Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 473 (2003) 65–70
www.elsevier.com/locate/ejphar

Enzyme activities of the nitric oxide–cGMP pathway in corpus cavernosum isolated from middle-aged rats

Naomi Hosogai*, Shoji Takakura, Toshitaka Manda, Seitaro Mutoh

Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka 532-8514, Japan

Received 20 February 2003; received in revised form 23 May 2003; accepted 3 June 2003

Abstract

Cyclic guanosine-3′,5′-monophosphate (cGMP)-mediated mechanisms play an important role in vasodilation and blood pressure regulation. We investigated basal activity of the nitric oxide (NO)–cGMP signal transduction pathway in corpus cavernosum from both middle-aged and young rats, and the electrical field stimulation-induced relaxation in the organ was also evaluated. In middle-aged rats, nitric oxide synthase (NOS) and cGMP-phosphodiesterase activities were significantly decreased; however, guanylate cyclase activity was similar. cGMP concentration, a secondary messenger of NO, remained almost the same level as compared with young rats. These results suggest that decrease in cGMP-phosphodiesterase activity is likely to account for the maintenance of cGMP concentration. In isolated corpus cavernosum from middle-aged rats, electrical field stimulation-induced relaxation was partially impaired. These results suggest that downregulation of the NOS and cGMP-phosphodiesterase activities are early events in the pathogenesis of erectile dysfunction.

Keywords: Phosphodiesterase; cGMP; Nitric oxide (NO) synthase; Guanylate cyclase; Corpus cavernosum

1. Introduction

Penile erection is a complex neurovascular process that involves relaxation of the corpus cavernosum smooth muscle (Andersson and Wagner, 1995). Relaxation of cavernosal smooth muscle leads to engorgement of blood in the corpus cavernosum which results in an increase in intracavernosal pressure and thus in tumescence. Many studies have shown that nitric oxide (NO) released from cavernous nerves and endothelium is a key factor for penile erection. The NO–cGMP signaling pathway is thought to play a critical role in regulation of corpus cavernosum response (Ignarro et al., 1990; Kim et al., 1991; Burnett, 1997; Gonzalez-Cadavid et al., 1999).

NO is formed from the conversion of L-arginine by nitric oxide synthase (NOS), which exists in three isoforms: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS). nNOS is expressed in penile neurons innervating the corpus cavernosum (Burnett et al., 1992), and eNOS protein expression has been identified primarily in both cavernosal smooth muscle and endothelium (Gonzalez et al., 2001). Recent evidence indicates that iNOS is constitutively expressed in penile cavernosal smooth muscle, although it is generally believed to be expressed in cells exposed to cytokines and up-regulated in pathophysiological conditions (Burnett, 1995; Garban et al., 1995; Rajasekaran et al., 1998; Podlasek et al., 2001). Neurally derived NO has been established as a mediator of smooth muscle cell relaxation in the penis, and it is thought that constitutive forms of NOS work to mediate the erection (Burnett et al., 1996; Burnett, 1997; Bivalacqua et al., 2000; Hurt et al., 2002). Released NO activates soluble guanylate cyclase, which catalyzes the conversion of guanosine-5′-triphosphate (GTP) to the intracellular second messenger cGMP in smooth muscle cells. An increase in cGMP modulates cellular events, such as relaxation of smooth muscle cells (Hobbs and Ignarro, 1996; Moro et al., 1996; Leitman et al., 1994). In general, intracellular cGMP concentration is regulated by not only soluble guanylate cyclase but also cGMP-phosphodiesterase, which hydrolyzes cGMP to 5′-GMP (Beavo, 1995; Soderling and Beavo, 2000).

Erectile dysfunction is defined as the consistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance (NIH Consensus Conference, 1993),

* Corresponding author. Tel.: +81-6-6390-1144; fax: +81-6-6304-5367.
E-mail address: naomi_hosogai@po.fujisawa.co.jp (N. Hosogai).

0014-2999/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01947-2

and is considered to be a natural process of aging (Feldman et al., 1994; Johannes et al., 2000). Studies have shown that erectile dysfunction is caused by inadequate relaxation of the corpus cavernosum with defect in NO production (Burnett, 1997; Azadzoi et al., 1998). There is experimental evidence for age-related erectile dysfunction and reduction in NOS activity in the corpus cavernosum (Garban et al., 1995; Bivalacqua et al., 2000). In a previous study, we showed that electrical field stimulation-induced relaxation was significantly decreased in corpus cavernosum isolated from middle-aged rats; however, the degree of alteration was small (Hosogai et al., 2001a). This result indicates that development of the functional change had already begun in the corpus cavernosum from middle-aged rat, namely these rats were considered to be in the early stage of erectile dysfunction, however, little is known about the change in the NO–cGMP signaling pathway.

In the present study, we focused on the basal NO–cGMP signaling pathway in corpus cavernosum from middle-aged rats. We compared NOS, soluble guanylate cyclase and phosphodiesterase activities plus cGMP concentration in corpus cavernosum isolated from middle-aged rats to those in young rats. In addition, electrical field stimulation-induced relaxation in isolated corporal smooth muscle strips from both middle-aged and young rats was examined.

2. Materials and methods

2.1. Animals

Young (2.5 months) and middle-aged (11–12 months) male Sprague–Dawley rats were purchased from Charles River Japan, and maintained under controlled light and temperature conditions until use. The animals were fed normal rat chow diet and had free access to water. The average body weight of middle-aged rats was heavier than that of young rats (middle-aged: 697.6 ± 35.3 g vs. young: 458.5 ± 18.6 g).

2.2. Biochemical assay

Rats were sacrificed by decapitation. Corpus cavernosum was removed, two strips of smooth muscle were dissected from each corpus cavernosum (Rajefer et al., 1992; Italiano et al., 1994), then immediately frozen in liquid nitrogen and stored at −80 °C. Homogenates were prepared in 1 ml of ice-cold 20 mM HEPES (pH 7.2) buffer containing 0.32 M sucrose, 0.5 mM EDTA, 1 mM dithiothreitol and protease inhibitors (3 μM leupeptin, 1 μM pepstatin A and 1 mM phenylmethyl sulfonylfluoride), using a Polytron homogenizer®. Cytosol fraction was separated from the particulate fraction by centrifugation at 12,500 × *g* for 60 min, at 4 °C. Protein concentration in the cytosol was assayed with Bio-Rad protein assay reagent®.

2.2.1. NOS activity

NOS activity was measured using the method of Lugg et al. (1995) as NO formation determined by the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline. The cytosol fraction was passed through Dowex AG50WX-8 (Na^+) resin to remove endogenous arginine, and 50 μl aliquots were incubated for 60 min at 37 °C in the presence of 2 μCi/ml L-[^{3}H]arginine, 2 mM NADPH, 0.45 mM $CaCl_2$, 100 μM L-arginine and 10 μg/ml calmodulin, with or without 2 mM *N*-nitro-L-arginine methyl ester (L-NAME) or 5 mM EGTA. Non-specific NOS activity was determined by L-NAME, an inhibitor of NOS, and calcium independency, a feature of inducible NOS, was ascertained by using EGTA, a chelator of calcium. After elimination of residual L-[^{3}H]arginine through the resin, L-[^{3}H]citrulline produced was measured with a scintillation counter. Protein concentrations in the cytosol were assayed with Bio-Rad protein assay reagent®. All values were expressed per mg of protein. Ca^{2+}-dependent NOS (constitutive NOS) activity was calculated from the difference between the amount of L-[^{3}H]citrulline formed in control tubes and the amount formed in tubes incubated with EGTA. iNOS activity was calculated from the difference between the amount of L-[^{3}H]citrulline formed in tubes incubated with EGTA and the amount formed in tubes incubated with EGTA plus L-NAME.

2.2.2. cGMP concentration

Cytosol fractions were acetylated, then cGMP levels were measured using a cyclic GMP [^{125}I] assay system® (Amersham International, IL, USA). The quantitative determination limit of this assay was 0.5 fmol. cGMP concentration was expressed per milligram protein.

2.2.3. cGMP-phosphodiesterase activity

cGMP-phosphodiesterase activity was determined using a modification of the two step radioisotope procedure (Thompson and Appleman, 1971). The reaction mixture (250 μl total volume) contained the cytosol fraction, 0.1 μM [^{3}H]cGMP, 30 mM $MgCl_2$, 1 mM dithiothreitol and 2 mM EGTA in 50 mM Tris–HCl (pH 8.0) buffer. The reaction was initiated by the addition of [^{3}H]cGMP and incubated in a water bath for 10 min at 30 °C, then stopped by boiling at 100 °C for 1 min. The reaction mixtures were then incubated with 2 mg/ml snake venom for 10 min at 30 °C to hydrolyze GMP to guanine. After addition of anion exchanger to remove residual [^{3}H]cGMP, the reaction mixture was vortex-mixed and centrifuged at 800 × *g* for 10 min. The resulting supernatant was transferred to Lumaplate® and the [^{3}H]5-mononucleotide formed by hydrolysis of cyclic nucleotide was determined using a Topcounter®.

2.2.4. Soluble guanylate cyclase activity

Soluble guanylate cyclase activity was determined by the method of Kimura et al. (1975). The reaction mixture contained 50 mM Tris–HCl (pH 7.6), 0.5 mM isobutylmethylxanthine, 3.5 mM creatine phosphate, 2.5 units/tube

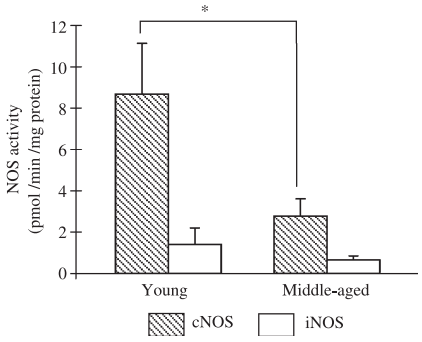

Fig. 1. NOS activity in corpus cavernosum from young and middle-aged rats. cNOS activity; filled columns, iNOS activity; open columns. Each data represents the mean ± S.E. $n = 5$. *Significantly different between the two groups, $P < 0.05$.

creatine phosphokinase and 50 μl of the cytosol fraction. This mixture was preincubated for 10 min at 37 °C, and the reaction of guanylate cyclase was initiated by addition of 4 mM $MnCl_2$ and 1 mM GTP in a final volume of 100 μl, then incubated for 15 min at 37 °C. The reaction was terminated by addition of 0.9 ml of 50 mM sodium acetate buffer (pH 4.0) to prevent nonenzymatic formation of cGMP, and heated at 90 °C for 3 min. cGMP amount was determined using a radioimmunoassay kit (cGMP [^{125}I] assay system®, Amersham International). Soluble guanylate cyclase activity was expressed per milligram protein.

2.3. Functional experiments

2.3.1. Tissue preparation

The corpus cavernosum was removed according to the same method described above and was immediately placed in Krebs solution: 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 10 mM glucose and 2.5 m $CaCl_2$. One strips of smooth muscle were dissected from each corpus cavernosum and mounted between two metal hooks in an organ bath chamber containing 25 ml of Krebs solution, maintained at 37 °C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. The isolated tissue was stretched to a resting force of 0.1 g, and isometric tension was recorded via a force–displacement transducer using a recorder (Nihon Kohden, Japan). The tissue was equilibrated for at least 1 h in this state. During this period,

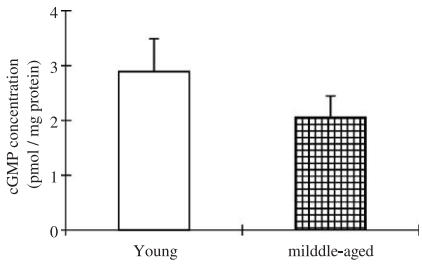

Fig. 2. cGMP concentration in corpus cavernosum from young and middle-aged rats. $n = 5$. Each data represents the mean ± S.E. There was no statistically significant difference between the two groups.

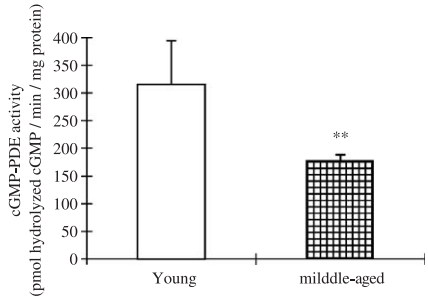

Fig. 3. cGMP-phosphodiesterase activity in corpus cavernosum from young and middle-aged rats. $n = 5$. Each data represents the mean ± S.E. **Significantly different from young rats, $P < 0.01$.

the tissue was washed with fresh solution every 15 min and the tension was readjusted if necessary.

2.3.2. Electrical field stimulation-induced relaxation

Atropine (1 μM) and guanethidine (5 μM) were routinely added to the bath to block muscarinic receptors and prevent the release of norepinephrine, respectively, in the last 30 min of the equilibration period in order to specify the relaxation response by excitation of non-adrenergic, non-cholinergic nerves. After the equilibration period, strips were contracted with 100 μM norepinephrine. After norepinephrine contractile responses had stabilized, tissues were subjected to electrical field stimulation-induced relaxation at 10 V (0.5 ms square wave pulse) using sequential frequencies of 1, 3, 5, 15, 30 and 40 Hz each delivered in 10 s trains. The percentage of relaxation response after electrical field stimulation was used as an index of corpus cavernosal responsiveness.

2.4. Drugs

Guanethidine, atropine, L-NAME, EGTA, dithiothreitol, leupeptin, pepstatin A, phenylmethyl sulfonylfluoride, L-arginine, NADPH, calmodulin, GTP, snake venom, isobutylmethylxanthine, creatine phosphate, creatine phosphokinase and sodium acetate were purchased from Sigma. Norepinephrine was purchased from Sankyo. [^{3}H]cGMP and L-[^{3}H]arginine were purchased from NEN Life Science Products. All other chemicals were of the purest commercially available grade.

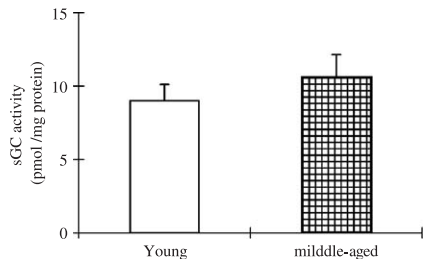

Fig. 4. Soluble guanylate cyclase activity in corpus cavernosum from young and middle-aged rats. $n = 5$. Each data represents the mean ± S.E. There was no statistically significant difference between the two groups.

2.5. Statistics

All data were expressed as mean ± S.E. Student's *t*-test was used to compare young and middle-aged groups.

3. Results

3.1. NOS, cGMP-phosphodiesterase and soluble guanylate cyclase activities plus cGMP concentration in corpus cavernosum

Total NOS activity in the corpus cavernosum isolated from middle-aged rats was significantly lower than that from young rats. cNOS activity decreased to 1/3 and iNOS activity to 1/2 of values in young rats (cNOS activity; young: 8.7 ± 2.5 pmol/min/mg protein vs. middle-aged: 2.8 ± 0.8 pmol/min/mg protein, iNOS activity; young rats: 1.4 ± 0.8 pmol/min/mg protein vs. middle-aged rats: 0.6 ± 0.2 pmol/min/mg protein; Fig. 1). cGMP concentration in the corpus cavernosum isolated from middle-aged rats was slightly lower than that of young rats (young: 2.89 ± 0.6 pmol/mg protein vs. middle-aged: 2.06 ± 0.39 pmol/mg protein; Fig. 2). As shown in Fig. 3, the cGMP-phosphodiesterase activity in the corpus cavernosum isolated from middle-aged rats was approximately half that from young rats (young: 315.7 ± 79.2 pmol hydrolyzed cGMP/min/mg protein vs. middle-aged: 177.4 ± 11.2 pmol hydrolyzed cGMP/min/mg protein; $P < 0.01$). There was no significant difference in soluble guanylate cyclase activity in the corpus cavernosum between the two groups (young: 9.0 ± 1.1 pmol /mg protein vs. middle-aged: 10.6 ± 1.54 pmol/mg protein; Fig. 4).

3.2. Electrical field stimulation-induced relaxation response

Isometric tension induced by 100 μM norepinephrine was not significantly different between the two groups (middle-aged: 0.14 ± 0.02 g vs. young: 0.17 ± 0.02 g). Both young and middle-aged tissues required the same concentration of papaverine (0.1 mM) to obtain full relaxation. Electrical field stimulation caused frequency-dependent relaxation in strips of the corpus cavernosum pre-contracted with norepinephrine. The degree of relaxation response in the corpus cavernosum isolated from middle-aged rats at 30 and 40 Hz was slightly but significantly smaller than that from young rats (Fig. 5).

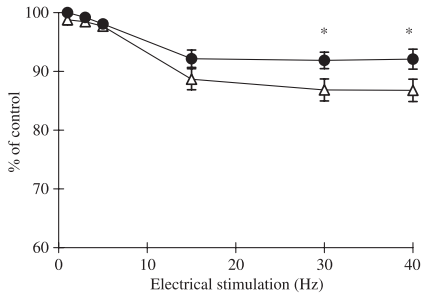

Fig. 5. Frequency–response curves to electrical field stimulation in corpus cavernosum from young and middle-aged rats. Young rats (△, $n = 10$), middle-aged rats (●, $n = 8$). Each data point represents the mean ± S.E. *: significantly different from young rats, $P < 0.05$.

4. Discussion

We found the following alterations in basal activity of the NO–cGMP pathway in corpus cavernosum from middle-aged rats compared to young rats: (1) cGMP concentration was only slightly reduced, despite a marked decrease in NOS activity; (2) cGMP-phosphodiesterase activity decreased by half; however, soluble guanylate cyclase activity was unchanged; and (3) NO-mediated relaxation of corpus cavernosum was only partially decreased. These data show that functional change was restricted, even though there were reciprocal alterations in enzyme activities in the corpus cavernosum from middle-aged rats.

In this study, both cNOS (nNOS and eNOS) and iNOS activity was decreased in the corpus cavernosum from middle-aged rats. Previous findings showed that the number of NOS containing nerves was not significantly lower in corpus cavernosum from intermediate (8.5 months)-aged Sprague–Dawley rat (Carrier et al., 1997). Therefore, our results might be attributed to decrease in both eNOS and iNOS activities. Further investigation may provide additional insight into the role of these isoenzymes in the corpus cavernosum from middle-aged rats.

Interestingly, we observed that cGMP concentration in corpus cavernosum isolated from middle-aged rats remained almost the same level as that from young rats, despite a marked decrease in the NOS activity in middle-aged rats. Intracellular level of cGMP, a secondary messenger of NO, is regulated by a balance between the rates of synthesis by guanylate cyclase and the rates of hydrolytic breakdown to GMP via cGMP-phosphodiesterase (Beavo, 1995; Soderling and Beavo, 2000). Two hypotheses are possible for maintaining cGMP concentration, even when there is a marked decrease in NOS activity. One is an increase in synthesis of cGMP from NO, that is, an increase in soluble guanylate cyclase activity. The other is a diminished degradation of cGMP, that is a decrease in cGMP-phosphodiesterase activity. There have been no reports studying simultaneous changes in cGMP concentration, guanylate cyclase activity and cGMP-phosphodiesterase activity in corpus cavernosum from middle-aged rats. A novel finding in the present study was that there was no difference in soluble guanylate cyclase activity; however, cGMP-phosphodiesterase activity was significantly decreased in corpus cavernosum isolated from middle-aged rats, suggesting that it might contribute to the maintenance of cGMP concentration. It is thought that the maintained cGMP concentration is brought about because decreases in NOS activity and

cGMP-phosphodiesterase activity occurred simultaneously in the corpus cavernosum isolated from middle-aged rats. These data suggest the NO–cGMP signaling pathway was impaired to a lesser extent, although NOS activity was significantly decreased in corpus cavernosum from middle-aged rats.

Recent studies demonstrated an autoregulation mechanism in the NO–cGMP pathway corresponding to a change in NO production (Hussain et al., 2001; Kim et al., 2001; Erandes et al., 2000). Decrease in NO level, induced by exposure to NOS inhibitor or disrupting the NOS gene, caused enhanced sensitivity to NO via an increase in guanylate cyclase activity and/or cGMP-phosphodiesterase activity (Hussain et al., 1999; Dowell et al., 1996; Hosogai et al., 2001b). These previous reports showed that cGMP concentration and guanylate cyclase activity increased, whereas cGMP-phosphodiesterase activity decreased, under conditions where NOS activity completely disappeared. However, our study showed that NOS activity decreased by 70%, cGMP concentration only slightly decreased, soluble guanylate cyclase activity did not change and cGMP-phosphodiesterase activity decreased by 50%. These data indicate a possibility that enzyme activities (soluble guanylate cyclase and phosphodiesterase) could change with degrees of decrease in NOS activity, in other words, there is reciprocal regulation of the NO–cGMP pathway. The down-regulation of cGMP-phosphodiesterase activity in corpus cavernosum from middle-aged rats probably represents a counter-regulatory effect from a decrease in NOS.

Electrical stimulation triggers a neurogenic response exclusively (Ignarro et al., 1990), that is electrical field stimulation-induced relaxation response is a standard and suitable method for evaluation of corpus cavernosum functions. Treatment with L-NAME, a NOS inhibitor, inhibited electrical field stimulation-induced relaxation and this inhibition was restored by addition of L-arginine (data not shown), suggesting that electrical field stimulation-induced relaxation is associated with the NO pathway. These result consistent with previous report (Ballard et al., 1998). Under these experimental conditions, electrical field stimulation-induced relaxation response was partially decreased in corpus cavernosum from middle-aged rats. This finding was consistent with cGMP concentration in the corpus cavernosum from middle-aged rats, although biochemical analyses were performed in non-stimulated tissue. Because the relaxation response was terminated in a few seconds, it is impossible to measure enzyme activities during relaxation. Even though the enzyme activities and the cGMP concentration were measured in non-stimulated tissue, they may be predictors of the situation of NO–cGMP pathway during electrical field stimulation. Aging is well known to alter penile function and age-related decrease in erectile function has been documented (Feldman et al., 1994; Johannes et al., 2000). Our findings, that electrical field stimulation-induced relaxation response was partially decreased in middle-aged rats, indicate that age-related functional change of corpus cavernosum has begun but is not developed completely in middle-aged rats. In addition, the corpus cavernosum isolated from young and middle-aged rats exhibited not only the same relaxation response to papaverine but also the same contractile response to norepinephrine, suggesting that there are no alteration in both contractile and relaxation abilities in corpus cavernosum from middle-aged rats. It is considered that the attenuated relaxant response might be associated specifically with alterations in the NO–cGMP pathway.

In summary, we have shown that basal cGMP concentration was not markedly decreased in corpus cavernosum isolated from middle-aged rats in which NOS activity was significantly decreased. This result is likely to be caused by decreased cGMP-phosphodiesterase activity. There seems to exist a reciprocal mechanism to maintain cGMP concentration in corpus cavernosum from middle-aged rats. In addition, NO-mediated relaxation of corpus cavernosum was only partially attenuated in middle-aged rats. Taken together, these results suggest that downregulation of the NOS and cGMP-phosphodiesterase activities are early events in the pathogenesis of erectile dysfunction.

References

Andersson, K.E., Wagner, G., 1995. Physiology of penile erection. Physiol. Rev. 75, 191–236.

Azadzoi, K.M., Goldstein, I., Siroky, M.B., Traish, A.M., Krane, R.B., Saenz De Tejada, I., 1998. Mechanisms of ischemia-induced cavernosal smooth muscle relaxation impairment in a rabbit model of vasculogenic erectile dysfunction. J. Urol. 160, 2216–2222.

Ballard, S.A., Gingell, C.J., Tang, K., Turner, L.A., Price, M.E., Naylor, A.M., 1998. Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. J. Urol. 159, 2164–2171.

Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75, 725–748.

Bivalacqua, T.J., Champion, H.C., Mehta, Y.S., Abdel-Mageed, A.B., Sikka, S.C., Ignarro, L.J., Kadowitz, P.J., Hellstrom, W.J., 2000. Adenoviral gene transfer of endothelial nitric oxide synthase (eNOS) to the penis improves age-related erectile dysfunction in the rat. Int. J. Impot. Res. 12 (Suppl. 3), S8–S17.

Burnett, A.L., 1995. Nitric oxide control of lower genitourinary tract functions: a review. Urology 45, 1071–1083.

Burnett, A.L., 1997. Nitric oxide in the penis: physiology and pathology. J. Urol. 157, 320–324.

Burnett, A.L., Lowenstein, C.J., Bredt, D.S., Chang, T.S., Snyder, S.H., 1992. Nitric oxide: a physiologic mediator of penile erection. Science 257, 401–403.

Burnett, A.L., Nelson, R.J., Calvin, D.C., Liu, J.X., Demas, G.E., Klein, S.L., Kriegsfeld, L.J., Dawson, V.L., Dawson, T.M., Snyder, S.H., 1996. Nitric oxide-dependent penile erection in mice lacking neuronal nitric oxide synthase. Mol. Med. 2, 288–296.

Carrier, S., Nagaraju, P., Morgan, D.M., Baba, K., Nunes, L., Lue, T.F., 1997. Age decreases nitric oxide synthase-containing nerve fibers in the rat penis. J. Urol. 157, 1088–1092.

Dowell, F.J., Henrion, D., Duriez, M., Michel, J.B., 1996. Vascular reactivity in mesenteric resistance arteries following chronic nitric oxide synthase inhibition in Wistar rats. Br. J. Pharmacol. 117, 341–346.

Erandes, R.P., Kim, D., Schmitz-Winnenthal, F.H., Amidi, M., Godecke, A., Mulsch, A., Busse, R., 2000. Increased nitrovasodilator sensitivity in endothelial nitric oxide synthase knockout mice: role of soluble guanylyl cyclase. Hypertension 35, 231–236.

Feldman, H.A., Goldstein, I., Hatzichristou, D.G., Krane, R.J., McKinlay, J.B., 1994. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J. Urol. 151, 54–61.

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

Gonzalez-Cadavid, N.F., Ignarro, L.J., Rajfer, J., 1999. Nitric oxide and the cyclic GMP system in the penis. Mol. Urol. 3, 51–59.

Gonzalez, C.M., Brannigan, R.E., Bervig, T., Zelner, D., Podlasek, C.A., McKenna, K.E., McVary, K.T., 2001. Protein and gene expression of nitric oxide synthase isoforms I and III in the rat penile shaft. J. Androl. 22, 54–61.

Hobbs, A.J., Ignarro, L.J., 1996. Nitric oxide-cyclic GMP signal transduction system. Methods Enzymol. 269, 134–148.

Hosogai, N., Ozaki, T., Seki, J., Goto, T., 2001a. Effect of FR226807, a selective phosphodiesterase 5 inhibitor, on the attenuated responsiveness of the corpus cavernosum in middle aged-rats and stretozotocin-induced diabetic rats. Jap. J. Pharmacol. 8 (Suppl. 1), P-894.

Hosogai, N., Seki, J., Goto, T., 2001b. Reciprocal regulation of cyclic GMP content by cyclic GMP-phosphodiesterase and guanylate cyclase in SHR with CsA-induced nephrotoxicity. Br. J. Pharmacol. 134, 995–1002.

Hurt, K.J., Musicki, B., Palese, M.A., Crone, J.K., Becker, R.E., Moriarity, J.L., Snyder, S.H., Burnett, A.L., 2002. Akt-dependent phosphorylation of endothelial nitric-oxide synthase mediates penile erection. Proc. Natl. Acad. Sci. 99, 4061–4066.

Hussain, M.B., Hobbs, A.J., MacAllister, R.J., 1999. Autoregulation of nitric oxide-soluble guanylate cyclase-cyclic GMP signalling in mouse thoracic aorta. Br. J. Pharmacol. 128, 1082–1088.

Hussain, M.B., MacAllister, R.J., Hobbs, A.J., 2001. Reciprocal regulation of cGMP-mediated vasorelaxation by soluble and particulate guanylate cyclases. Am. J. Physiol. 280, H1151–H1159.

Ignarro, L.J., Bush, P.A., Buga, G.M., Wood, K.S., Fukuto, J.M., Rajfer, J., 1990. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem. Biophys. Res. Commun. 170, 843–850.

Italiano, G., Calabro, A., Pagano, F., 1994. A simplified in vitro preparation of the corpus cavernosum as a tool for investigating erectile pharmacology in the rat. Pharmacol. Res. 30, 325–334.

Johannes, C.B., Araujo, A.B., Feldman, H.A., Derby, C.A., Kleinman, K.P., McKinlay, J.B., 2000. Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male aging study. J. Urol. 163, 460–463.

Kim, N., Azadzoi, K.M., Goldstein, I., Saenz, D.E., Tejada, I., 1991. A nitric oxide-like factor mediates nonadrenergic-noncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle. J. Clin. Invest. 88, 112–118.

Kim, D., Rybalkin, S.D., Pi, X., Wang, Y., Zhang, C., Munzel, T., Beavo, J.A., Berk, B.C., Yan, C., 2001. Upregulation of phosphodiesterase 1A1 expression is associated with the development of nitrate tolerance. Circulation 104, 2338–2343.

Kimura, H., Mittal, C.K., Murad, F., 1975. Activation of guanylate cyclase from rat liver and other tissues by sodium azide. J. Biol. Chem. 250, 8016–8022.

Leitman, D.C., Waldman, S.A., Murad, F., 1994. Regulation of particulate guanylate cyclase by natriuretic peptides and Escherichia coli heat-stable enterotoxin. Adv. Pharmacol. 26, 67–86.

Lugg, J.A., Rajfe, J., Gonzalez-Cadavid, N.F., 1995. Dihydrotestosterone is the active androgen in the maintenance of nitric oxide mediated penile erection in the rat. Endocrinology 136, 1495–1501.

Moro, M.A., Russel, R.J., Cellek, S., Lizasoain, I., Su, Y., Darley-Usmar, V.M., Radomski, M.W., Moncada, S., 1996. cGMP mediates the vascular and platelet actions of nitric oxide: confirmation using an inhibitor of the soluble guanylyl cyclase. Proc. Natl. Acad. Sci. 93, 1480–1485.

NIH Consensus Conference, 1993. Impotence. NIH Consensus Development Panel on Impotence. JAMA 270, 83–90.

Podlasek, C.A., Zelner, D.J., Bervig, T.R., Gonzalez, C.M., McKenna, K.E., McVary, K.T., 2001. Characterization and localization of nitric oxide synthase isoforms in the BB/WOR diabetic rat. J. Urol. 166, 746–755.

Rajasekaran, M., Mondal, D., Agrawal, K., Chen, I.L., Hellstrom, W., Sikka, S., 1998. Ex vivo expression of nitric oxide synthase isoforms (eNOS/iNOS) and calmodulin in human penile cavernosal cells. J. Urol. 160, 2210–2215.

Rajefer, J., Aronson, W.J., Bush, P.A., Dorey, F.J., Ignarro, L.J., 1992. Nitric oxide as a mediator on relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N. Engl. J. Med. 326, 90–94.

Soderling, S.H., Beavo, J.A., 2000. Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. Curr. Opin. Cell Biol. 12, 174–179.

Thompson, W.J., Appleman, M.M., 1971. Multiple cyclic nucleotide phosphodiesterase activities from rat brain. Biochemistry 10, 311–316.