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The effect of chronic renal failure on phosphodiesterase inhibitor-induced relaxation responses in rabbit cavernosal strips

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Abstract

Erectile dysfunction is common in men with chronic renal failure. Previously nitrergic and endothelium-dependent relaxation responses have been shown to be reduced in chronic renal failure rabbits. We have therefore investigated the efficacy of phosphodiesterase inhibitors on the corpora cavernosa obtained from uremic rabbits. Uremia was induced with 5/6 nephrectomy and 4 weeks later cavernosal tissue strips were isolated. The relaxant effect of phosphodiesterase 5 inhibitors, zaprinast (1–300 μM) and sildenafil (0.01–300 μM), phosphodiesterase 3 inhibitor amrinone (1–100 μM) and non-specific phosphodiesterase inhibitor papaverine (1–300 μM) were investigated on phenylephrine (10 μM)-induced tone. We found a shift in the dose–response curve of only phosphodiesterase 5 inhibitors. These results suggest that the decreased production or availability of endogenous nitric oxide in chronic renal failure animals leads to decreased efficacy of phosphodiesterase 5 inhibitors to induce relaxation.

Keywords: Impotence; Renal failure; Phosphodiesterase inhibitor; (Rabbit)

1. Introduction

Penile erection is a hemodynamic process involving relaxation of smooth muscle of the corpus cavernosum. This relaxation process results in an increased flow of blood into the trabecular spaces of the corpora cavernosa (Lue and Tanagho, 1987; Andersson and Wagner, 1995). Although the control of cavernosal smooth muscle tone is complex, it is clear that the most important chemical mediator of cavernosal relaxation is nitric oxide (NO), released directly from non-adrenergic non-cholinergic nerve endings and from the endothelium. Within the smooth muscle cell, NO stimulates the enzyme soluble guanylyl cyclase to convert guanosine triphosphate (GTP) into the active second messenger cyclic guanosine monophosphate (cGMP) (Rajfer et al., 1992; Bush et al., 1992). Results of a number of recent studies have suggested that the cyclic nucleotides cAMP and cGMP are important second messengers in mediating the relaxation of various smooth muscle cells, including cavernous smooth muscle (Lincoln, 1989; Sparwasser et al., 1994). Phosphodiesterases are enzymes responsible for the hydrolysis of cGMP and cAMP to monophosphates. Of the phosphodiesterase isozyme families, phosphodiesterase 5,6,9 are specific for cGMP as a substrate. Phosphodiesterase 4,7,8 are specific for cAMP, and phosphodiesterase 1,2,3,10,11 hydrolyse both cGMP and cAMP (Beavo et al., 1994). Recently, experimental studies showed that phosphodiesterase 3,5 and 11 isoenzymes play an important role in smooth muscle tone regulation of the corpus cavernosum (Taher et al., 1997; Stief et al., 1998). Therefore, drugs that inhibit phosphodiesterase potentiate the action of cGMP and thus facilitate penile erectile activity. This proposal is supported by reports that various more specific phosphodiesterase inhibitors enhance the NO-induced relaxation of human and rabbit corpus cavernosal tissue in vitro (Rajfer et al., 1992; Bush et al., 1992). Sildenafil, a selective inhibitor of type 5 cGMP phosphodiesterase (Boolell et al., 1996a; Jeremy et al., 1997; Ballard et al., 1998) has been proved to be effective in the treatment of erectile dysfunc-

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tion after oral administration in men (Boolell et al., 1996b; Goldstein et al., 1998). The incidence of erectile dysfunction among patients with renal failure is significantly higher than that of the general population (Procci et al., 1981; Rodger et al., 1984; Palmer, 1999). Erectile dysfunction in men with chronic renal failure has been reported to occur in 20–100% of the cases (Karacan et al., 1978; Campese and Liu, 1990). Clinical complaints include diminished frequency of intercourse, decreased libido, and abnormal ability to initiate and maintain an erection (Glass et al., 1987). Studies investigating the pathophysiology of impotence in uremia have concluded that the etiology is multifactorial (Glass et al., 1987; Jünemann et al., 1990). Psychological factors have received widespread research attention (Karacan et al., 1978; Campese and Liu, 1990; Glass et al., 1987). Organic factors have focused on uremic neuropathy, hyperparathyroidism, hyperprolactinemia, antidepressant and antihypertensive medication, dysfunction of the autonomic nervous system, low testosterone levels, depletion of serum zinc and anemia due to erythropoietin deficiency (Karacan et al., 1978; Campese and Liu, 1990; Glass et al., 1987; Jünemann et al., 1990).

In our previous study, chronic renal failure in rabbits led to alteration in the (non-adrenergic, non-cholinergic) NANC-mediated and carbachol-induced (endothelium-dependent) relaxation of corpus smooth muscle in the rabbits and it is conceivable that this impairment of nitrergic responses may contribute to erectile dysfunction (Bagcivan et al., in press).

In the present study, we examined the effects of various more specific phosphodiesterase inhibitors on corpus cavernosum tissue from rabbits with chronic renal failure and compared the effects with those in controls.

2. Materials and methods

In this study, 20 male New Zealand white rabbits weighing 2.5–3 kg were used. The rabbits were divided into two groups. All rabbits were anaesthetized by an intramuscular injection with xylazine (5 mg/kg) and ketamine (20 mg/kg). Operations were performed with the rabbit in supine position and through a midline abdominal incision. Ten rabbits underwent a surgical procedure for the induction of uremia (2/3 partial excision of the left kidney and followed 2 weeks later with right nephrectomy) as previously described (chronic renal failure rabbits); the remaining 10 animals underwent sham operations (controls) (Gotloib et al., 1982). We accepted increases of both blood urea and creatinine levels as diagnostic of renal failure. Two weeks after the induction of uremia, the effect of chronic renal failure and sham operation on rabbit corpus cavernosum was assessed (Oreopoulos et al., 1992). Total penectomy was performed after 4 weeks in each group under general anesthesia by intravenous pentobarbital injection and exsanguination.

2.1. Preparation of corpus cavernosum tissue

All control and experimental rabbits were killed and the entire penis was removed at end of the 4-week period. A ventral incision was made on the right and left corpora, the tunica was dissected and the corpus cavernosum tissue was exposed and immediately placed in organ chambers. Each rabbit provided four strips of corpus cavernosum smooth muscle that were studied separately.

2.2. Organ chamber experiments

Strips of corpus cavernosum tissue measuring approximately $2 \times 2 \times 15$ mm were studied in 20-ml water-jacketed tissue baths containing physiological salt solution for isometric tension measurement. The strips were tied with silk to a force transducer (Grass FT 03, Quincy, MA) at one end and fixed with silk ties to a glass support at the other end. The transducer output was recorded on a Grass polygraph model 79E. The solution was gassed with 95% O_2 and 5% CO_2 during the study and the temperature was maintained at 37 °C by a thermoregulated water circuit. Resting load was set at 2 g, a value which was previously found to be optimal for measurement of changes in tension of rabbit corpus cavernosum preparations. The preparations were allowed to equilibrate in Krebs' bicarbonate for 1 h and during this time, Krebs' bicarbonate was replaced every 15 min with fresh solution. Potassium chloride (124 mmol/l) was added at the beginning of the experiments. After the tissues were washed, they were contracted with phenylephrine in submaximal concentration. After equilibration, the strips were contracted with phenylephrine (10^{-6}–3×10^{-6} M). These concentrations produced 70–80% of the maximal response to phenylephrine. After the phenylephrine-induced contraction had reached a plateau, the concentration–response relationships for papaverine, a non-specific phosphodiesterase inhibitor, (10^{-6}–3×10^{-4} M), sildenafil, a specific phosphodiesterase 5 inhibitor, (10^{-8}–3×10^{-4} M), zaprinast, a specific phosphodiesterase 5 inhibitor, (10^{-6}–3×10^{-4} M) or amrinone, a specific phosphodiesterase 3 inhibitor, (10^{-6}–10^{-4} M), were obtained by adding one of these agents to the bath in a cumulative manner. The tissues were washed for 15 min before adding vehicle. Only one agonist was tested in each of the strips.

2.3. Electrical-field stimulation

The strips were stimulated for 10 s with two parallel platinum electrodes at sequential frequencies of 2, 4, 8, 16, 32 and 64 Hz as square wave pulses of 50 V (0.8 ms) delivered by a current amplifier and a stimulator (S 88, Grass). The strips were allowed to return to the baseline precontractile tension between the tests at each frequency. In all studies, the duration of the electrical stimulation was 5 s. Before electrical-field stimulation, the tissue was treated

Table 1
General characteristics of chronic renal failure rabbits and controls with normal renal function (each group comprising 10 rabbits)

	Controls	Chronic renal failure rabbits
Body weight (kg)	2.60 ± 0.04	2.13 ± 0.01[a]
Serum creatinine (mg/dl)	1.23 ± 0.15	4.53 ± 0.12[a]
Serum urea (mg/dl)	26.33 ± 1.52	64.00 ± 2.00[b]
Total calcium (mg/dl)	14.03 ± 0.73	17.40 ± 0.15[b]
Serum phosphate (mg/dl)	4.03 ± 0.30	5.63 ± 0.70[b]
Serum sodium (mmol/l)	140 ± 1.00	145 ± 2.30
Serum potassium (mmol/l)	5.23 ± 0.15	5.30 ± 0.30
Serum cholesterol (mg/dl)	48 ± 1.32	50 ± 1.12
Serum triglycerides (mg/dl)	65 ± 0.13	64.23 ± 0.15
VLDL-c (mg/dl)	20.14 ± 1.00	21.18 ± 0.76
HDL-c (mg/dl)	18.34 ± 0.12	20.11 ± 0.56
Prolactine (ng/ml)	0.50 ± 0.07	0.50 ± 0.03
FSH (ml U/l)	0.13 ± 0.05	0.13 ± 0.01
LH (ml U/ml)	0.13 ± 0.07	0.11 ± 0.08
Testosterone (pg/ml)	86.33 ± 2.59	37.00 ± 2.56[b]
T3 (pg/ml)	0.43 ± 0.32	6.60 ± 0.37[a]
T4 (ng/ml)	0.10 ± 0.00	0.82 ± 0.05[a]
TSH (μU/ml)	0.10 ± 0.01	0.10 ± 0.2
Parathyroid hormone (pg/ml)	0.72 ± 0.12	5.00 ± 0.61[a]

Values are means ± S.D.
[a] $P<0.01$.
[b] $P<0.05$.

with an adrenergic nerve blocker, guanethidine (10^{-5} M), and a muscarinic receptor antagonist, atropine (10^{-6} M) for 30 min to eliminate the adrenergic and cholinergic components, and to determine the relaxation responses to the stimulation of non-adrenergic, non-cholinergic nerves. The complete blockade of electrically stimulated relaxation of corpus smooth muscle with the addition of an autonomic ganglion blocker, hexamethonium (10^{-4} M), is consistent with the hypothesis that electrically elicited relaxation is mediated by a NANC neuronal pathway. The relaxation responses elicited by electrical-field stimulation were abolished with 1 μM tetrodotoxin, suggesting that the relaxant stimulation was neurogenic. Relaxation was elicited with electrical-field stimulation after submaximal contraction with phenylephrine. The technique used for obtaining the responses to electrical-field stimulation was described previously (Bush et al., 1992). One strip from each rabbit was contracted with phenylephrine (10^{-5} M) and the second strip was kept unstimulated. Relaxant responses to electrical-field stimulation were compared to responses obtained from chronic renal failure rabbits.

2.4. Analysis of data and statistics

Experimental values were expressed as the means ± S.E.M. Relaxant effects of agonists were expressed as percentages of the precontraction in response to phenylephrine. In order to evaluate the effects of agonists; maximum responses (E_{max}) and pD_2 values (apparent agonist affinity constants; $-\log ED_{50}$) were calculated. The concentration–response data obtained in each experiment were plotted as the response–concentration (y) against the response (x). This produced a straight line relationship in each experiment, as predicted from the Scatchard equation for drug–receptor interaction.

Statistical comparisons between groups were performed using an unpaired Student's t-test. Probabilities of less than 5% ($P<0.05$) were considered significant.

2.5. Solution and drugs

The ionic composition of the Krebs' solution was as follows (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2. Fresh solutions were prepared on the day of the experiments. Sildenafil citrate was obtained from Fako, Turkey. Phenylephrine hydrochloride, amrinone, zaprinast, papaverine hydrochloride guanethidine, hexometonyum, L-arginine and N^G-nitro-L-arginine methyl ester were obtained from Sigma, St. Louis, MO, USA. Papaverine was dissolved in distilled water but amrinone, sildenafil and zaprinast were dissolved in dimethylsulfoxide. All drugs were prepared daily.

3. Results

Body weights, blood and hormone values for both groups prior to the operations and at the end of 4 weeks are shown in Table 1. The mean body weight of chronic renal failure rabbits was significantly lower than that of the controls ($P<0.05$). Serum creatinine, urea and serum calcium, and phosphate levels were significantly elevated in chronic renal failure rabbits ($P<0.05$). Other biochemical

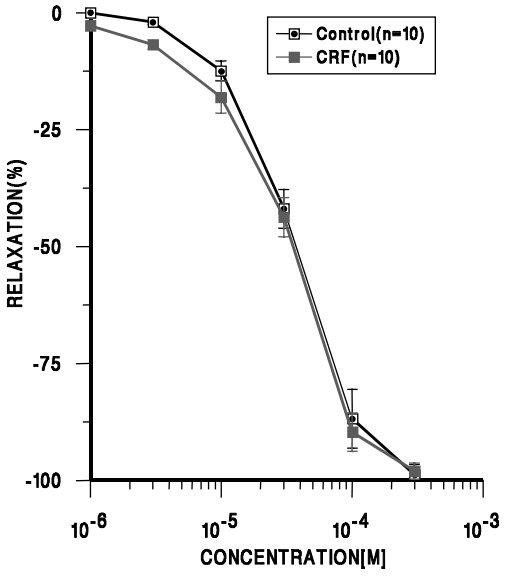

Fig. 1. Papaverine caused concentration-dependent relaxation in control and chronic renal failure rabbit corpus cavernosum strips precontracted with phenylephrine (10 μmol/l). Papaverine relaxed the cavernous tissue without a significant change in the E_{max} and pD_2 values in chronic renal failure and in control rabbits.

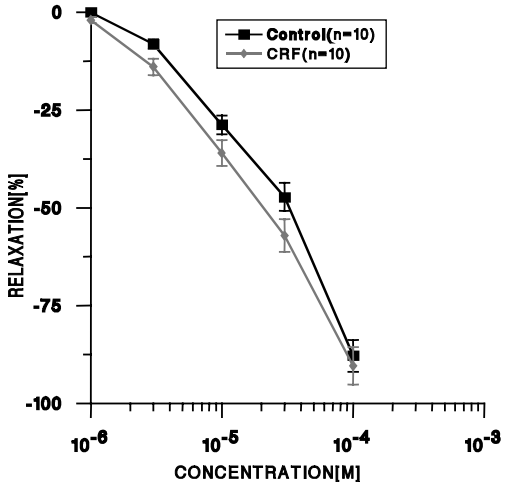

Fig. 2. Amrinone caused concentration-dependent relaxation in control and chronic renal failure rabbit corpus cavernosum strips precontracted with phenylephrine (10 μmol/l). Amrinone relaxed the cavernous tissue without a significant change in the E_{max} and pD_2 values in chronic renal failure and in control rabbits.

values were not changed. T3, T4, parathyroid hormone levels were significantly increased and testosterone levels were significantly decreased in chronic renal failure rabbits ($P<0.05$). Prolactin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH) levels were not changed. Plasma lipid levels remained in the normal range in both groups.

When tissues were contracted with 124 mmol/l KCl, similar tensions were achieved in both groups, with means (S.E.M.) contractions of 2148 ± 261 and 2281 ± 260 mg in controls and chronic renal failure rabbits.

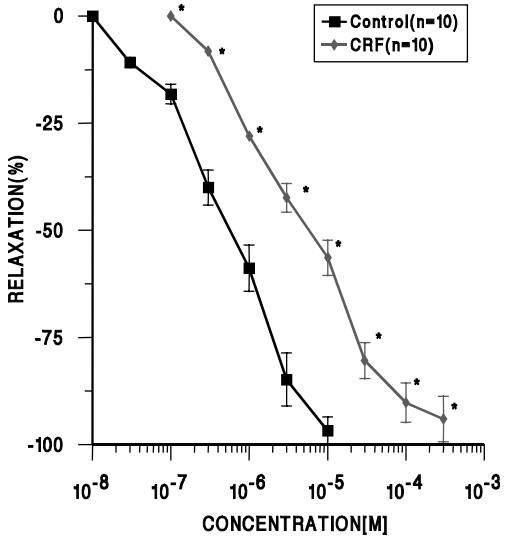

Fig. 3. Sildenafil caused concentration-dependent relaxation curves in control and chronic renal failure rabbit corpus cavernosum strips precontracted with phenylephrine (10 μmol/l). There was a significant decrease in the pD_2 values but no change in E_{max} values in the relaxation responses induced with sildenafil in the precontracted cavernosal tissues of chronic renal failure rabbits.

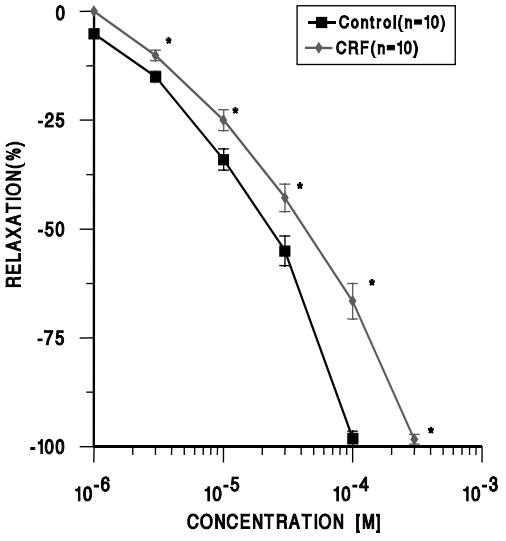

Fig. 4. Zaprinast caused concentration-dependent relaxation in control and in chronic renal failure rabbit corpus cavernosum strips precontracted with phenylephrine (10 μmol/l). Zaprinast decreased the pD_2 values but the effect was not statistically significant in chronic renal failure rabbits.

Papaverine, amrinone, sildenafil and zaprinast produced concentration-dependent relaxation in corpus cavernosum strips precontracted with phenylephrine in the controls and chronic renal failure rabbits (Figs. 2–4). In the control group, sildenafil was found to be most effective for relaxation. Zaprinast, amrinone and papaverine produced relaxation less potently did than sildenafil. Papaverine and amrinone relaxed the cavernosum tissue precontracted with phenylephrine without a significant change in the E_{max} and pD_2 values in chronic renal failure and control rabbits (Figs. 1 and 2, and Table 2) ($P>0.05$). There was a significant decrease in the pD_2 values but no change in E_{max} values for the relaxation responses induced with sildenafil was observed in the precontracted cavernosum tissues of chronic renal failure rabbits (Fig. 3 and Table 2) ($P<0.05$). Zaprinast decreased the pD_2 values but it was not significantly so

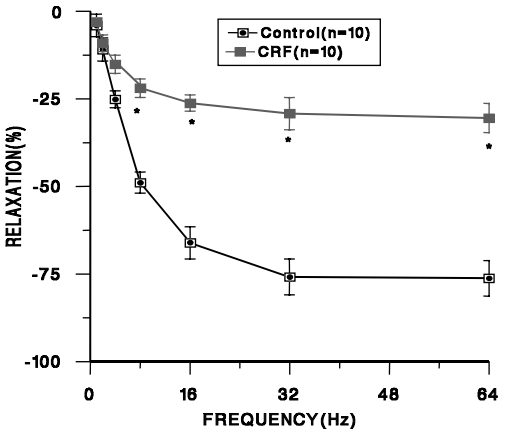

Fig. 5. Electrical-field stimulation caused frequency-dependent relaxation in control and chronic renal failure rabbit corpus cavernosum strips precontracted with phenylephrine (10 μmol/l).

in chronic renal failure rabbits (Fig. 4) ($P>0.05$). The application of N^G-nitro-L-arginine methyl ester (3×10^{-5} M), a potent inhibitor of NO synthesis, impaired the relaxation responses elicited by electrical-field stimulation and led to an increase in basal tension. This impairment of the relaxation response was restored by L-arginine (3×10^{-4} M) and it was concluded that the relaxation responses elicited by electrical-field stimulation were a nitrergically mediated activation. There were significant differences between control and chronic renal failure rabbits in electrical-field stimulation induced frequency-dependent relaxations (Fig. 5).

4. Discussion

As previously reported, the 5/6 nephrectomized (unilateral total nephrectomy plus contralateral 2/3 nephrectomy) chronic renal failure rabbit is a suitable model to investigate the effects of a uremic status (Oreopoulos et al., 1992). In our study, serum urea and creatinine levels were significantly elevated in chronic renal failure rabbits and neither parameter changed in the control group. T3, T4, parathyroid hormone levels were significantly increased and testosterone levels were significantly decreased in chronic renal failure rabbits. The results now presented demonstrate that, in corpus cavernosum tissue precontracted with phenylephrine, the relaxation responses to papaverine and amrinone were similar in the chronic renal failure rabbits and the control groups, but concentration–response curves obtained with sildenafil and zaprinast were shifted to the right and pD_2 values were decreased in chronic renal failure rabbits.

The effects of many hormones and neurotransmitters are mediated through specific receptors coupled to adenylate cyclase and guanylate cyclase (Azadzoi et al., 1992; Willis et al., 1981; Schoeffier and Stoclet, 1985; Trigo-Rocha et al., 1993) Phosphodiesterases inactivate cyclic nucleotides, and the three different phosphodiesterase isozymes in human erectile tissue, phosphodiesterase 3, 4 and 5 have been characterized (Taher et al., 1997). Theoretically, agents that enhance the NO-cGMP and/or cAMP signal transduction pathway such as nitrovasodilators or phosphodiesterase inhibitors may prove beneficial in the treatment of erectile dysfunction. However, papaverine, a non-specific phosphodiesterase inhibitor, causes pain during injection and may also cause prolonged erections and cavernous fibrosis in a significant number of patients (Truss et al., 1997). Recent basic research suggests that the concept of isoenzyme-selective phosphodiesterase inhibition may be applicable in the treatment of erectile dysfunction (Boolell et al., 1996a). Recently, a specific phosphodiesterase 5 inhibitor, sildenafil, was introduced in clinical studies. In studies of human corpus cavernosum, sildenafil enhanced the relaxation in response to electrical stimulation. It has a very definite effect on erectile activity in humans and sildenafil promotes penile erection through increased intracellular cGMP in response to sexual stimulation potentiating smooth muscle relaxation (Jeremy et al., 1997; Goldstein et al., 1998). Medina et al. (2000) suggested that the relaxation induced by sildenafil in the internal mammary artery, radial artery, and forearm vein does not support the proposal that the action of sildenafil is only dependent on preexisting activation of the NO-cGMP levels. They observed that the relaxation induced by sildenafil was evident in the absence of an NO donor in all four vessels and that the relaxation was amplified by adding sodium nitroprusside to the organ bath. In our study, despite chronic renal failure-induced impairment in the nitrergic system (Bagcivan et al., in press), sildenafil and zaprinast in chronic renal failure rabbit corporal strips caused the maximum relaxation responses with higher doses as compared to the controls indicating that the effect of these compounds is not solely dependent on preexisting activation of the NO-cGMP levels.

Zaprinast, cGMP-specific phosphodiesterase inhibitor, relaxed corpus cavernosum with pD_2 values similar to those for papaverine (Thompson, 1991). In dogs, intracavernous injection of zaprinast enhanced the tumescence induced by pelvic nerve stimulation and was found to enhance the relaxations caused by electric field stimulation (Lincoln, 1989; Trigo-Rocha et al., 1993). These observations suggest that the relaxant effect of zaprinast is related with the NO pathway.

In the present study, when we compared the relaxant responses to papaverine and amrinone in the cavernosum tissue strips precontracted with phenylephrine in the control and chronic renal failure rabbits in vitro, there was no difference between the two groups. However, sildenafil and zaprinast concentration–response curves were shifted to the right and pD_2 values were decreased in chronic renal failure rabbits. Our previous study with the corpus caverno-

Table 2
Maximum relaxation response (E_{max} % of phenylephrine-induced contraction) and pD_2 values on exposure to agonists in strips of corpus cavernosum obtained from the chronic renal failure rabbits and controls

	Control ($n=10$)	Chronic renal failure rabbits ($n=10$)
Sildenafil		
E_{max}	98.6 ± 6.20	97.2 ± 5.80
pD_2	6.15 ± 0.044	5.09 ± 0.071
Zaprinast		
E_{max}	98.3 ± 4.5	98.1 ± 4.0
pD_2	4.84 ± 0.056	4.51 ± 0.065
Amrinone		
E_{max}	87.8 ± 5.1	90.4 ± 4.8
pD_2	4.68 ± 0.055	4.46 ± 0.080
Papaverine		
E_{max}	98.0 ± 2.3	97.2 ± 1.75
pD_2	4.60 ± 0.063	4.68 ± 0.070
Electrical-field stimulation		
E_{max}	76.2 ± 5.1	30.5 ± 4.2[a]

[a] $P<0.05$.

sum smooth muscle indicated that chronic renal failure impaired NANC-mediated and carbachol-induced (endothelium-dependent) relaxation of corporal smooth muscle, whereas relaxation with sodium-nitroprusside, an endothelium-independent agent, was not impaired in chronic renal failure rabbits (Bagcivan et al., in press). These results of a previous study suggested that the NO-cGMP pathway was involved in chronic renal failure rabbits. The impairment of endothelium-mediated and NANC-mediated relaxation of cavernosum smooth muscle seems to play a more important role in the pathogenesis of uremic impotence rather than does the problem of the smooth muscle itself.

In this study, sildenafil and zaprinast concentration–response curves were shifted to the right and pD_2 values were decreased in chronic renal failure rabbits but decreased pD_2 values with zaprinast were not statistically significant. It can be speculated that these results may arise from the impairment of spontaneous NO release in tissue because of significant impairment in NANC-mediated and carbachol-induced (endothelium-dependent) relaxation responses of corpus smooth muscle in chronic renal failure rabbits. In the present study, there were no changes in E_{max} values with sildenafil and zaprinast in chronic renal failure rabbits (Figs. 3 and 4, and Table 2). These results suggest that the decreased production or availability of endogenous NO in chronic renal failure animals leads to decreased efficacy of phosphodiesterase 5 inhibitors to induce relaxation.

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