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Coenzyme Q10 protect against ischemia/reperfusion induced biochemical and functional changes in rabbit urinary bladder

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Abstract *Purpose* Ischemia, reperfusion, and free radical generation have been recently implicated in the progressive bladder dysfunction. Coenzyme Q10 (CoQ10) is a pro-vitamin like substance that appears to be efficient for treatment of neurodegenerative disorders and ischemic heart disease. Our goal was to investigate the potential protective effect of CoQ10 in a rabbit model of in vivo bilateral ischemia and ischemia/reperfusion (I/R). *Material and Methods* Six groups of four male New Zealand White rabbits each were treated with CoQ10 (3 mg/kg body weight/day—dissolved in peanut oil) (groups 1–3) or vehicle (peanut oil) (groups 4–6). Groups 1 and 4 (ischemia-alone groups) had clamped bilateral vesical arteries for 2 h; in groups 2 and 5 (I/R groups), bilateral ischemia was similarly induced and the rabbits were allowed to recover for 2 weeks. Groups 3 and 6 were controls (shams) and were exposed to sham surgery. The effects on contractile responses to various stimulations and biochemical studies such as citrate synthase (CS), choline acetyltransferase (ChAT), superoxide dismutase (SOD), and catalase (CAT) were evaluated. The protein peroxidation indicator, carbonyl group, and nitrotyrosine contents were analyzed by Western blotting. *Results* Ischemia resulted in significant reductions in the contractile responses to all forms of stimulation in vehicle-fed rabbits, whereas there were no reductions in CoQ10-treated rabbits. Contractile responses were significantly reduced in vehicle-treated I/R groups, but significantly improved in CoQ10-treated rabbits. Protein carbonylation and nitration increased significantly in ischemia-alone and I/R bladders; CoQ10 treatment significantly attenuated protein carbonylation and nitration. CoQ10 up-regulated SOD and CAT activities in control animals; the few differences in CoQ10-treated animal in SOD and CAT after ischemia and in general increase CAT activities following I/R. *Conclusions* CoQ10 supplementation provides bladder protection against I/R injury. This protection effect improves mitochondrial function during I/R by repleting mitochondrial CoQ10 stores and potentiating their antioxidant properties.

Keywords Bladder · Coenzyme Q10 · Obstruction · Ischemia

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Introduction

Increasing evidences have shown that ischemia and reperfusion (I/R) are major etiologic factors in the progression of bladder dysfunction induced by partial outlet obstruction, bladder hyperactivity, arterial atherosclerosis, and diabetes [1–4]. It has been shown that during bladder emptying, the increased intra-wall pressure results in blood vessel compression and results in decreased blood flow and tissue hypoxia [5, 6]. This phenomenon is more prominent

in the obstructed hypertrophied bladder for increase in bladder wall thickness and intravesical pressure after bladder outlet obstruction [6].

Both in vivo and in vitro ischemia studies showed that ischemia caused significant bladder dysfunction and led to decreased contractile responses to all forms of stimulation [7–10]. Part of this damage is due to an alternation of ionic homeostasis, promoting H and Ca build up; generation of free radicals; and the resultant cellular and subcellular membrane peroxidation. Mitochondria play a predominant role in cellular energy production. In the absence of oxygen supply, mitochondria respiration is disturbed and ATP synthesis decreased. Mitochondria are highly vulnerable to ischemic changes and may involve in oxidative stress-induced apoptosis. Thus, clinical applications had focused on free radical reduction and mitochondria protection for the treatment of I/R injury.

CoQ10 is a liquid-soluble endogenous pro-vitamin found naturally in the mitochondria. Primary dietary sources of CoQ10 include oily fish, organ meats, and whole grains. CoQ10 carries out important biochemical functions in cells, especially in inner mitochondria membrane, with 2 major functions. One is to serve as an electron and proton carrier for energy coupling. CoQ10 transfers electrons and protons to form an electrical gradient across the cell membrane, which in turn drives the formation of adenosine triphosphate [11, 12]. The second major function is to serve as an antioxidant, acting as a primary scavenger of free radicals [11].

CoQ10 has been used as a therapeutic agent in several cardiovascular, degenerative neurologic and neuromuscular diseases, based upon its fundamental role in mitochondrial function and cellular bioenergetics [13–17]. No absolute contraindications are known for CoQ10 and adverse effects with CoQ10 are rare, making it a safe supplementation. However, CoQ10 has never been used as a treatment for bladder ischemia/reperfusion injury. Based on these reports, the present study is designed to determine the effect of CoQ10 on the rabbit urinary bladder to bilateral ischemia and ischemia followed by 2 weeks of reperfusion.

Material and methods

These studies were approved by the Institutional Animal Care and Use Committee of the Stratton Affairs Medical Center, Albany, NY.

Six groups of four male New Zealand White rabbits each were treated with CoQ10 (3 mg/kg body weight/day oral feeding) [18] (groups 1–3) or vehicle (peanut oil) (groups 4–6). All rabbits were treated daily for 2 weeks before surgery, continuing until the end of the experiment. After 2 weeks, rabbits were initially sedated with ketamine-xylazine (25 mg/kg ketamine/5 mg/kg xylazine, i.m.) and maintained in anesthesia with isofluorane (1–3%) inhalation. In groups 1 and 4 (ischemia-only groups), vesical arteries were clamped for 2 h and then sacrificed the same day. In groups 2 and 5 (I/R groups), bilateral ischemia was similarly induced for 2 h, at which time the clamps were removed and the rabbits allowed to recover for 2 weeks. Groups 3 and 6 were controls (shams) and were exposed to sham surgery. Sham surgery consisted of isolating the vessels entering the bladder base and closing the incision. Gentamicin (1 mg/kg) and buprenorphine hydrochloride (0.3 mg/kg) were given i.m. to all rabbits on the first and second day after surgery.

Bladder strip preparation and contractile responses

After the bladders were removed and weighed, three longitudinal strips were obtained. The bladder strips included muscle and mucosa. Each strip was mounted in a separate 2-ml bath containing Tyrode's solution (124.9 mM NaCl, 2.6 mM KCl, 23.8 mM $NaHCO_3$, 0.5 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 1.8 mM $CaCl_2$, and 5.5 mM dextrose) maintained at 37°C and equilibrated with a mixture of 95% oxygen and 5% carbon dioxide. An initial resting tension of 2 g was applied for 30 min, and the responses were recorded isometrically using a force-displacement transducer. Electrical field stimulation (FS) was achieved by supramaximal voltages (80 V) at 2, 8, and 32 Hz. After FS, the maximal responses were determined sequentially for ATP, carbachol (20 μM), and KCl (120 mM). A series of three washes, at 15-min intervals, with Tyrode's solution followed each of the pharmacologic stimulations.

Western blot for measurement of nitrotyrosine and protein carbonylation

Frozen tissue of bladder muscle wall and mucosa was homogenized on ice in a homogenization buffer (50 mM Tris, pH 7.5, 5% Tiron) containing the Halt Protease inhibitor Cocktail (Pierce, Rockford, IL) at 100 mg/ml using a Kinematica GmBH (Switzerland) homogenizer on setting 7 with a 12 mm generator. Each homogenized sample was centrifuged at 2500 rpm for 10 min at 4°C in a Labnet Hermle Z233 MK-2 centrifuge (Labnet, Woodbride, NJ). The supernatant was centrifuged at 10,000 rpm for 30 min in the same centrifuge at 4°C again. The supernatant was then centrifuged at 40,000 rpm for 1 h at 4°C. One ml of the final supernatant was saved and used for analysis.

After addition of SDS (final concentration, 1%), the sample was boiled for 4 min and centrifuged at 10,000 rpm for 15 min. Protein concentration in the supernatant was measured using the Pierce BCA protein assay kit. Membranes were blocked with 5% nonfat milk in 0.05% Tween 20 in PBS for 1 h at room temperature and then incubated with primary antibody, monoclonal antibody to nitrotyrosine (Alexis Biochemicals, San Diego, CA), and goat anti-2,4-Dinitrophenylhydrazine (DNP) (Bethyl laboratories, Montgomery, TX). After treatment with the primary antibody, the membranes were washed with 20 mM Tris, 500 mM NaCl, and 0.05% Tween 20 and incubated with secondary antibody (goat anti-mouse IgG at 1:10,000). All incubations were done for 45 min at 37°C. The unbound antibodies were removed by washing 5 times in TTBS for 5 min each at room temperature. The substrate was visualized using ECL-Plus (Amersham Pharmacia Biotech, Buckinghamshire, England) for 2 min and membrane sealed in a hybridization bag, scanned and analyzed with a Kodak Image Station 440CF and Kodak ID image analysis software (Scientific Image System, Rochester, NY). Standard curves were constructed using BSA pre-labeled with DNP to establish that the protein concentrations used for analysis fell within the linear range.

Measurement of citrate synthase (CS), choline acetyl transferase (ChAT), superoxide dismutase (SOD), and catalase

SOD activity

SOD (total) activity was determined by the method of Flohe and Otting, using a cytochrome C reduction test. In this model, oxygen free radicals are generated by xanthine oxidase reactions with ferricytochrome C. SOD activity is calculated from the degree of inhibition of this reaction and recorded as the change in optical density (mOD) at 550 nm (using a spectrophotometer) per milligram of protein.

Specifically, bladder tissue was homogenized in a 50-mmol/l phosphate buffer (pH 7.8) at 200 mg/ml. The homogenate was centrifuged at 18 000 *g* for 10 min. The pellet was eliminated and the supernatant used for the following assay: 2 ml of solution A (0.76 mg xanthine in 10 ml of 1 mmol/l NaOH, added to 50 mg cytochrome C + 3.7 mg EDTA in 100 ml 50 mmol/l phosphate buffer) at 25°C was incubated with 50 µl of the tissue sample or SOD standards in a 3-ml cuvette; 200 µl of solution B (5.63 µl xanthine oxidase in 1 ml 0.1 mmol/l EDTA) was used to start the reaction. After mixing, the absorbance change indicating cytochrome C reduction was measured using a spectrophotometer at 550 nm for 2 min. The change in absorbance with time over the first 2 min for all preparations was linear and used in the plots shown. SOD activity is calculated from the inhibition of this reaction by increasing concentrations of SOD in the tissue homogenate and recorded as the change in optical density (mOD) at 550 nm. The concentration of tissue homogenate that inhibits 25% of activity is presented in Fig. 7 and represents the enzymatic activity of SOD in the sample. We use IC_{25} rather than IC_{50} because some of the samples do not reach 50% inhibition. Purified SOD (Sigma Chemical Co., St Louis, MO) was prepared at 25 ng/ml and diluted 1:1 to 0.39 ng/ml for a standard curve, with specificity confirmed by heating both preparations for 10 min at 90°C, which eliminated all activity.

Catalase assay

Following the method of Aebi [19], for the catalase experiments we initiated the reaction by adding H_2O_2 in 50 mM phosphate buffer (pH 7.0) in the presence of one concentration of tissue extract. The degradation of H_2O_2 was monitored at 240 nm and 25°C, and an extinction coefficient of 43.6 mol^{-1} cm^{-1} was used to calculate units of activity. The enzyme activity is expressed in units per milligram protein (1 unit = 1 mol of H_2O_2 degraded for 1 min). The slope of the enzyme reaction was utilized as a quantitative measure of CAT activity in units of change in optical density per minute. The slope of the curve for each tissue was graphed using the best-fit curve by Sigmaplot, and Sigmastat was used for statistical analyses.

Citrate synthase assay

A sample aliquot (100 µl) is added to a 0.5 cm cuvette, along with 1.0 ml 0.05 M Tris buffer (pH 7.6), 50 µl 0.2–10 mM oxaloacetate (substrate), 30 µl 12.3 mM acetyl-coenzyme A, 100 µl 1 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), and 100 µl 10% Triton X-100. The free coenzyme-A generated by CS activity reacts with DTNB to form a colored compound that is quantified at 412 nm. Absorbance is recorded every 30 s for 6 min (reaching steady state) using a Hitachi spectrophotometer. Protein concentration is determined using the Lowry method.

Choline acetyl transferase (ChAT) activity

Frozen tissue samples are homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) containing 10 mM EDTA and centrifuged at 20,000 *g* for 30 min to remove the cell membranes, mitochondria, and nuclei. Aliquots are incubated at 37°C for 10 and 20 min with 200 µl of reaction

mixture consisting of 0.2–10 mM acetyl-coenzyme A (acetyl-CoA) (substrate), 0.2 mM ^{3}H-acetyl-CoA (200 mCi/mmol), 8 mM choline, 50 mM sodium phosphate, 0.3 M NaCl, 20 mM EDTA, and 96 nM physostigmine. After incubation, each solution is diluted with 5 m1 of 0.01 M sodium phosphate and the reaction stopped with 2 ml of acetonitrile containing 5 mg/ml tetraphenylboron. Then, the contents of each reaction tube are transferred to a 20 ml scintillation vial and 10 ml of Betamax scintillation fluid is added slowly to each vial. The vials are shaken lightly. Samples stand for 1 h while the phases separate, extracting acetylcholine (^{3}H-Ach) into the Betamax phase, while ^{3}H-acetyl-CoA stays in the aqueous phase. The aqueous phase is removed and ^{3}H-ACh is measured using scintillation spectroscopy. ChAT activity is reported as fmoles Acetyl-Co-A generated per min per mg protein/30 min.

In regard to the biochemical analyses, the assays for citrate synthase, choline acetyltransferase, superoxide dismutase, and catalase are quantitative studies which provide maximal activities. DNP and nitrotyrosine are quantitative Western blot analyses that provide relative densities compared to control = 100%.

Statistical analysis

For all studies, analysis of variance was used with the Neuman-Keuls test for individual group comparisons. Statistical significance was judged by a P value of < 0.05.

Results

The contractility response to field stimulation after ischemia alone and I/R is shown in Fig. 1. The response of field stimulation significantly decreased in ischemia-alone and I/R groups. Treatment with CoQ10 can significantly attenuate the contractile responses. CoQ10 administration also resulted in a significant increase in the contractile response to ATP, carbachol, and KCl above control rabbit bladders in I/R group (Fig 2). Surprisingly, ischemia alone has little effect on contractile response to ATP, carbachol, and KCl.

Figure 3 represents the mitochondria marker enzyme citrate synthase activities in bladder wall. CoQ10 mediated a significant increase of citrate synthase activity in I/R group. There are no significant changes on citrate synthase activities in control and ischemia-alone groups. The effect of CoQ10 on ChAT activity is presented in Fig. 4. CoQ10 administration had no significant effect on the ChAT activity of the bladder wall in ischemia-alone group. The activities of ChAT in I/R group were significantly reduced whereas CoQ10 administration significantly increase

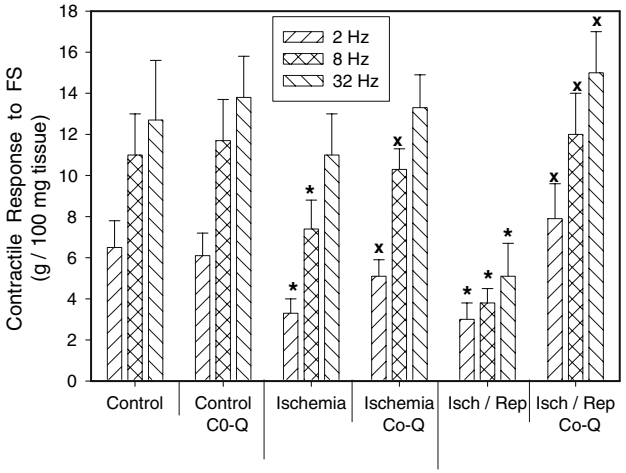

Fig. 1 Effects of CoQ10 on contractile responses of detrusor strips to field stimulations. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; ×, significantly different from ovariectomy group; $P < 0.05$

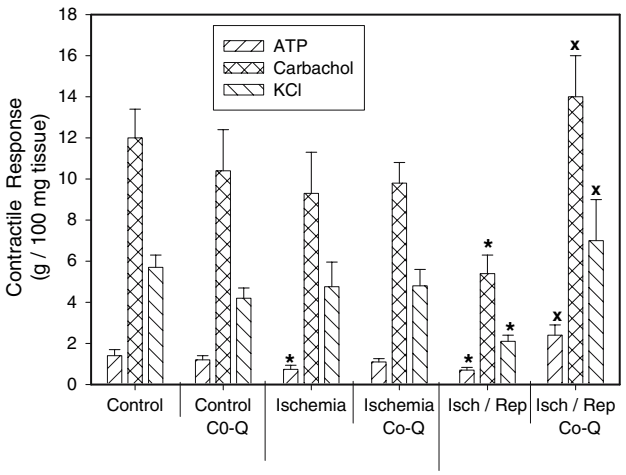

Fig. 2 Effects of CoQ10 on contractile responses of detrusor strips to 1 mM ATP, 20 μM carbachol, and 120 mM KCl. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; ×, significantly different from ovariectomy group; $P < 0.05$

ChAT activities compared to vehicle group, indicating a protection effect of CoQ10 over cholinergic nerve.

Figure 5 shows that protein carbonylation increased significantly after ischemia/reperfusion for the muscle and mucosa layers; both attenuate to control level by CoQ10 administration. Ischemia alone also increases protein carbonylation, though it does not reach statistical significance. Figure 6 shows the expression of nitrotyrosine level in both bladder wall and mucosa. There was no significant difference between ischemia-alone and control group, both with or without CoQ10 administration. However, there was a significant increase in protein nitration in muscle and mucosa layers in I/R group. CoQ10 administration

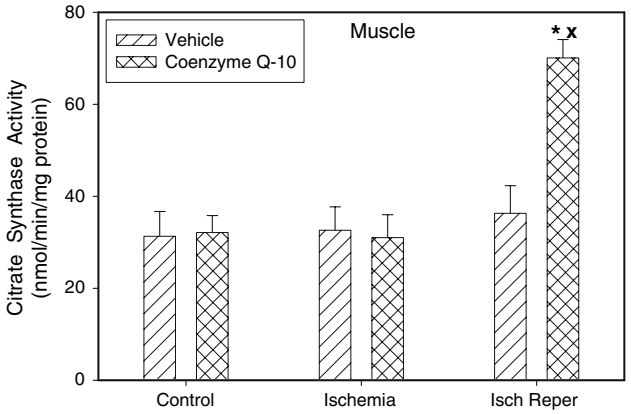

Fig. 3 Effects of CoQ10 on citrate synthase activities in rabbit bladder wall. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; $P < 0.05$

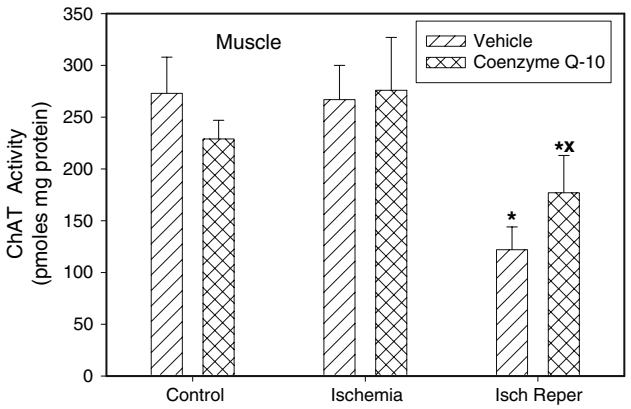

Fig. 4 Effects of CoQ10 on choline acetyl-transferase activities in rabbit bladder wall. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; $P < 0.05$

significantly decreases the protein nitration level, especially in the mucosa layer.

Figure 7 shows the effect of CoQ10 on SOD activities (IC25) of bladder muscle and mucosa under control, ischemia, and I/R conditions, respectively. It should be noted that the lower IC25 is, the higher enzyme activities. The SOD activity of the control muscle is higher than control mucosa. The SOD activities of the control muscle and mucosa were significantly higher in CoQ10-treated rabbits than in the vehicle-treated rabbits. While ischemia alone resulted in a significant increase in SOD activity in the vehicle-treated rabbits (both muscle and mucosa), there was a mild difference in SOD activity in the CoQ10-treated rabbits. Reperfusion resulted in significant decreases in SOD activity when compared to the ischemia-alone groups for muscle compartment and slight decrease in mucosa layer. CoQ10 administration significantly increased SOD activities in I/R compared to vehicle group in bladder mucosa.

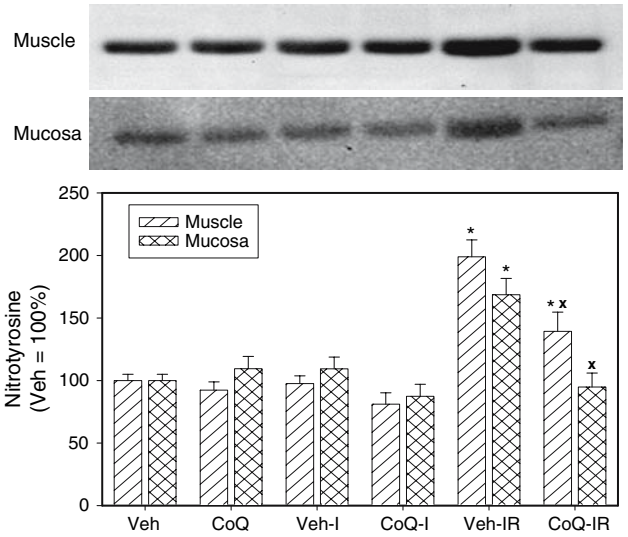

Fig. 5 (**a**) Representative Western blots of rabbit bladder homogenate probed for nitrotyrosine expression. (**b**) Average expression of nitrotyrosine in different samples. Y-axis numbers represent the values compared to control group, control is 100%. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; ×, significantly different from vehicle group; $P < 0.05$

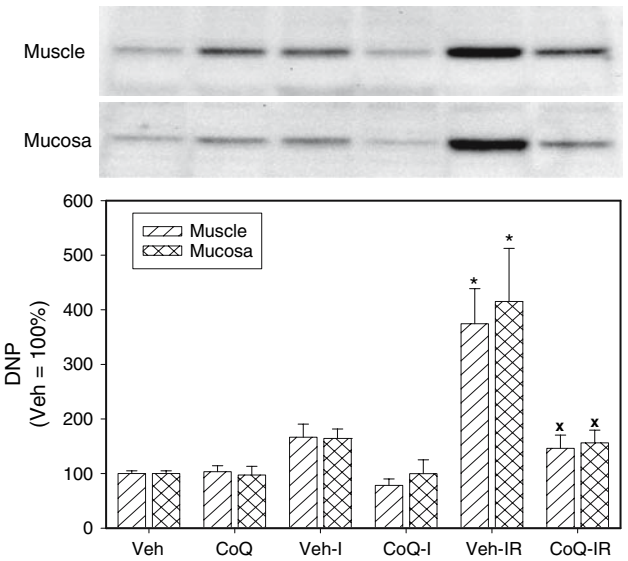

Fig. 6 (**a**) Representative Western blots of rabbit bladder homogenate probed for carbonyl group expression. (**b**) Average expression of carbonyl group in different samples. Y-axis numbers represent the values compared to control group, control is 100%. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; ×, significantly different from vehicle group; $P < 0.05$

Similar to SOD, the CAT activities in the control muscle and mucosa were significantly higher in the CoQ10-treated rabbit bladders than in the vehicle-treated bladders. Ischemia alone resulted in decreased activities in the vehicle-treated bladders in the mucosa layer. Reperfusion resulted in significant decreases in CAT activities in the

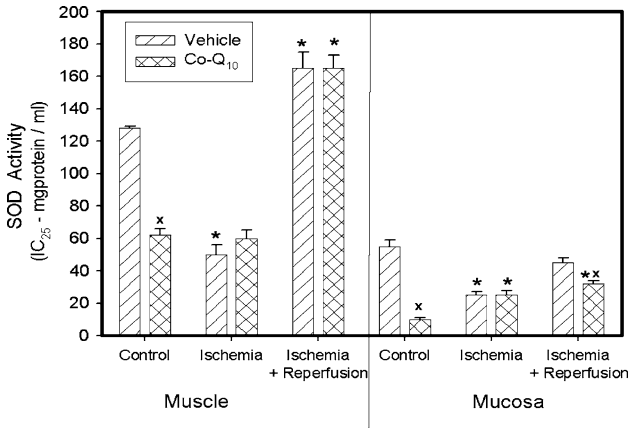

Fig. 7 Effects of CoQ10 on SOD IC_{25} (25% of concentration that reacted) activities in both rabbit bladder wall and mucosa. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; ×, significantly different from vehicle group; $P < 0.05$

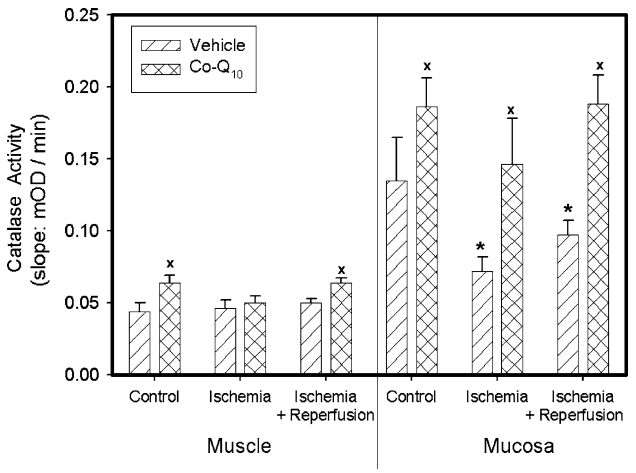

Fig. 8 Effects of CoQ10 on catalase activities in both rabbit bladder wall and mucosa. Each bar is the mean ± SEM for $N = 4$. *, significantly different from control group; ×, significantly different from vehicle group; $P < 0.05$

vehicle group and CAT activities significantly increase in CoQ10 treated bladder when compared to vehicle-treated group, both in the muscle and mucosa layers (Fig. 8).

Discussion

The results of the present study have demonstrated that CoQ10 can protect against ischemia and I/R-induced functional and biochemical changes in the rabbit urinary bladder. The process of bladder contraction can be separated into two phases, an initial peak phase and a second tonic phase. The initial peak response is supported energetically by extant cellular ATP stores, whereas the tonic phase requires active mitochondrial oxidation of substrates to generate energy. Ability of the bladder to sustain contraction is directly related to the availability of energy produced by mitochondrial electron transport and oxidative phosphorylation [20]. Decompensation of bladder from extended obstruction may be mediated by breakdown of mitochondrial function, with a resultant decreased contractile function.

This study supports our previous observation that the destructive effects of reperfusion are worse than ischemia alone [21]. Murin et al. noted that lipid peroxidation and protein oxidation occurred mainly following reperfusion as early as 15 min following reperfusion in rat brain I/R model [22]. The free radical damages following reperfusion are mainly caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Both ROS and RNS are generated following reperfusion. Oxidation and nitration damage the membrane L-type calcium channels function; dysregulate Ca^{2+} homeostasis; increase activities of Ca^{2+}-dependent enzymes, mainly phospholipase and calpain; and mediate the progressive denervation of bladder wall.

Consistent with previous studies, this experiment showed clearly that the responses to field stimulation (neurogenic stimulation) are more sensitive to ischemia alone and I/R than contractile responses to carbachol, ATP, and KCl. Many researchers have provided biochemical evidence of reperfusion injury. Lin et al. observed that perfusion following the relief of acute bladder distention was associated with a time-dependent increase in lipid peroxidation, which leads to nerve and smooth muscle membrane damage [7]. For the indicator of cholinergic innervation, choline acetyl-transferase activity significantly decreased after reperfusion and was partially reversed by CoQ10 administration. This evidence suggests that nerve membranes or synapses are the structures most sensitive to I/R injury. Our findings also demonstrated that CoQ10 has a significant protective effect against I/R-induced damage to both nerves and autonomic receptors that are related to the protection response to field stimulation and carbachol. CoQ10 can offer neuroprotection at the mitochondrial level in the apoptosis pathway against oxidative stress [23]. CoQ10 may act in the mitochondria by enhancing electron transport, preventing ROS generation from mitochondria, increasing mitochondrial ATP production, and stabilizing mitochondria membrane. It should be pointed out that this is an acute ischemia model, which may cause diffuse bladder wall fibrosis and loss of smooth muscle [4]. In contrast to acute ischemia, chronic bladder ischemia may enhance cyclooxygenase protein expression, alter leukotriene and prostaglandin production, thus inducing neural excitation and smooth muscle overactivity [4, 24, 25].

Mitochondrial function was evaluated by the mitochondrial marker enzyme, citrate synthase. Citrate synthase activities significantly increase after CoQ10 administration in I/R group. Studies showed that in patients with ischemic heart disease, plasma CoQ10 concentrations were lower than in normal subjects [26]. Physiologically CoQ10 concentration in mitochondrial membrane is not saturated; addition of exogenous CoQ10 increases the rate of electron transfer in the respiratory chain, thus improving the efficiency of oxidative phosphorylation and mitochondrial coupling [27]. These observations suggest that the main mechanisms responsible for CoQ10 protective effects are the supplementation of depleted mitochondrial CoQ10 stores. Recovery of ATP stores, increase of phosphocreatine concentration, and protection of creatine kinase can restore mitochondria function and protect bladder during reperfusion.

CoQ10 significantly attenuates protein carbonylation and nitration, indicating an antioxidant protective effect of CoQ10 from oxidative damage in both ischemia-alone and I/R groups. The antioxidant function of CoQ10 may be explained by two mechanisms: first, CoQ10 provides hydrogen equivalents to reduce peroxyl and/or alkoxyl radicals; and second, CoQ10 can also regenerate the reduced vitamin E, another powerful antioxidant, to coordinate the antioxidant effects [28]. The present study also measured natural antioxidant mechanisms, namely SOD and CAT. SOD and CAT are chief defenses in the cells. Cytoplasmic SOD converts superoxide to peroxide, which in turn is brokendown by CAT to water and oxygen. The intermediate peroxide is a dangerous molecule; if its production exceeds the ability of CAT to degrade it, it could cause damage to the cell. Thus, it is important for SOD and CAT to work in conjunction and counterbalance. Feeding rabbits with CoQ10 resulted in a significant increase in activities of both SOD and CAT in control muscle and mucosa layers, thus providing an initial protection against cellular and subcellular damages caused by ischemia and I/R. Other studies demonstrated that CoQ10 treatment increased antioxidant parameters like superoxide dismutase, catalase, and glutathione and reduced lipid peroxidation in the liver homogenates of diabetic rats [29]. Ischemia alone also resulted in increase of SOD and CAT activities. This could be the natural defense mechanism of bladder cells to ischemia. CoQ10-administration could upregulate SOD activities to protect cells from ischemia. However, I/R resulted in a decrease of SOD activities, both in vehicle and CoQ10 feeding rabbits, especially in the smooth muscle layer. This could probably be the result of free radical damage to the enzyme itself.

This ischemia/reperfusion model can be applied not only to elderly partial outlet obstruction bladder but also to atherosclerotic processes in the elderly. By using laser Doppler flow probe, it has been proved clinically that decreased bladder blood flow correlated strongly with decreased bladder wall compliance [30]. Furthermore, increases of oxidative stress are also reported to be associated with several bladder dysfunctions, such as interstitial cystitis, diabetes cystopathy, and bladder cancer [2, 31–33]. CoQ10 supplementation may provide neuroprotection and serve as detrusor cells' free radical scavengers, which may benefit patients with bladder outlet obstruction as well as older adults with acute and probably chronic urinary retention.

Conclusion

This study confirms that CoQ10 supplementation provides bladder protection against ischemia-alone and I/R injury. Reduction of oxidative stress in conjunction with increased antioxidant reserve in CoQ10-fed bladder suggests that this protection effect is achieved by improving mitochondrial function through replacing mitochondrial CoQ10 stores, potentiating antioxidant properties, and acting as an oxygen-derived free radical scavenger in the bladder ischemia.

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