Journal of Andrology, Vol. 26, No. 2, March/April 2005

Rho-kinase Inhibition Improves Erectile Function in Aging Male Brown-Norway Rats

MAHADEVAN RAJASEKARAN, STEVEN WHITE, ANGELO BAQUIR, AND NATHAN WILKES

From the Division of Urology, University of California San Diego Medical Center, San Diego, California, and the Urology Research Laboratory, Veterans Medical Research Foundation, La Jolla, California.

ABSTRACT: Physiological aging is a significant risk factor in the onset of male erectile dysfunction (ED) and an imbalance in factors that modulate cavernosal smooth-muscle tone may play a role in these altered penile hemodynamic mechanisms. To evaluate the association between aging and male erectile function, we monitored neurogenic erectile response and its correlation to systemic arterial pressure changes in old (21–23 months of age) vs young (6–9 months of age) Brown-Norway (BN) rats. We tested the hypothesis that age-associated ED is due to unregulated vasoconstrictive tone, contributed in part by an increased Rho-kinase activity, and that antagonism of Rho-kinase activity attenuates the age-related decline in male erectile function. We also examined the hypothesis that a combination of Rho-kinase antagonism and phosphodiesterase-5 (PDE-5) inhibition has a synergistic effect in improving the erectile response in these aging animals. Erectile function in old BN rats was evaluated before and after intracavernosal injection of a specific inhibitor of Rho-kinase (Y-27632) alone or in combination with zaprinast, a PDE-5 inhibitor. Erectile capabilities of the young and old BN rat groups were significantly different in corpus cavernosum pressure response after electrical-field stimulation of the major pelvic ganglion. Y-27632 administration attenuated the aging-related changes in male erectile function seen in BN rats. Rho-kinase antagonism and PDE-5 inhibition had a synergistic effect in improving erectile function in old rats. Our data indicate that aging leads to impairment in the neurogenic erectile response in BN rats involving a possible derangement in penile hemodynamic mechanisms of the erectile tissue. Rho-kinase inhibition may be of value in treating age-related ED.

Key words: Muscle, smooth, drug effects, penis physiology, biological aging, penile erection.

J Androl 2005;26:182–188

Aging is a well-recognized risk factor for male erectile dysfunction (ED), a highly prevalent problem that affects 10% to 52% of men (Feldman et al, 1994). An age-associated decline in male hormone levels, decreased nitric oxide (NO) production, increased contractility of the smooth muscle of the penile corpora, a decreased relative percentage of smooth-muscle fibers, and increased collagen content are considered possible etiologic factors (Andersson, 2003). Normal erectile function is characterized by a delicate in vivo balance between vasoconstricting and vasorelaxing mediators on corporal smooth-muscle tone (Taub et al, 1993). Endothelium-derived NO and endothelin-1 (ET-1) have been recognized to modulate erectile function. NO is a key modulator of cavernosal smooth-muscle relaxation, whereas ET-1 is believed to maintain penile flaccidity (Saenz de Tejada et al, 1991; Burnett, 1995). Evidence suggests there are age-related alterations in the levels of these modulators of erectile function (Garban et al, 1995b; Carrier et al, 1997; Dahiya et al, 1999; Rajasekaran et al, 2002). While most studies examining the role of aging on erectile function have focused on the impact of aging on NO signaling pathways, few studies have examined the role of other regulators in maintaining penile flaccidity.

Besides the well-established noradrenergic contraction mechanisms in the penis, an additional mechanism involving increased sensitivity to ionic calcium has recently been proposed (Chitaley et al, 2001; Wang et al, 2002). This pathway involves RhoA, a small monomeric G-protein that activates Rho-kinase. Activated Rho-kinase phosphorylates the regulatory subunit of smooth-muscle myosin phosphatase (SMPP-IM). Inhibitory phosphorylation of SMPP-IM leads to sensitization of myofilaments to Ca^{2+} (Somlyo and Somlyo, 2000). An age-related increase in RhoA expression has been documented in rat vascular tissues and has been felt to be responsible for age-associated vascular disorders (Miao et al, 2001). A specific inhibitor of Rho-kinase, (+)-(R)-transB (1-amino-ethyl)-N-(4-pyridyl) cyclohexacarboxamide dihydrochloride monohydrate (Y-27632), has been shown to relax vascular and nonvascular smooth muscle, and intracavernosal injection of Y-27632 in rats has been shown to induce penile erection (Chitaley et al, 2001). We have recently demonstrated that hypertension, another vascular

Supported in part by a new investigator award (M.R.) from the Stein Institute of Research on Aging (SIRA), University of California, San Diego, and by the American Foundation for Urologic Disease/American Urological Association summer scholarship award (S.W.).

Correspondence to: Dr Mahadevan Rajasekaran, c/o Miriam Seva, Division of Urology, UCSD Medical Center, 200 West Arbor Dr (8897), San Diego, CA 92103-8897 (e-mail: mseva@ucsd.edu).

Received for publication July 14, 2004; accepted for publication September 20, 2004.

risk factor for ED, is associated with elevated penile RhoA levels and that inhibition of Rho-kinase activity with Y-27632 was beneficial in attenuating the decline in erectile function in hypertensive rats (Wilkes et al, 2004). In addition, Rho-kinase inhibition has been shown to be effective in reversing ED in castrated hypogonadal rats (Wingard et al, 2003). The impact of Rho-kinase inhibition in age-related ED has not been explored.

The present study was designed to monitor age-related alterations in male erectile function and specifically to evaluate the role of Rho-kinase pathway in the Brown-Norway (BN) rat, a sensitive animal model of reproductive aging. We also examined the hypotheses that antagonism of Rho-kinase activity attenuates the age-related decline in male erectile function and that a combination of Rho-kinase antagonism and phosphodiesterase-5 (PDE-5) inhibition has a synergistic effect in improving the erectile response in these aging animals.

Materials and Methods

Animals and Experimental Design

Our Institutional Animal Care and Use Committee approved this experimental protocol. Male young (age 6–9 months) and old (21–23 months) BN rats were obtained from the National Institute on Aging animal facilities and employed as a model for aging. The rats were anesthetized using an intraperitoneal injection of a mixture of ketamine (97 mg/kg) and xylazine (13 mg/kg); an adequate level of anesthesia was maintained by supplemental doses of ketamine. Arterial blood pressure (AP) was monitored by cannulating the carotid artery with Intramedic PE 50 tubing (Becton, Dickinson, and Company, Franklin Lakes, NJ) connected to a pressure transducer. Corpus cavernosum pressures (CCP) were recorded by cannulating either of the corporal bodies approximately 3 to 5 mm above the base of the penis. This cannulation was performed using a 25-gauge IV butterfly needle filled with 100 U/mL heparinized saline (Vacutainer; Becton, Dickinson, and Company), which was also connected to a pressure transducer. Pressures were measured using 2 UFI pressure transducers (UFI, Morrobay, Calif) in series with a UFI amplifier. The data were acquired by connecting the amplifier to a PC running Labview (National Instruments Corporation, Austin, Tex). Pressure transducers were calibrated daily.

The major pelvic ganglion (MPG) was identified by dissecting the fibrous capsule posterior to the intersection of the lateral lobes of the prostate and the vas deferens with cotton tips. The MPG was stimulated with a voltage via 2 platinum electrodes delivered from a Grass S48 stimulator (Grass-Telefactor, West Warwick, RI). Stimulus levels of 1.5, 3, and 4.5 V were used for durations of 1 minute each; the rats were given a 5-minute rest period between stimulations. CCP levels during stimulation were recorded directly into a computerized data acquisition program.

$$MAP = \frac{1}{T}\sum_{0}^{T} P(t)\Delta t;$$

In this formula, $P(t)$ denotes the pressure wave data as a function of time and Δt is the time increments between data points. T is the period of the pressure wave. The Σ symbol is used to denote that the pressure data is incremental and not continuous.

We employed 3 protocols to test our hypotheses.

Protocol 1—The impact of aging as well as the correlation of blood pressure to electrical field stimulation (EFS)-induced erectile response was evaluated in young and old rats by stimulating the MPG at 1.5, 3.0, and 4.5 V and recording AP and CCP as described above.

Protocol 2—To test the hypothesis that the specific Rho-kinase antagonist Y-27632 (Calbiochem, San Diego, Calif) attenuates the age-related decline in male erectile function, old animals were subjected to Protocol 1 followed by the intracavernosal administration of Y-27632 (50 nmol). After 10 to 15 minutes, Protocol 1 was repeated.

Protocol 3—To test the hypothesis that a combination of Rho-kinase antagonism and PDE-5 inhibition may have a synergistic effect in improving the erectile response in old animals, we subjected the old animals to Protocol 1 and then to the intracavernosal administration of the PDE-5 inhibitor zaprinast (Sigma Chemicals, St Louis, Mo) (100 μg) followed by Y-27632 (50 nmol). After 10 to 15 minutes, Protocol 1 was repeated.

All drug solutions were stored in a freezer in amber bottles; working solutions were prepared on a frequent basis and kept cold until injection time. In both old and young animals, the drugs were administered intracavernosally in small volumes (5 μL) when CCP was at a baseline value. The effect of a single injection agent on CCP was measured until CCP returned to the preinjection level. The next injection was made at least 10 to 15 minutes after CCP had returned to a stable baseline. An injection of 5 μL of saline vehicle had no significant sustained effect on CCP (Wilkes et al, 2004).

Statistical Analysis

Data were presented as mean ± standard error of the mean. Statistical differences were determined by ANOVA followed by Newman-Keuls multiple comparison test, where relevant, and by 2-tailed unpaired Student's *t* test. A *P* value of less than .05 was considered significant.

Results

Comparison of EFS-Induced Erectile Response in Young and Old BN Rats

We selected voltages of 1.5, 3, and 4.5 V for the present evaluations based on our preliminary experiments in BN rat species; voltages lower than 1.5 V failed to produce a consistent response. During resting periods in the absence of stimulation, the baseline CCP remained stable throughout the experiment. The CCP and CCP/mean arterial pressure (MAP) data are summarized in the Table. Representative tracings of CCP and MAP in young and old rats are depicted in Figure 1a and b. The effects of EFS of the MPG on CCP changes are illustrated in Figure 2a and b.

Our results showed a significant difference between old

*CCP and CCP/MAP changes in young and old Brown-Norway rats**

Species/Treatment	1.5 V		3 V		4.5 V	
	CCP cm H_2O	CCP/MAP	CCP cm H_2O	CCP/MAP	CCP cm H_2O	CCP/MAP
Young/EFS	41.07 ± 8.18	0.806 ± 0.138	64.66 ± 5.08	0.816 ± 0.072	70.50 ± 7.10	0.875 ± 0.082
Old/EFS	27.19 ± 6.61†	0.227 ± 0.094†	42.33 ± 5.74†	0.5384 ± 0.065†	33.20 ± 4.16†	0.586 ± 0.023†
Old + Y-27632/EFS	51.14 ± 7.87‡	0.678 ± 0.123‡	64.51 ± 1.23‡	0.762 ± 0.051‡	65.58 ± 12.87‡	0.852 ± 0.050‡
Old + ZAP + Y-27632/EFS	66.21 ± 8.52§	0.85 ± 0.01§	84.69 ± 10.34§	0.97 ± 0.07§	76.19 ± 6.53§	0.95 ± 0.03§

* CCP indicates corpus cavernosum pressures; MAP, mean arterial pressure; EFS, electrical field stimulation; and ZAP, zaprinast.
† $P < .05$ when compared with young group.
‡ $P < .05$ when compared with untreated old group.
§ $P < .05$ when compared with untreated old group.

and young rats in ganglionic stimulation-induced CCP changes. A striking difference in maximum CCP value as well as in the ratio of CCP to MAP was noticed between the groups at all stimulation levels (the Table; Figure 2a and b). At 1.5 V, the young rats achieved about 34% higher CCP than the old group (41.07 ± 8.18 and 27.19 ± 6.60 in the young and old groups, respectively; $P <$ .05). At 3 V, the mean CCP in the young group was 64.66 ± 5.08 when compared with 42.33 ± 5.74 cm H_2O in old rats (the young group achieved 35% higher CCP at this stimulation than their older counterparts; $P < .05$). Stimulations at 4.5 V showed a more pronounced difference than the 3-volt stimulation. The mean CCP in young rats was 53% greater than that in old rats (the CCPs were 70.50 ± 7.10 and 33.20 ± 4.16 for the young and old groups, respectively; $P < .05$) (Figure 2a).

A similar trend was observed for the CCP/MAP index. The differences in CCP/MAP ratio between young and old animals were significant ($P < .05$) at all the indicated stimulation levels and ranged between 33% and 71% (Table; Figure 2b).

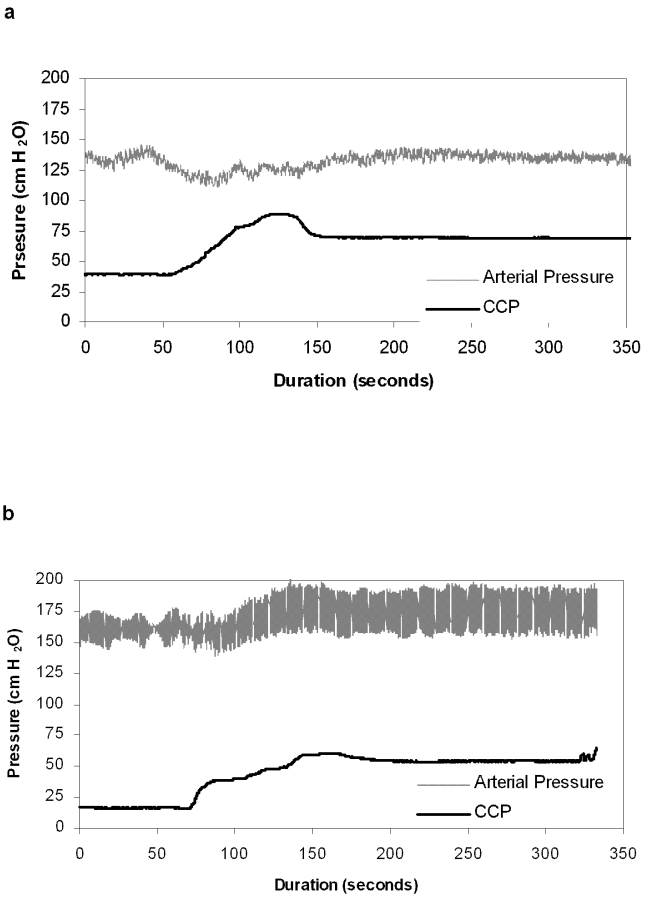

Figure 1. Representative tracings of corpus cavernosum pressures (CCP) and mean arterial pressure (MAP) changes in response to electrical field stimulation (EFS) of the major pelvic ganglion (MPG) in **(a)** young and **(b)** old Brown-Norway (BN) rat species at 3-V stimulation. Duration of erectile response (seconds) is indicated in the X-axis and CCP change (cm H_2O) is shown in the Y-axis.

Efficacy of Rho-Kinase and PDE-5 Inhibition on Neurogenic Erectile Response in Aging Rats

The effect of Rho-kinase inhibition on EFS-induced erectile response in old rats as measured by changes in CCP is illustrated in Figure 3a and b. In old rats, the impact of Rho-kinase inhibition following intracavernosal Y-27632 could be seen immediately. A significant improvement in erectile response was observed at all voltage levels of stimulation (Figure 3a; Table). In Y-27632-injected old rats, at 1.5 V, the CCP was 51.14 ± 7.87 and the mean CCP/MAP was 0.678 ± 0.123 as compared with the predrug stimulation values of 27.19 ± 6.61 and 0.227 ± 0.094 ($P < .05$). At 3 V, the Y-27632-treated old rats achieved a CCP of 64.51 ± 1.23 and a CCP/MAP of 0.762 ± 0.051 ($P < .05$ when compared with the predrug stimulation values). At 4.5 V, the Y-27632-treated rats had a mean CCP of 65.58 ± 12.87 and a mean CCP/MAP of 0.852 ± 0.050 ($P < .05$ when compared with their predrug stimulation values).

A combination of Rho-kinase inhibition with Y-27632 and PDE-5 inhibition with zaprinast resulted in a synergistic improvement in neurogenic erectile response in old rats (Figure 3a and b). Representative tracings of CCP changes are shown in Figure 4. This combination produced a consistent 12% to 27% improvement in erectile response when compared with the Rho-kinase inhibition

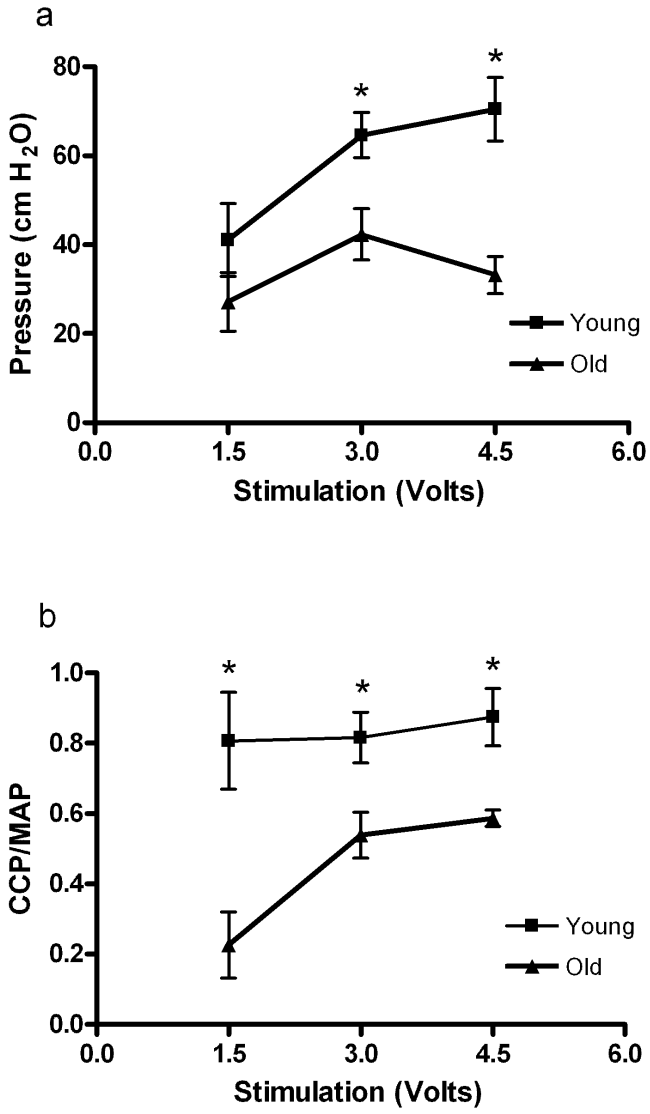

Figure 2. **(a)** The effects of electrical field stimulation (EFS) of the major pelvic ganglion (MPG) on the erectile response shown as corpus cavernosum pressures (CCP) changes (cm H_2O) in young and old rats and reported as mean ± standard error of responses (n = 7–12 experimental animals/group). Significant differences between young and old rats in EFS-induced CCP changes were observed at 3- ($P < .05$) and 4.5- ($P < .05$) V stimulations. **(b)** The effects of EFS of the MPG on the erectile response shown as the ratio of CCP to mean arterial pressure (MAP) (cm H_2O) changes in young and old rats and reported as mean ± standard error of responses (n = 3–6 experimental animals/group). Significant differences in CCP/MAP ratios in young vs old rats were observed at 1.5-, 3- ($P < .01$), and 4.5- ($P < .01$) V stimulations.

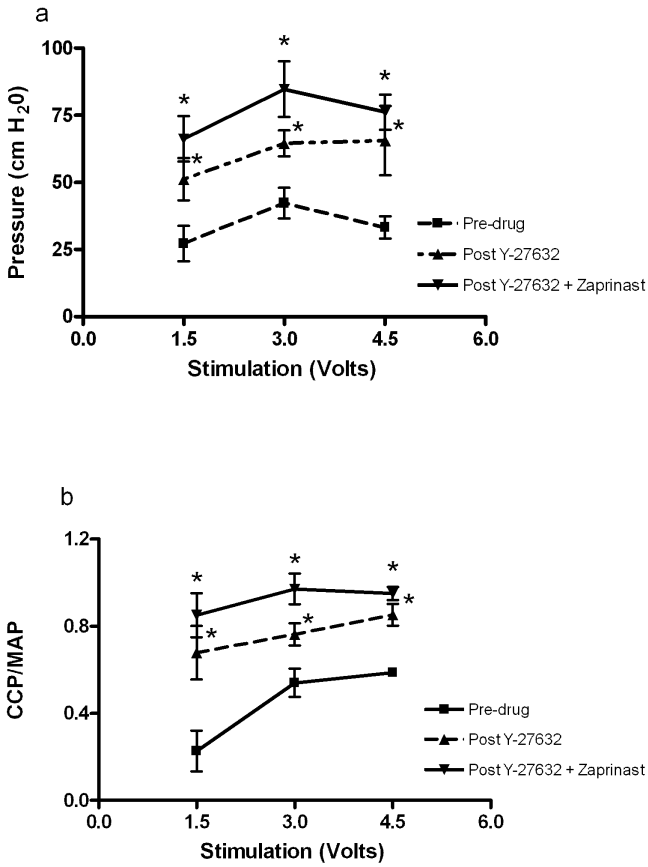

Figure 3. Effects of intracavernosal injection of Y-27632 (50 nmol) and zaprinast (100 μg) on neurogenic erectile response following electrical field stimulation (EFS) of the major pelvic ganglion (MPG) in old rats represented as changes in **(a)** corpus cavernosum pressures/mean arterial pressure (CCP)/(MAP) (mm Hg) and **(b)** CCP, reported as mean ± standard error of responses (n = 7–12 experimental animals/ group). Significant differences in EFS-induced CCP changes in young vs old rats were observed at 1.5-, 3-, and 4.5-V stimulations.

alone (Figure 3a and b; Table). The mean increase in CCP and the index of CCP/MAP were significant at all the levels of stimulation ($P < .05$).

Evaluation of Systemic Arterial Pressure Changes

MAP in old rats ranged from 120 to 150 cm H_2O, whereas young rats had a MAP range of 110 to 140 cm H_2O. Changes in MAP following MPG stimulation are illustrated in Figure 5a. There was a voltage-dependent decrease in MAP in both groups of animals; however, there was no significant difference in these changes between the groups at any voltage (Figure 5a). Intracavernosal injection of Y-27632 and zaprinast resulted in a marginal (20–25 cm H_2O) drop in MAP in old rats (not significant; Figure 5b).

Discussion

The findings in this study demonstrate that aging is associated with significant impairment of erectile function in a rat model of reproductive aging. We found 1) that there are differences in erectile response to ganglionic stimulation between young and old BN rats, 2) improvement of EFS-induced erectile response after RhoA/Rho-kinase inhibition, and 3) synergistic potentiation of erec-

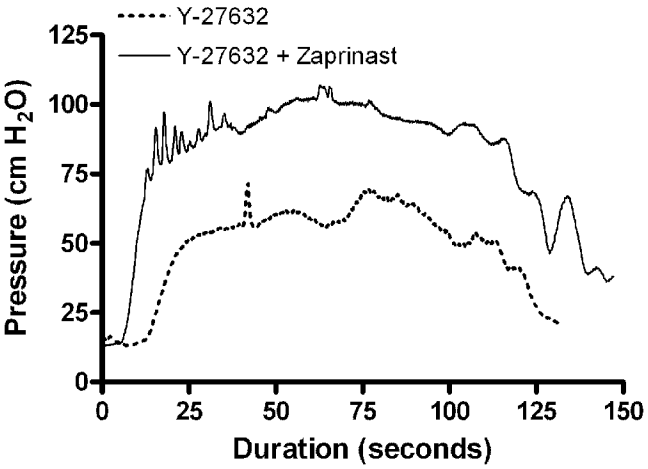

Figure 4. Representative tracings of corpus cavernosum pressures (CCP) and mean arterial pressure (MAP) changes in response to electrical field stimulation (EFS) of the major pelvic ganglion (MPG) at 3-V stimulation following the intracavernosal injection of Y-27632 (50 nmol) and zaprinast (100 μg) in old rats. Duration of erectile response (seconds) is indicated in the X-axis and CCP change (cm H_2O) is shown in the Y-axis.

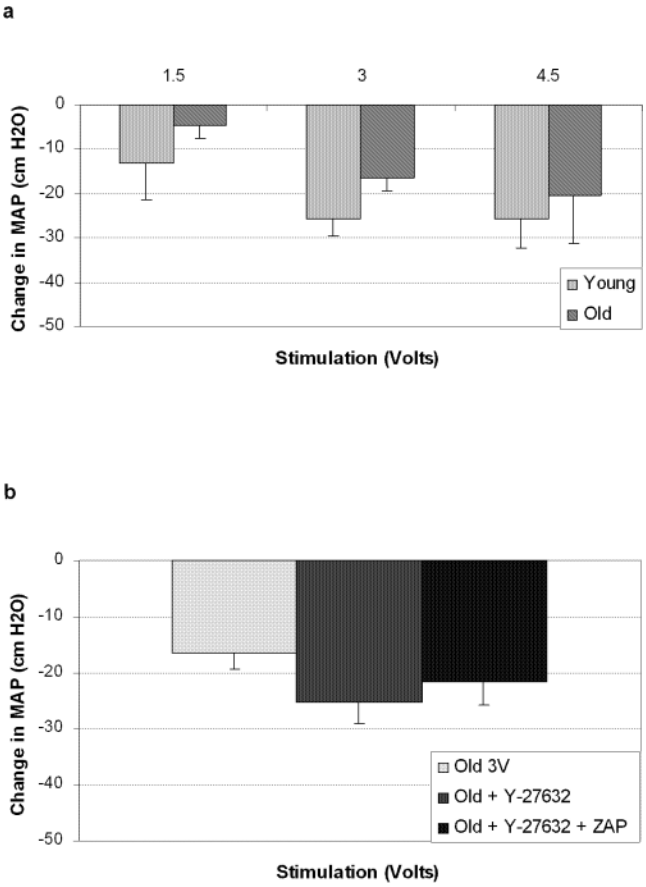

Figure 5. **(a)** Mean changes in mean arterial pressure (MAP) from baseline MAP in response to electrical field stimulation (EFS) of the major pelvic ganglion (MPG) in young and old rats. Each bar represents the mean ± standard error of 7 to 12 individual measurements. **(b)** Maximal changes in MAP in response to EFS of the MPG before and after intracavernosal injection of Y-27632 (50 nmol) and zaprinast (100 μg) in old rats. Each bar represents the mean ± standard error of 7 to 12 individual measurements.

tile response following RhoA/Rho-kinase inhibition and PDE-5 antagonism in old rats.

The impact of aging on male sexual function has been the focus of several previous investigations (Garban et al, 1995a,b; Carrier et al, 1997; Champion et al, 1999; Dahiya et al, 1999; Rajasekaran et al, 2002; Bivalacqua et al, 2003). These studies suggest that age-related physiological changes in the penis, such as a decline in male hormone levels, decreased NO production, increased contractility of the smooth muscle of the penile corpora, a decreased relative percentage of smooth-muscle fibers, and increased collagen content, may contribute to development of ED in this population. While most of the previous studies on the role of aging on erectile function have focused on the NO-cGMP pathway, little emphasis has been placed on other regulators of erectile function. We were particularly interested in exploring the contribution of a novel calcium-sensitizing RhoA/Rho-kinase pathway to the aging-related decline in erectile function.

The RhoA signaling pathway, through activation of Rho-kinase leading to inhibition of myosin phosphatase resulting in an increase in myosin light chain (MLC) phosphorylation and force in smooth muscle, has been recognized to play a role in several vascular disorders (Chrissobolis and Sobey, 2001; Mukai et al, 2001). Inhibiting the RhoA/Rho-kinase pathway has been shown to cause erection in rats (Chitaley et al, 2001; Mills et al, 2001). Y-27632 has been shown to selectively inhibit the contractile activity associated with RhoA/Rho-kinase without stimulating vasorelaxation directly (Mills et al, 2002). NO has recently been shown to inhibit RhoA-mediated vasoconstriction and produce an erectile response in rats, possibly through inhibition of RhoA migration into the smooth muscle (Mills et al, 2002). Lower bioavailability of NO in the aging penis would lead to a reduction in RhoA migration to the membrane as well as cGMP-mediated Rho-kinase inactivation. The net result would be an increased penile smooth-muscle tone, which may be responsible for the impaired erectile response seen in the aging rats. Inhibition of the RhoA/Rho-kinase pathway has been shown to improve erectile function in rat models associated with other ED risk factors such as hypertension and hypogonadism. We have recently demonstrated that inhibition of Rho-kinase activity with Y-27632 was beneficial in attenuating the decline in erectile function in hypertensive rats (Wilkes et al, 2004). Wingard et al (2003) have shown that Rho-kinase inhibition was effective in reversing ED in castrated hypogonadal rats. An age-related increase in RhoA expression has been

recently documented in rat vascular tissues, and this has been implicated in age-associated vascular disorders (Miao et al, 2001). Our results in the present study confirm these observations on the role of the Rho-kinase pathway in male erectile function and further suggest that Rho-kinase inhibitors could be of benefit in the treatment of age-related ED.

As aging is known to be associated with alterations in the NO-cGMP pathway as well as the Rho-kinase pathway (Garban et al, 1995b; Carrier et al, 1997; Miao et al, 2001; Rajasekaran et al, 2002), we extended our studies to examine whether a combination of PDE-5 inhibition, which would prevent cGMP degradation, and Rho-kinase antagonism, which would reduce the penile smooth-muscle tone, could result in a synergistic improvement of erectile response in aging BN rats. Furthermore, recent evidence suggests interplay between the NO-cGMP-cAMP and RhoA/Rho-kinase pathways (Kim et al, 2000; Abdel-Latif, 2001; Sauzeau et al, 2000; Sakai et al, 2003). Sakai et al (2003) reported that accumulation of cAMP in cells inhibited the RhoA activation in tracheal smooth muscle. Using vascular smooth-muscle cells, Sauzeau et al (2000) demonstrated that cGMP inhibited RhoA-dependent calcium sensitization. This inactivation of the RhoA/Rho-kinase pathway has been proposed to be mediated via cyclic nucleotide-dependent protein kinases through phosphorylation of RhoA (Sauzeau et al, 2000; Abdel-Latif, 2001). These observations suggest that interactions between cyclic nucleotide signaling systems and the Rho-kinase pathways may be responsible for the observed drug synergism between zaprinast and Y-27632. These results further suggest that this combination warrants exploration for its potential to improve the erectile response in severe cases of ED, where a modest 41% improvement has been reported with PDE-5 inhibition alone (Jarow et al, 1999). In addition, Rho-kinase inhibition may be explored as an alternate form of therapy for patients to whom PDE-5 inhibitors cannot be administered; for example, those on antihypertensive medications such as nitrites. This novel experimental drug Y-27632 has a significant potential to evolve as an alternative medication to oral medications, such as Viagra, for a distinct segment of the large, aging-ED patient population.

In conclusion, the BN rat strain may serve as an ideal animal model for the evaluation of age-associated ED in humans. Rho-kinase inhibition may be a novel therapeutic target in age-related male ED. Combination therapy with PDE-5 and Rho-kinase inhibition also merits therapeutic exploration.

Acknowledgment

The authors wish to acknowledge Daniel Cosgrove for his technical support.

References

Abdel-Latif AA. Cross talk between cyclic nucleotides and polyphosphoinositide hydrolysis, protein kinases, and contraction in smooth muscle. *Exp Biol Med (Maywood).* 2001;226:153–163.

Andersson KE. Erectile physiological and pathophysiological pathways involved in erectile dysfunction. *J Urol.* 2003;170:S6–S13; discussion S13–S14.

Bivalacqua TJ, Armstrong JS, Biggerstaff J, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ, Champion HC. Gene transfer of extracellular SOD to the penis reduces O2-* and improves erectile function in aged rats. *Am J Physiol Heart Circ Physiol.* 2003;284:H1408–H1421.

Burnett AL. Role of nitric oxide in the physiology of erection. *Biol Reprod.* 1995;52:485–489.

Carrier S, Nagaraju P, Morgan DM, Baba K, Nunes L, Lue TF. Age decreases nitric oxide synthase-containing nerve fibers in the rat penis. *J Urol.* 1997;157:1088–1092.

Champion HC, Bivalacqua TJ, Hyman AL, Ignarro LJ, Hellstrom WJ, Kadowitz PJ. Gene transfer of endothelial nitric oxide synthase to the penis augments erectile responses in the aged rat. *Proc Natl Acad Sci U S A.* 1999;96:11648–11652.

Chitaley K, Wingard CJ, Clinton Webb R, Branam H, Stopper VS, Lewis RW, Mills TM. Antagonism of Rho-kinase stimulates rat penile erection via a nitric oxide-independent pathway. *Nat Med.* 2001;7:119–122.

Chrissobolis S, Sobey CG. Evidence that Rho-kinase activity contributes to cerebral vascular tone in vivo and is enhanced during chronic hypertension: comparison with protein kinase C. *Circ Res.* 2001;88:774–779.

Dahiya R, Chui R, Perinchery G, Nakajima K, Oh BR, Lue TF. Differential gene expression of growth factors in young and old rat penile tissues is associated with erectile dysfunction. *Int J Impot Res.* 1999;11:201–206.

Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J Urol.* 1994;151:54–61.

Garban H, Marquez D, Cai L, Rajfer J, Gonzalez-Cadavid NF. Restoration of normal adult penile erectile response in aged rats by long-term treatment with androgens. *Biol Reprod.* 1995a;53:1365–1372.

Garban H, Vernet D, Freedman A, Rajfer J, Gonzalez-Cadavid N. Effect of aging on nitric oxide-mediated penile erection in rats. *Am J Physiol.* 1995b;268:H467–H475.

Jarow JP, Burnett AL, Geringer AM. Clinical efficacy of sildenafil citrate based on etiology and response to prior treatment. *J Urol.* 1999;162:722–725.

Kim NN, Huang Y, Moreland RB, Kwak SS, Goldstein I, Traish A. Cross-regulation of intracellular cGMP and cAMP in cultured human corpus cavernosum smooth muscle cells. *Mol Cell Biol Res Commun.* 2000;4:10–14.

Miao L, Calvert JW, Tang J, Parent AD, Zhang JH. Age-related RhoA expression in blood vessels of rats. *Mech Ageing Dev.* 2001;122:1757–1770.

Mills TM, Chitaley K, Lewis RW, Webb RC. Nitric oxide inhibits RhoA/Rho-kinase signaling to cause penile erection. *Eur J Pharmacol.* 2002;439:173–174.

Mills TM, Chitaley K, Wingard CJ, Lewis RW, Webb RC. Effect of Rho-kinase inhibition on vasoconstriction in the penile circulation. *J Appl Physiol.* 2001;91:1269–1273.

Mukai Y, Shimokawa H, Matoba T, Kandabashi T, Satoh S, Hiroki J, Kaibuchi K, Takeshita A. Involvement of Rho-kinase in hypertensive vascular disease: a novel therapeutic target in hypertension. *FASEB J.* 2001;15:1062–1064.

Rajasekaran M, Kasyan A, Jain A, Kim SW, Monga M. Altered growth

factor expression in the aging penis: the Brown-Norway rat model. *J Androl*. 2002;23:393–399.

Saenz de Tejada I, Carson MP, de las Morenas A, Goldstein I, Traish AM. Endothelin: localization, synthesis, activity, and receptor types in human penile corpus cavernosum. *Am J Physiol*. 1991;261:H1078–H1085.

Sakai J, Oike M, Hirakawa M, Ito Y. Theophylline and cAMP inhibit lysophosphatidic acid-induced hyperresponsiveness of bovine tracheal smooth muscle cells. *J Physiol*. 2003;549:171–180.

Sauzeau V, Le Jeune H, Cario-Toumaniantz C, et al. Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca2+ sensitization of contraction in vascular smooth muscle. *J Biol Chem*. 2000;275:21722–21729.

Somlyo AP, Somlyo AV. Signal transduction by G-proteins, Rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *J Physiol*. 2000;522(pt 2):177–185.

Taub HC, Lerner SE, Melman A, Christ GJ. Relationship between contraction and relaxation in human and rabbit corpus cavernosum. *Urology*. 1993;42:698–704.

Wang H, Eto M, Steers WD, Somlyo AP, Somlyo AV. RhoA-mediated Ca2+ sensitization in erectile function. *J Biol Chem*. 2002;277: 30614–30621.

Wilkes N, White S, Stein P, Bernie J, Rajasekaran M. Phosphodiesterase-5 inhibition synergizes Rho-kinase antagonism and enhances erectile response in male hypertensive rats. *Int J Impot Res*. 2004;16:187–194.

Wingard CJ, Johnson JA, Holmes A, Prikosh A. Improved erectile function after Rho-kinase inhibition in a rat castrate model of erectile dysfunction. *Am J Physiol Regul Integr Comp Physiol*. 2003;284: R1572–R1579.