and ileum.[24]

Carbachol also binds directly to muscarinic receptors, but it is not decomposed by AChE like the acetylcholine, and its effect is long-lasting.[25] Carbachol-induced contraction is more in the control than the diabetic bladder. This result proves that there is dysfunction in muscarinic receptors during diabetes.

The lower urinary tract complication of diabetes mellitus is diabetic cystopathy or diabetic bladder dysfunction. Neurogenic changes occur after the onset of diabetes.[16] Previous studies of the effect of diabetes on detrusor contractility showed decrease force production in the diabetic rat. Changes in muscarinic receptor population are also linked to altered contractility.[26-28]

Therefore, we can conclude that diabetes mellitus decreases the effect of muscarinic receptors and increased the presence and/or activity of cholinesterase in streptozotocin-diabetic bladder tissue compared with control. In diabetes mellitus, cholinesterase modulation (increase) may play a role in the development of inadequate bladder contraction seen in chronic diabetic bladder dysfunction. Both effects have great impact on the cholinergic system in diabetic bladder. These results

clarify the need for new drugs that have dual effects to stimulate muscarinic receptors and at the same time inhibit the AChE enzyme.

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