Effect of Diabetes on the Ion Pumps of the Bladder

Seham Mustafa

OBJECTIVE To establish whether the activities of Na^+/K^+-adenosine triphosphatase (ATPase) and Ca^{2+}-ATPases ion pumps in bladder smooth muscle are altered as a consequence of diabetes and, if so, how this might contribute to bladder cystopathy. Urinary bladder dysfunction is a common occurrence in patients with diabetes. Pressure generation requires calcium and cytosolic ATP. Activities of these pumps are responsible for calcium homeostasis.

METHODS Rat urinary detrusor muscle strips were suspended in organ baths containing Krebs solution for isometric tension recording. Tissue responses to the Na^+/K^+-ATPase pump inhibitor, ouabain, the plasma membrane Ca^{2+} ATPase inhibitor, vanadate, and the sarcoplasmic reticulum Ca^{2+} ATPase inhibitor, cyclopiazonic acid (CPA), were examined from normal and streptozocin-induced diabetic rats for 2, 4, and 12 weeks.

RESULTS Ouabain, vanadate, and CPA caused concentration-dependent contractions of bladder strips from diabetic and normal rats. The degree of contraction of diabetic bladder muscle was lower than that of controls. This reduction was a function of duration of diabetes. For ouabain, the reduction peaked at 2 weeks, with partial restoration to normal after diabetes induction. For vanadate and CPA, the reduction increased with the duration of diabetes.

CONCLUSION The ion pumps are important modulators of bladder smooth muscle tone, and in a rat model of streptozotocin-induced diabetes, the activity of these pumps is impaired. Although this is only a single model of diabetes, these findings suggest that a defect in these pumps may be an important component of the development of diabetic bladder cystopathy. UROLOGY 81: 211.e17–211.e21, 2013. © 2013 Elsevier Inc.

The urinary bladder is a smooth muscle organ whose function is to collect and store urine at low intravesical pressure and then to expel the urine periodically by means of a highly coordinated sustained contraction.[1] That diabetes mellitus results in urinary bladder dysfunction is well established. A large atonic bladder with urinary retention, urgency, urinary incontinence, and overactive bladder are common occurrences in patients with long-term diabetes.[2-4] Although these are not life-threatening conditions, they can affect a patient's life, impairing social, physical, and psychological activity, productivity at work, and sexual health.[5,6]

Bladder contraction requires calcium, and the availability of metabolic energy breakdown of cytosolic adenosine triphosphate (ATP). Activities of Na^+/K^+-ATPase and Ca^{2+}-ATPases in bladder smooth muscle are responsible for calcium homeostasis. Bladder function also depends on the state of neural innervation, the structure of the organ as a whole, and the sensitivity of receptors to certain agonists or to an altered number of receptors and postreceptor events.[1,7] Calcium-related mechanisms, phosphoinositides, adenylate cyclase, and ionic transport mechanisms are the major postreceptor events. Most of these parameters have not been investigated in smooth muscles.

Smooth muscle contraction is regulated by an elevation of cytosolic Ca^{2+}, which is controlled by the balance between Ca^{2+} entry into the cell/release from intracellular stores, and Ca^{2+} sequestration/extrusion from the cell.[8] The most prominent mechanisms that transport Ca^{2+} are plasma membrane and sarcoplasmic membrane Ca^{2+}ATPase pumps and the Na^+/Ca^{2+} exchange system.[9] The electrogenic Na^+/K^+ATPase pump plays a critical role in the maintenance of the cellular ionic milieu and membrane potential and is thought to influence smooth muscle tone.[10] Ca^{2+}-ATPases and Na^+/K^+ATPase require direct coupling of metabolic energy (ATP) because they work against the concentration gradient; however, they have not been studied in diabetic smooth muscles. The aim of the present study was to evaluate ion pump activities in bladder smooth muscle of the rat as a contributor item in the development of bladder dysfunction in diabetes.

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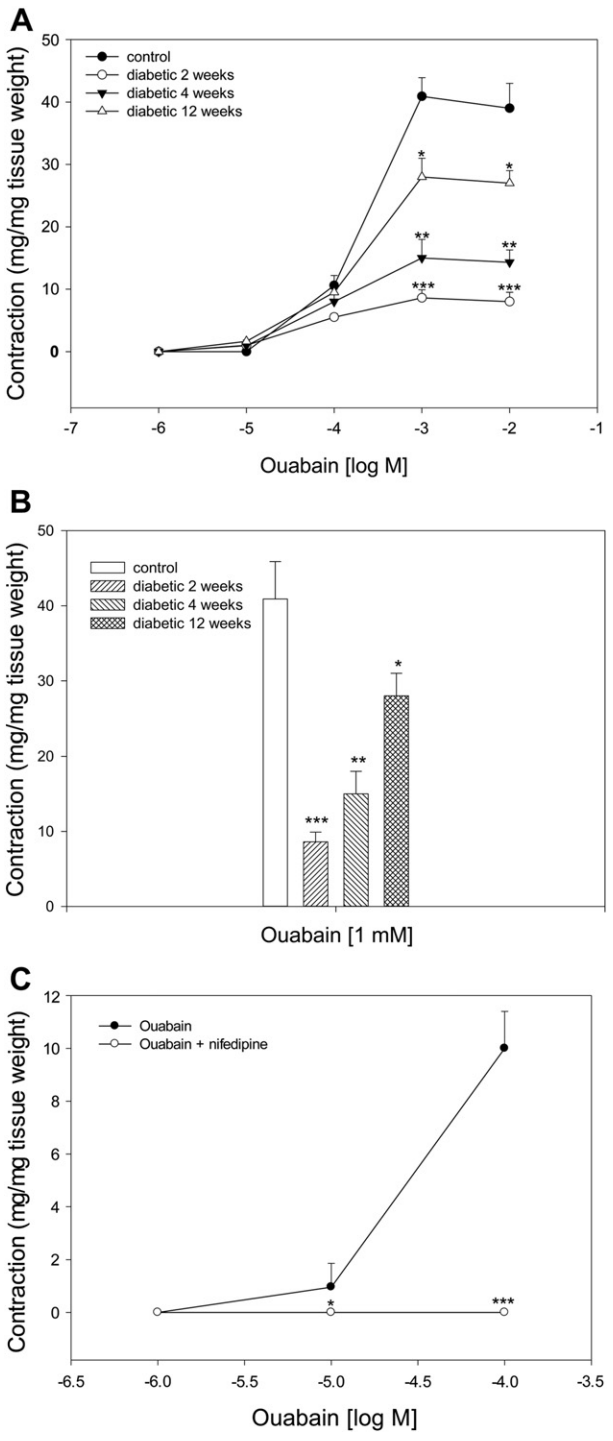

Figure 1. (A) Effect of diabetes on concentration-response curves of ouabain-induced contraction in bladder strips from control and diabetic rats for 2, 4, and 12 weeks. Results are means ± SEM of 6 experiments. Significant difference between normal and diabetic groups: $*P < .05$; $**P < .01$; $***P < .001$. **(B)** Effect of duration of diabetes on ouabain (1 mM)-induced contraction in bladder strips from control and diabetic rats for 2, 4, and 12 weeks. Results are mean ± SEM of 6 experiments. Significant difference between normal and diabetic groups: $*P < .05$; $**P < .01$; $***P < .001$). **(C)** Concentration-response curves of ouabain-induced contraction in bladder strips from diabetic rats for 4 weeks in the absence and presence of nifedipine (1 μM). Results are mean ± SEM of 4 experiments. $*P < .05$; $***P < .001$.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats weighing approximately 200 g were housed individually on a 12-hour light/dark cycle (lights on from 0600 to 1800 hours). 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 by intravenous injection of streptozotocin (STZ; 55 mg/kg body weight) diluted in 0.05 M sodium citrate (pH 4.5); control rats received buffer alone by the same route. Induction of diabetes was ascertained by determination of blood glucose concentrations.

Preparation of Bladder Strips

Rats were fasted for 6 h before being killed by decapitation. Blood was collected into heparinized tubes, centrifuged at 3000 g for 15 minutes, and the plasma was 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 (pH 7.4). The bladder was cut longitudinally into 2 equal strips from control rats and into 4 equal preparations from the diabetic rats, which were suspended in 10 mL organ baths containing Krebs solution and 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 Electronik) connected to a Gould recorder. The strips were initially loaded to a tension of 1 gram and allowed to equilibrate for 60 minutes, during which time they were washed twice. At the end of each experiment the muscle was weighed and responses were calculated as mg × mg^{-1} tissue weight.

Drugs

Carbachol hydrochloride, sodium orthovanadate, and ouabain octahydrate were obtained from Sigma Chemicals, St. Louis, MO. Nifedipine and cyclopiazonic acid (CPA) were obtained from Research Biochemicals International, Natick, MA. All drugs were dissolved in distilled water, except nifedipine and cyclopiazonic acid, which were dissolved in ethanol.

Calculation

Data are presented as mean ± SEM of (n) experiments. Where necessary, differences between 2 mean values were compared using the paired or unpaired Student *t* test, 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

Bladder weight increased markedly in the diabetic rats compared with the control group. The average weights

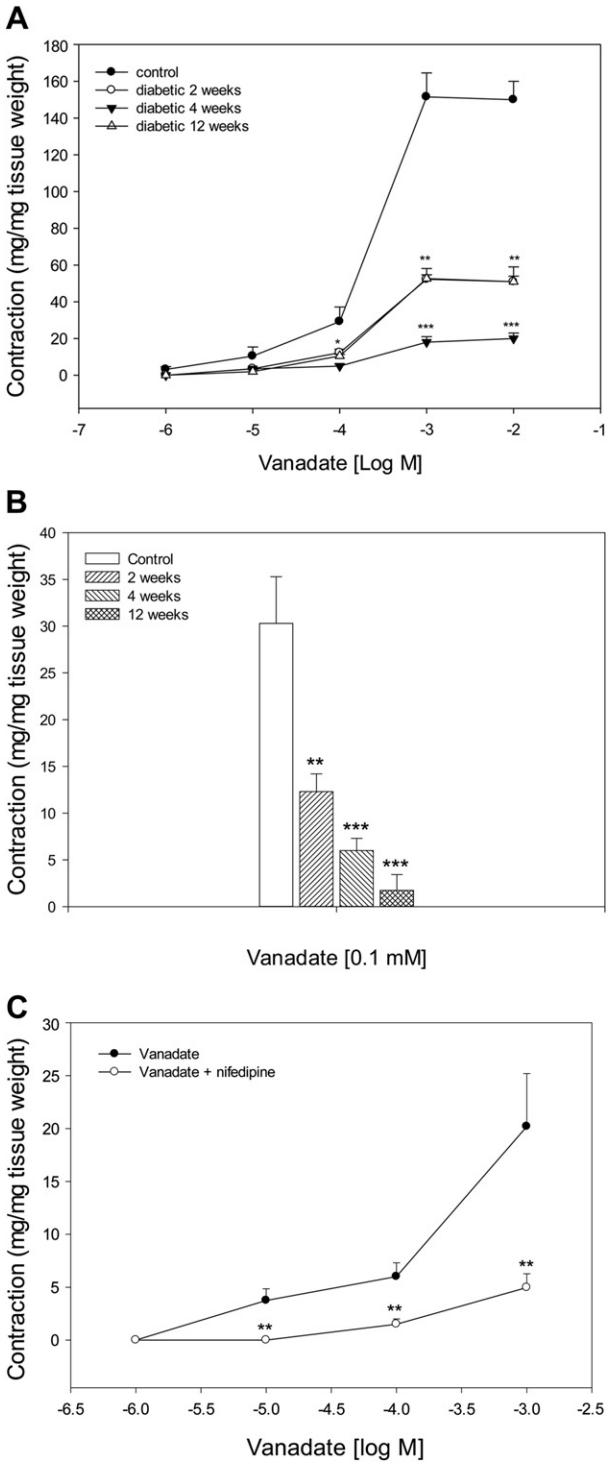

Figure 2. (A) Effect of diabetes on concentration-response curves of vanadate-induced contraction in bladder strips from control and diabetic rats for 2, 4, 12 weeks. Results are means ± SEM of 4 experiments. Significant difference between normal and diabetic groups: $*P < .05$; $**P < .01$; $***P < .001$. **(B)** Effect of duration of diabetes on vanadate (0.1 mM)-induced contraction in bladder strips from control and diabetic rats for 2, 4, and 12 weeks. Results are mean ± SEM of 4 experiments. Significant difference between normal and diabetic groups: $**P < .01$; $***P < .001$. **(C)** Concentration-response curves of vanadate-induced contraction in bladder strips from diabetic rats for 4 weeks in the absence and presence of nifedipine (1μM). Results are mean ± SEM of 4 experiments. Significant difference between the groups: $**P < .01$.

were 0.1000 g for control rats and 0.2384 g for 12-week diabetic rats (n = 6; $P > .001$). There was also lumen enlargement in diabetic rat bladders; therefore, contractility data were normalized as milligrams of tension generated per milligram of tissue weight.

Effect of Ouabain on the Bladder Smooth Muscle

Ouabain (1 μM-10 mM), an inhibitor of the Na^+/K^+ ATPase pump, caused concentration-dependent contractions of bladder strips from normal and diabetic rats (Fig. 1A). Its contractions peaked within 2 minutes. Ouabain-induced bladder contraction was significantly reduced after the induction of diabetes. The maximum reduction occurred 2 weeks after inducing diabetes; however, there was a tendency toward partial recovery to normal with increasing duration of diabetes (4 and 12 weeks), as shown in Figures 1A and 1B. Ouabain-induced contractions in 4-week diabetic strips were abolished by the treatment with nifedipine (1 μM), the L-type Ca^{2+} channel blocker, as shown in Figure 1C, indicating the important role for extracellular calcium.

Effect of Vanadate on the Bladder Smooth Muscle

Vanadate (1 μM-10 mM), an inhibitor of the plasma membrane Ca^{2+} ATPase pump, caused concentration-dependent contractions of bladder strips from normal and diabetic rats, which peaked within 3 minutes (Fig. 2A). The degree of contraction of diabetic bladder muscle was lower than that of controls. This reduction was a function of duration of diabetes, as shown in Figure 2B. Pretreatment with nifedipine (1 μM) inhibited vanadate-induced contractions in 4-week diabetic strips, indicating a role for extracellular calcium, as shown in Figure 2C.

Effect of CPA on the Bladder Smooth Muscle

CPA (1 μM-1 mM), an inhibitor of the sarcoplasmic membrane Ca^{2+} ATPase pump, caused concentration-dependent contractions of bladder strips from normal and diabetic rats, which peaked within 3 minutes. CPA-induced bladder contraction was significantly reduced after the induction of diabetes for 12 weeks, as shown in Figures 3A and 3B.

DISCUSSION

This study showed that bladder weight increased significantly in the diabetic rats compared with the control group. Previous studies reported that there was increase in bladder weight of diabetic rats that corresponds to a time-dependent increase in smooth muscle mass. They also

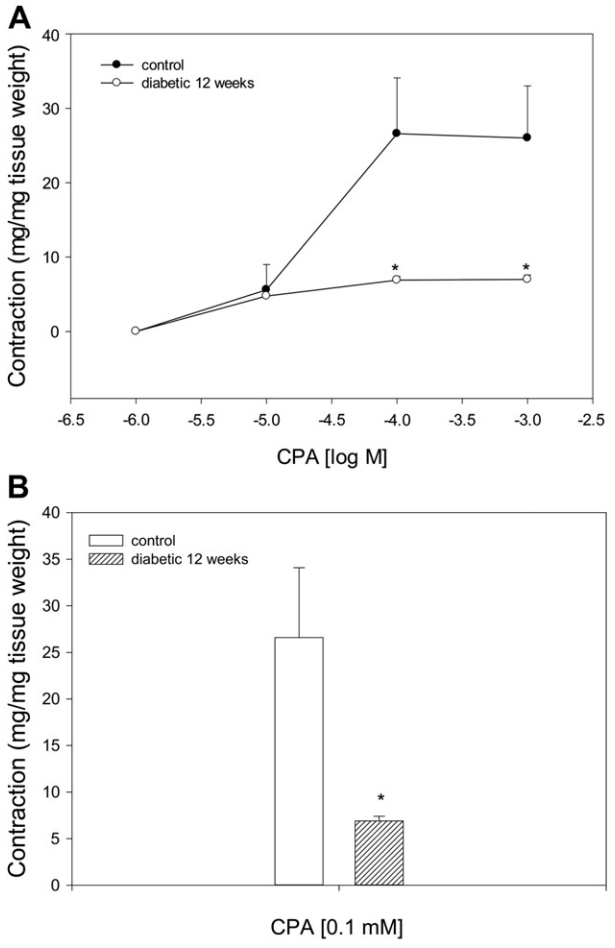

Figure 3. (A) Effect of diabetes on concentration-response curves of cyclopiazonic acid (CPA)-induced contraction in bladder strips from control and diabetic rats for 12 weeks. Results are mean ± SEM of 4 experiments. Significant difference between normal and diabetic groups: $*P < .05$. **(B)** Effect of duration of diabetes on CPA (100 μM)-induced contraction in bladder strips from control and diabetic rats for 12 weeks. Results are mean ± SEM of 4 experiments. Significant difference between normal and diabetic groups: $*P < .05$.

showed that lumen enlargement and hypertrophy are 2 obvious responses to polyuria in diabetes.[11,12]

Diminished Na^+/K^+ ATPase activity has been reported in a number of tissues, including aorta, heart ventricular muscle, and peripheral nerves of STZ-induced diabetic rats.[13-15] $Ca2^+$-ATPases and Na^+/K^+ATPase require direct coupling of metabolic energy (ATP) because they work against the concentration gradient. They have not been adequately studied in bladder models of diabetic smooth muscle cystopathy. $Ca2^+$ ATPases activity have not been studied in an animal model of diabetic cystopathy nor has the effect of the duration of type I diabetes on these pumps.

The present study suggests that the diminution of Na^+/K^+ ATPase and Ca^{2+} ATPases activity may play a significant role in the development of bladder dysfunction in diabetes. This is supported by the use of agents that inhibit the pumps. Ouabain, an inhibitor of Na^+/K^+ ATPase, vanadate, an inhibitor of plasma membrane Ca^{2+} ATPase, and CPA, an inhibitor of the sarcoplasmic membrane Ca^{2+} ATPase pumps, reproduced the depressor effects of diabetes on bladder contractility. These inhibitors caused concentration-dependent contractions, suggesting that Na^+/K^+ ATPase and Ca^{2+} ATPases activity is an important modulator of smooth muscle tone in the bladder.

Inhibition of Na^+/K^+ ATPase activity by ouabain would cause depolarization and contraction of bladder smooth muscle as a result of an increase in the intracellular Ca^{2+} concentration via the opening of voltage-sensitive Ca^{2+} channels, as suggested in cardiac and vascular tissue and in the trachea.[10,16-18] Alternatively, Na^+ pump inhibitors may increase Na^+/Ca^{2+} exchange activity as a result of elevated intracellular Na^+ concentration.[10,19] In the bladder smooth muscle, however, Na^+/Ca^{2+} exchange activity does not seem to play a major role in ouabain-induced contractions[20] because they were completely prevented by nifedipine, the L-type Ca^{2+} channel blocker. Therefore, ouabain-induced contraction is entirely dependent on extracellular Ca^{2+}. The inhibitory effect of diabetes on rat bladder contractility is in agreement with another report on diabetic rabbit bladder.[20] This present study showed that bladder strips from diabetic rats exhibited a lower degree of contraction in response to ouabain compared with the control animals. This reduction peaked in diabetic rats for 2 weeks, with partial restoration occurring after 10 weeks. This restoration may be due to the increase in the activity of the Na^+/Ca^{2+} exchange system to compensate the inhibition of the pump. In addition, intracellular Ca^{2+} concentration is known to be increased by time in the diabetic rat.[14] Diabetes has also been shown to increase the number of voltage-operated Ca^{2+} channels in the bladder.[21] Therefore, elevation of the Ca^{2+} level due to the previous data may be the reason for the partial restoration to normal.

Vanadate, an inhibitor of the plasma membrane Ca^{2+} ATPase pump, inhibits Ca^{2+} ATPase activity, leading to accumulation of intracellular Ca^{2+}.[9,22] Similar results were obtained in the trachea.[23] Bladder strips from diabetic rats exhibited lower degree of contraction in response to vanadate than those from control animals. The reduction increased with the duration of diabetes. This reduction reflects the continuous decrease of the Ca^{2+}-ATPase pumps activity by time. Nifedipine inhibited vanadate-induced contraction in diabetic strips but did not abolish it, suggesting that it is due to extracellular as well as intracellular Ca^{2+}, the influx of Ca^{2+} is through voltage-operated channels, and prevention of Ca^{2+} efflux is through the Ca^{2+} ATPase pumps.

CPA is an inhibitor of the sarcoplasmic membrane Ca^{2+} ATPase pump.[24] Inhibition of Ca^{2+} sequestration will increase the intracellular Ca^{2+} concentration. Inhibition of sarcoplasmic-Ca^{2+} ATPase will lead to the

bladder contraction. CPA induced concentration-dependent contractions of bladder strips from normal and diabetic rats, which were lower in diabetic rats. The reduction increased with the duration of diabetes. This reduction reflects the continuous decrease of the Ca^{2+}-ATPase pumps activity by time.

Clinically, diabetes mellitus is frequently associated with a "failing" detrusor function, as characterized by increased bladder capacity hyporeflexia and the presence of residual urine.[20] The findings of this study show that diabetes diminishes ATPase activity and subsequently attenuates the contraction mediated by the inhibitors of the pumps. These effects are due to the induction of diabetes after a single dose of STZ, which is known to have no effect on other organs, including the bladder.

Because ATP is derived from glycogen, the production of ATP could be decreased in the diabetic tissues, and this would affect all ATPase enzymes, which are responsible for the activity of the pumps in the body. Calcium-transport ATPases are members of a class of transmembrane proteins that are able to transduce the energy liberated by ATP hydrolysis into the transport of ions against a steep electrochemical gradient.[23]

CONCLUSIONS

The rat model of STZ-induced type I diabetes diminishes Na^+/K^+ ATPase and Ca^{2+} ATPases activity in bladder smooth muscle, disrupting the normal calcium homeostasis. This finding suggests that diabetic cystopathy may be a direct result of altered calcium regulation in the bladder smooth muscle. Whether this finding exists in other models of diabetes remains to be determined.

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