ORIGINAL ARTICLE

EFFECT OF SODIUM ORTHOVANADATE ON THE URINARY BLADDER RINGS ISOLATED FROM NORMAL AND HYPERGLYCEMIC RATS

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ABSTRACT
Effect of sodium orthovanadate on the urinary bladder rings isolated from normal and hyperglycemic rats was investigated. Vanadate concentrations of 0.1, 0.5 and 1 mM produced a concentration-dependant increase in isolated urinary bladder tension in both normal and hyperglycemic tissues. In normal urinary bladder rings, the response to increasing concentrations of vanadate were 12.8 ± 0.6, 20.1 ± 0.74 and 32.5 ± 1.2 g tension /g tissue, respectively. Hyperglycemia significantly potentiated the response of bladder rings to vanadate. In hyperglycemic rats, the response of the urinary bladder to the same concentrations of vanadate were 21.3 ± 0.78, 30 ± 1.1 and 50.5 ± 1.6 g tension/g tissue, respectively. The responses were reversible and further contractions could be elicited at 30 minutes intervals. The contractions of normal urinary bladder rings to vanadate were not altered by pretreatment with atropine or L-NAME. Melatonin, nifedipine as well as indomethacin significantly reduced the response of normal and hyperglycemic bladder rings to vanadate. On the other hand, ascorbic acid significantly enhanced the response of these rings to vanadate. Hydrogen peroxide (H_2O_2)-induced increase in the urinary bladder tension was very similar and comparable to contractions induced by vanadate. Similarly, H_2O_2-induced contraction in the urinary bladder rings was significantly reduced after incubation of the bladder rings with melatonin, indomethacin or nifedipine and the response was not altered by ascorbic acid. The results of the present study indicate that vanadate produced marked contraction in the normal urinary bladder rings and this contraction was significantly enhanced by hyperglycemia. The present data shows also that the contractile effect of vanadate on isolated urinary bladder rings is partially dependent on extracellular calcium and generation of free radicals. The present results suggested a key role of H_2O_2 in mediating the contraction of urinary bladder rings induced by vanadate.

Keywords: Sodium orthovanadate, urinary bladder, H_2O_2**,** melatonin, hyperglycemia.

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INTRODUCTION

Vanadium is widely distributed in the environment and can exert toxic and carcinogenic effects especially in workers in petrochemical, steel and mining industries. It originates mainly from the combustion of fossil fuels, especially residual oil (Huang *et al*., 2000). Although most foods contain low concentration of vanadium, food is the major source of exposure to vanadium for the general population. Good dietary sources include black pepper, parsley, mushrooms, spinach, oysters, shellfish, cereals, fish, sunflower, carrot and cabbage (O'Connell, 2001). No specific biochemical function has yet been identified for vanadium.

Recently, the ability of vanadium to mimic insulin action is considered as a potential pharmacological effect for vanadate (Orvig *et al*., 1995). Vanadium alters lipid and glucose metabolism by enhancing glucose oxidation, glycogen synthesis and inhibits liver gluconeogenesis (Poucheret *et al*., 1998). Vanadium modulates and mimics many of the metabolic actions of insulin both *in-vivo* and *in-vitro* (Cam *et al*., 2000; Wang *et al*., 2001).

Vanadate contracts several smooth muscles such as isolated vas deferens of rat (Garcia *et al*., 1981), cat gastric smooth muscle (Sim and Kim, 1998), rat gall bladder (Alcon *et al*., 2000), isolated rat aorta, (Zhou *et al*., 1997), guinea-pig trachea and lung parenchymal strips (Nayler and Sparrow, 1983) and rat pulmonary arterial rings (Nagi *et al*., 2002). However, the effect of vanadate on the smooth muscles of the urinary bladder is not examined yet.

The exact mechanism(s) responsible for the vanadate-induced contractions in smooth muscles is not yet certain. However, several mechanisms have been proposed. Protein tyrosine phosphorylatin has been reported to mediate vanadate-induced contractions in smooth muscles (Laniyonu *et al*., 1994). Protein tyrosine phosphorylation requires a protein tyrosine kinase (PTK) as well as a protein tyrosine phosphatase (PTP). Vanadate is a potent inhibitor of PTP activity (Huyer *et al*., 1997) and thus, activates tyrosine kinase pathway (Alcon *et al*., 2000). Inhibition of Na^+ / K^+ ATPase (Goldwaser *et al*., 2000) as well as inhibition of Ca^{2+} dependent ATPase activity (Aureliano and Madeira, 1994) and increases the extracellular calcium influx (Alcon *et al.,* 2000) were also proposed as mechanisms for vanadate's action.

The present study was undertaken to investigate the effect of vanadate on the isolated urinary bladder rings of normal and diabetic rats as well as the possible mechanism(s) of action.

MATERIALS AND METHODS

Drugs and Chemicals

Sodium orthovanadate was purchased from BDH (Poole, England). Acetylcholine chloride, atropine sulphate, streptozotocin (STZ) and melatonin were purchased from Sigma Chemical Co. (St. Louis, MO., USA). $^{\omega}$N-nitro-L-arginine-methyl ester hydrochloride (L-NAME) was purchased from Fluka Chemie Steinheim, Switzerland. Nifedipine, indomethacin and ascorbic acid were obtained as a generous gift from Pharco Pharmaceuticals Co. (Alexandria, Egypt). Hydrogen peroxide (H_2O_2) was obtained from ADWIC Co. as 30% w/v solution.

STZ was dissolved in citrate buffer at pH =4.5 (Palmer *et al.,* 1998). Other chemicals used in this study were of highest analytical grade. Drugs were freshly prepared just before use. All drugs were dissolved in normal saline, melatonin and nifedipine were dissolved first in least amount of ethanol and then desired dilutions were made in distilled water. Indomethacin was dissolved in distilled water containing trace amount of $NaHCO_3$.

Experimental animals

Adult Sprague-Dawley rats of both sexes weighing 200-250 g, obtained from local breeders, were housed six per cage in a light-controlled room with an alternating 12 hour light/dark cycle. The animals were kept at temperature of 25 ± 1 ^{0}C and relative humidity range of 45-80 %. Animals were allowed free access to standard laboratory food and water ad libitum. The animals were handled in accordance with the strict guiding principles of the NIH for experimental care and use of animals.

Preparation of urinary bladder rings

Rats were killed by an overdose of ether and the urinary bladder was immediately excised and placed in warm physiological salt solution (PSS). The composition of PSS (in mM) was 118 NaCl; 4.7 KCl; 2.5 $CaCl_2.2H_2O$; 1.2 KH_2PO_4; 1.2 $MgSO_4.7H_2O$; 25 $NaHCO_3$ and 10 glucose (El-Kashef, 1996). The urinary bladder was trimmed and cut into rings. The rings were mounted vertically between a clamp and a force transducer in an organ bath filled with 50 ml of the PSS.

The PSS was kept at 37º C and continuously bubbled with carbogen. The suspended urinary bladder rings were allowed to equilibrate for 30 minute under a resting load of 1 g. During this time, the bath solution was replaced every 5 min. The isometric tension generated by bladder smooth muscle was monitored by a displacement transducer (model 50-7905, Harvard Apparatus LTD., South Natick, MA, USA) and recorded on a two-channel oscillograph (model 50-8622, Harvard Apparatus LTD., USA). The response of the urinary bladder rings to different agents was recorded and calculated as g tension/ g tissue.

Experimental design

Concentration-response curves to vanadate were constructed. The rings were exposed to different concentrations of vanadate (0.1, 0.5, 1 mM) in a non

cumulative manner. Exposure to each concentration of vanadate was maintained until the maximal response to that concentration was reached (about 7-8 min). The rings were then repeatedly washed with several changes of the PSS .The rings were allowed to return to baseline before the next vanadate concentration was added. At least 30 min was allowed to elapse for the rings to return to baseline before the addition of next concentration.

Eight groups of urinary bladder rings of normal rats, each of 6 rings were used. Group (1), control group, concentration-response curves for vanadate were constructed before and 20 min after the addition of saline to the organ bath. In the other groups, concentration-response curves for vanadate were constructed before (control) and following the addition of each of the following receptor antagonists or enzyme inhibitors prior to each concentration of vanadate:

Group (2) 1 μM of atropine, a muscarinic antagonist for 5 min., (Laniyonu *et al*., 1994). Group (3) 1 mM of free radical scavenger melatonin, for 20 min. (Nagi *et al.,* 2002). Group (4) vehicle of melatonin. Group (5) nitric oxide synthase inhibitor, L-NAME for 20 min. (1 mM, Colasanti *et al.,* 1999). Group (6) calcium channel blocker, nifedipine for 15 min. (1 μM, Sim and Kim, 1998). Group (7) cyclo-oxygenase inhibitor, indomethacin for 20 min (10 μM, Persson *et al.,* 1996). Group (8) ascorbic acid for 20 min. (5 mM, Gitto et al., 2001).

Effect of hydrogen peroxide

Four groups of normal urinary bladder rings, each of 6 rings were used. Hydrogen peroxide (500 μM, Gonzalez-Pacheco *et al.,* 2002) was added to the bath before and 20 min after incubation of the rings with melatonin (1 μM) in the first group; 15 min after incubation with nifedipine (1 μM) in the second group; 20 min after incubation of the rings with indomethacin (10 μM) in the third group and 15 min after incubation of the rings with ascorbic acid (5 mM) in the fourth group.

Induction of experimental hyperglycemia

Hyperglycemia was induced in 200-250 g Spraque-Dawley rats by I.P injection of 50 mg/Kg streptozotocin (Adachi, 1995). Eighteen rats fasted for 16-18 hours were injected with STZ and 2 days later, blood samples were collected for detection of diabetes by measuring serum glucose levels. Animals with serum glucose level more than 300 mg/dl were used in this study. After 2 weeks from induction of diabetes, diabetic rats were sacrificed, urinary bladder was immediately excised and placed in warm physiological salt solution (PSS). Four groups of urinary bladder rings, each of five rings, were used. Group (1), concentration-response curves for vanadate were constructed before and 20 min after incubation of the rings with melatonin (1mM), before the addition of each concentration of vanadate. Group (2), concentration-response curves for vanadate were constructed before and 15 min after incubation of the rings with nifedipine (1 μM), before the addition of each concentration of vanadate. Group (3), concentration-response curves for vanadate were constructed before and 20 min after incubation of the rings with indomethacin (10 μM), before the addition of each concentration of vanadate. Group (4), concentration-response curves for vanadate were constructed before and 15 min after incubation of the rings with ascorbic acid (5 mM), before the addition of each concentration of vanadate.

STATISTICAL ANALYSIS

Data were presented as means ± S.E.M. Significance was calculated at $p<0.05$. Statistical analysis of the results was done using Student's t-test for unpaired and paired data (Daniel, 1991).

Statistical calculations were carried out using Instat-2 computer program (Graph Pad Software Inc., V2.04, San Diego, CA, USA).

RESULTS

Vanadate concentrations of 0.1, 0.5 and 1 mM, added to the isolated urinary bladder rings, produced a dose-related increase in urinary bladder tension in both normal and hyperglycemic tissues (fig 1). There was a significant difference between the effect of vanadate on both normal and hyperglycemic urinary bladder rings. The effect was reproducible in both normal and hyperglycemic bladder rings.

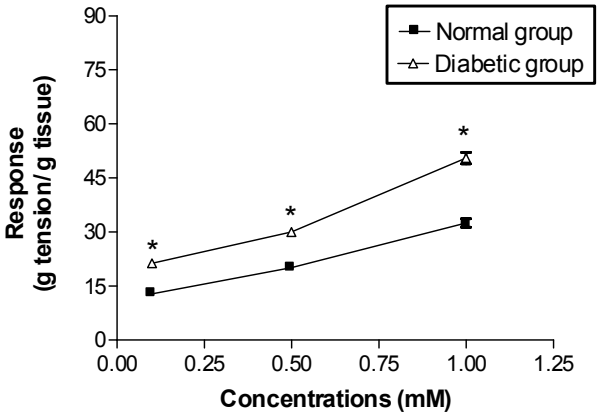

Fig. 1: Effect of different concentrations of sodium orthovanadate on the isolated urinary bladder rings of normal and diabetic rats. Data are expressed as means ± S.E.M; n =52 and 23 for normal and hyperglycemic rings, respectively.
*Significantly different from respective normal value at $p < 0.05$ using Student's "t" test for unpaired data.

Atropine, 1μM, or L-NAME (1mM) did not significantly alter the response of normal urinary bladder rings to the

effect of vanadate. On the other hand, melatonin 1 mM, significantly reduced the contractile response of normal urinary bladder rings to vanadate (fig. 2). Similarly, nifedipine, 1 μM produced a significant reduction in the response of urinary bladder rings to vanadate (fig. 2).

Addition of indomethacin, 10 μM, to normal urinary bladder rings produced a significant reduction in the response of the rings to vanadate (Fig. 2), while addition of ascorbic acid, 5 mM, significantly enhanced the contractile response of the normal urinary bladder rings to vanadate. Lower concentrations of ascorbic acid, 0.05 or 0.5 mM, did not alter the response of the rings to vanadate (fig. 2).

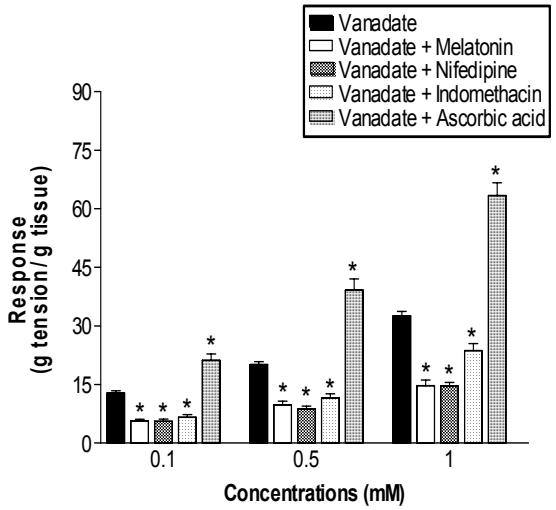

Fig. 2: Effect of melatonin (1 mM), nifedipine (1μM), indomethacin (10 μM) and ascorbic acid (5 mM) on vanadate-induced increase in urinary bladder tension of normal rats. Data are expressed as means ± S.E.M; n = 6.
*Significantly different from respective vanadate value when given alone at $p < 0.05$ using Student's "t" test for paired data.

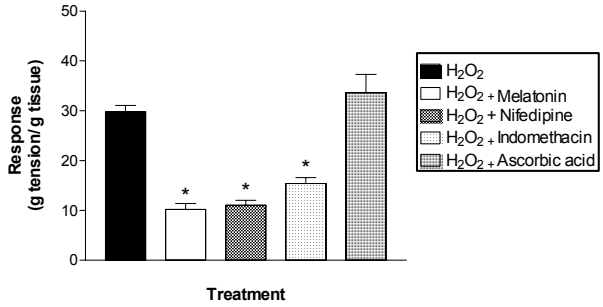

Fig. 3: Effect of 1mM melatonin, 1μM nifedipine, 10 μM indomethacin and 5 mM ascorbic acid on H_2O_2 (500 μM) induced-increase in urinary bladder tension of normal rats. Data are expressed as means ± S.E.M; n = 6.
*Significantly different from H_2O_2 alone at $p < 0.05$ using Student's "t" test for paired data.

Hydrogen peroxide 500 μM, produced a significant contraction in the urinary bladder rings. This contraction was significantly reduced by incubation of the rings with melatonin (1 mM), nifedipine (1 μM) or by 10 μM indomethacin (fig. 3). On the other hand, ascorbic acid, when added in a concentration of 5 mM did not alter the response of the isolated normal urinary bladder rings to hydrogen peroxide (fig. 3).

Melatonin, when added in a concentration of 1 mM produced a significant reduction in the response of isolated bladder rings to vanadate. Incubation of the hyperglycemic urinary bladder rings with nifedipine (1 μM) produced a significant decrease in the response of these rings to vanadate (fig. 4).

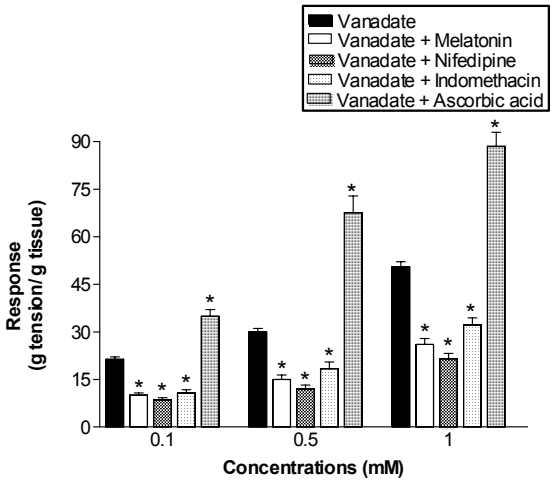

Fig. 4: Effect of melatonin (1 mM), nifedipine (1μM), indomethacin (10 μM) and ascorbic acid (5 mM) on vanadate-induced increase in urinary bladder tension of hyperglycemic rats. Data are expressed as means ± S.E.M: n = 5.
*Significantly different from respective vanadate value when given alone at $p < 0.05$ using Student's "t" test for paired data.

Indomethacin, when added in a concentration of 10 μM, produced a significant reduction in the response of hyperglycemic urinary bladder rings to vanadate as seen in Fig. 4. While, ascorbic acid added in a concentration of 5 mM produced a significant increase in the response of these rings to vanadate (fig. 4).

DISCUSSION

In the present study, sodium orthovanadate induced contraction of the isolated rat urinary bladder rings in a concentration-dependent manner. This observation is consistent with the results of previous studies, showed that vanadate contracts several smooth muscles (Garcia *et al.,*

1981; Alcon *et al.,* 2000; Zhou *et al.,* 1997; Nayler & Sparrow, 1983; Nagi *et al.,* 2002; Laniyonu *et al.,* 1994; Candura *et al.,* 1994; Murphy *et al.,* 2002; Li *et al.,* 2004).

In the present study, vanadate-induced contraction in isolated urinary bladder rings was gradual, reaching maximal response after about 8 minutes from addition of vanadate, indicates that the effect of vanadate is probably mediated through release of endogenous substance(s). This proposed hypothesis is further supported by the observation that another addition of vanadate did not produce immediate contraction in the isolated urinary bladder rings, but takes at least 20-30 min interval to initiate new contraction. It seems that after the first addition of vanadate, the endogenous substance(s) released to mediate the effect of vanadate were depleted and time is needed to resynthesis new mediator(s).

In this study, atropine did not significantly alter vanadate-induced contraction in normal urinary bladder rings, indicating that muscarinic receptors are not involved. This observation is in agreement with the studies of others in rat vascular and gastric smooth muscle (Laniyonu *et al.,* 1994) and in human isolated bronchus (Cortijo *et al.,* 1997).

The results of the present study showed that melatonin significantly reduced vanadate-induced contraction of urinary bladder rings isolated from normal and hyperglycemic rats. This observation agreed with the findings of Nagi *et al.* (2002) that the contractile response of the isolated rat pulmonary arterial rings to vanadate is mediated via free radicals and/or H_2O_2. Melatonin, the principal hormone of the pineal gland, is known to be a free-radical scavenger as well as a H_2O_2 scavenger (Karbownik & Lewinski, 2003).

L-NAME, a nitric oxide synthase (NOS) inhibitor, did not significantly alter vanadate-induced contraction of urinary bladder rings of normal rats. This observation agreed with the study of Zheng *et al*. (1999) showing that tyrosine phosphatase signal pathway may be irrelevant for the induction of functional NOS in gastric circular muscle tissue. Also, vanadate induced pulmonary artery constriction was attributed, at least in part, to the loss of vasodilator activity provided by endothelial NO, this was supported by the attenuation of the constriction by NO donor and augmentation by NOS inhibitor, L-NAME (Li *et al.,* 2004).

In this study, vanadate-induced contractions of urinary bladder rings of normal and hyperglycemic rats were significantly attenuated by nifedipine. This is in agreement with the findings of others (Zhou *et al.,* 1997; Murphy *et al.,* 2002) showing that vanadate-induced contractions were dependent on extracellular calcium. Thus, it seems that the contractile effect of vanadate in urinary bladder rings of both normal and hyperglycemic rats is largely dependent on the availability of extracellular Ca^{2+}.

Indomethacin, a non-selective cyclo-oxygenase (COX) inhibitor, significantly reduced vanadate-induced contraction of urinary bladder rings isolated from normal and hyperglycemic rats, indicating the involvement of prostanoids in the vanadate-induced contraction. A number of reports have shown that COX is involved in vanadate-induced contraction (Alcon *et al.,* 2000; Laniyonu *et al.,* 1994). Furthermore, thromboxane A_2 (TXA_2) receptor antagonists and TXA_2 synthase inhibitors significantly suppressed the stimulatory release of lipoprotein lipase by vanadate in isolated rat fat pads (Morita *et al.,* 1995). These results suggest that TXA_2 generated from PLA_2-COX-TXA_2 synthase pathway may serve as the mediator for vanadate-induced contraction.

The present results showed that high concentration of ascorbic acid significantly increased vanadate-induced contraction of urinary bladder rings of normal and hyperglycemic rats. This observation indicates that ascorbate, an antioxidant, failed to provide any protection against vanadate-induced contraction probably due to the interference of its pro-oxidant potential with its antioxidant activity (Younes *et al.,* 1991). In *in-vitro* system, oxidation of NAD(P)H requires NAD(P)H, vanadate, ascorbate and phosphate. Ascorbate provides the reducing equivalents necessary to reduce vanadate to vanadyl. Vanadyl autoxidizes producing superoxide anion which initiates a free radical chain reaction resulting in oxidation of NAD(P)H with the net production of H_2O_2 (Yoshino *et al.,* 1989).

The exact mechanism(s) responsible for the vanadate induced-contractions in smooth muscles is not yet certain. However, several mechanisms have been proposed, including protein tyrosine phosphorylation that is regulated by the balanced action of protein tyrosine kinases (PTKs), which catalyze phosphorylation of specific tyrosine residues, and protein tyrosine phosphatases (PTPs) that facilitate dephosphorylation of tyrosine residues (Parfenova *et al.,* 1999). Vanadate is a potent inhibitor of PTP activity **(**Huyer *et al.,* 1997) and therefore, activates tyrosine kinase pathway (Alcon *et al.,* 2000).

Moreover, redox regulation of tyrosine phosphorylation has also been reported and PTP was a selective target of oxidation by H_2O_2 (Gabbita *et al.,* 2000). Furthermore, activation of tyrosine kinase has also been proposed to mediate the H_2O_2–induced vascular contractions and that inhibitors of tyrosine kinase significantly blocked contractions evoked by H_2O_2 (Jin & Rhoades, 1997; Yang *et al.,* 1998). It seems that contractions in smooth muscles produced by vanadate or H_2O_2 are mediated indirectly via inhibition of PTP. Thus, a possible role of H_2O_2 in mediating the vanadate-induced contractions in isolated urinary bladder rings was looked at thoroughly.

H_2O_2 induced an increase in the urinary bladder tension that was very comparable to contractions induced by vanadate. This is in agreement with other report indicating that, at least in part, H_2O_2 may be involved in mediating the contractile effect of vanadate in isolated pulmonary arterial rings of rat (Nagi *et al.,* 2002). Furthermore, vanadate or H_2O_2–induced contractions in the urinary bladder rings were significantly reduced in a similar way after incubation of the rings with melatonin, indomethacin and nifedipine. H_2O_2 is a by-product of oxidative metabolism, in which energy activation and electron reduction are involved (Xiao *et al.,* 2002). In the present study H_2O_2, the two-electron reduction product of oxygen, has been used as a model of oxidative stress that induced contraction of isolated rat urinary bladder rings in a concentration-dependent manner. This observation is consistent with the results of previous studies, showed that H_2O_2 contracts several smooth muscles (Nagi *et al.,* 2002; Xiao *et al.,* 2002; Gao & Vanhoutte, 1993; Rabe *et al.,* 1995; Rodriguez-Martinez *et al.,* 1998; Gao & Lee, 2001; Tzeng *et al.,* 2003).

In the present study, melatonin significantly reduced H_2O_2-induced contraction of the isolated urinary bladder rings of normal rats in a way very similar to the reduction of vanadate-induced contraction. This is in agreement with others confirming that melatonin is a H_2O_2 and free radical scavenger and that contraction of isolated pulmonary arterial rings of rats to vanadate was induced by H_2O_2 (Nagi *et al.,* 2002). Melatonin is an effective H_2O_2 scavenger due to its ability to inhibit lipid peroxidation induced by H_2O_2 that mediates contraction of smooth muscle (Gulcin *et al.,* 2003).

In addition, nifedipine significantly reduced H_2O_2-induced contraction of the isolated urinary bladder rings of normal rats. This observation is in agreement with other reports indicating the importance of extracellular calcium for H_2O_2-induced contraction in isolated rat aorta (Yang *et al.,* 1998; Tzeng *et al.,* 2003). Similarly, indomethacin significantly reduced H_2O_2-induced contraction of the isolated urinary bladder rings of normal rats. This observation is consistent with other reports showed that H_2O_2-induced contractions are mediated via generation of products of the cyclo-oxygenase pathway (Xiao *et al.,* 2002; Gao & Vanhoutte, 1993; Gao & Lee, 2001).

On the other hand, ascorbic acid did not significantly alter H_2O_2-induced contraction of urinary bladder rings of normal rats. Increasing the intracellular accumulation of vitamin C promotes mitochondrial events leading to peroxynitrite-dependent formation of H_2O_2 and resulting in a rapid necrotic response (Guidarelli *et al.,* 2004). Mitochondria do not contain catalase and are, therefore largely dependent on reduced glutathione (GSH) and glutathione peroxidases for its antioxidant protection. Therefore, the results of the present study suggest that the contractile effect of vanadate on the isolated rat urinary bladder could be, at least in part, mediated through H_2O_2 release.

Hyperglycemia induced endothelial dysfunction and consequently pathogenesis of diabetic complications through augmentation of inflammatory responses by upregulating COX_2 and its inflammatory products (Kiritoshi *et al.,* 2003). It has been reported that hyperglycemia enhanced production of reactive oxygen species (ROS), especially H_2O_2 and this increase contributes to the increased endothelial generation of prostaglandin H synthase (PGHS)-mediated vasoconstriction in diabetes (Kobayashi & Kamata, 2002).

Moreover, the epithelium from hyperglycemic rat urinary bladders was thicker and heavier and the absolute amount of endogenous prostaglandins E_2 and $F_{2\alpha}$ was higher than for control animals. The prostaglandin-release impairment may be responsible, in part, for bladder abnormalities observed in pathological conditions, such as diabetes (Pinna *et al.,* 2000). Therefore, the greater activation of COX_2, the most important PGHS, and induction of PGE_2, $F_{2\alpha}$, TXA_2 synthesis and/or isoprostanes via hyperglycemia-increased ROS production (especially H_2O_2) appeared to be partly responsible for the enhanced contraction to vanadate in diabetes.

Endothelial cells play a negative modulator role on the vasoconstriction or contraction of smooth muscles elicited by H_2O_2 through generation of vasoactive agents such as nitric oxide (Mohazzab *et al.,* 1996) and prostacyclin (Wesseles & Hempel, 1996). Oxidative stress may promote endothelial dysfunction through increased production of reactive oxygen species that inactivate nitric oxide. This endothelial nitric oxide plays a critical role in the cytoprotection against endothelial oxidative stress mediated by H_2O_2 in normoglycemic rats (Rodriguez-Martinez *et al.,* 1998). Therefore, in diabetes in which endothelial cells are altered, the release or action of NO as well as its protective role is altered (Marin & Rodriguez-Martinez, 1997) and higher sensitivity to oxidative stress induced by H_2O_2 could be expected (Sotnikova *et al.,* 1999).

In conclusion, the data reported herein indicate that the contractile effect of sodium orthovanadate in urinary bladder rings is partially dependent on extracellular calcium and may be mediated via free radicals and or arachidonic acid metabolites. The role of H_2O_2 in mediating the contraction of urinary bladder rings to vanadate can not ignored.

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