Cell Tissue Res (1995) 282:109–116

Cell & Tissue Research

Neural and endothelial nitric oxide synthase activity in rat penile erectile tissue

William G. Dail, Vera Barba, Leonard Leyba, Raphael Galindo

Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA

Received: 6 May 1994 / Accepted: 25 April 1995

Abstract. NADPH-diaphorase (NADPH-D) activity and immunoreactivity for neural and endothelial nitric oxide synthase (nNOS and eNOS, respectively) were used to investigate nitric oxide (NO) regulation of penile vasculature. Both the histochemical and immunohistochemical techniques for NOS showed that all smooth muscles regions of the penis (dorsal penile artery and vein, deep penile vessels, and cavernosal muscles) were richly innervated. The endothelium of penile arteries, deep dorsal penile vein, and select veins in the crura and shaft were also stained for NADPH-D and eNOS. However, the endothelium of cavernous sinuses was unstained by both techniques. Fewer fibers were seen in the glans penis, those present being associated with small blood vessels and large nerve bundles near the trabecular walls. All penile neurons in the pelvic plexus, located by retrograde transport of a dye placed in the corpora cavernosa penis, were stained by the NADPH-D method. Essentially similar results were obtained with an antibody to nNOS. These data suggest that penile parasympathetic neurons comprise a uniform population, as all seem capable of forming nitric oxide. However, in contrast to the endothelium of penile vessels, the endothelium lining the cavernosal spaces may not be capable of nitric oxide synthesis.

Key words: Penis – Nitric oxide synthase – NADPH diaphorase – Endothelium – Pelvic plexus – Rat (Sprague Dawley)

Introduction

Several lines of evidence indicate that nitric oxide (NO) may be the inhibitory substance released by vasodilator nerves to the penis. This suggestion is based on organ-bath studies of penile smooth muscle; these demonstrate that stimulation of the nonadrenergic-noncholinergic (NANC) pathway increases the biosynthesis and release of NO (Ignarro et al. 1990) and that compounds that block the synthesis of NO interfere with neurogenic relaxation (Holmquist et al. 1991; Kim et al. 1991; Knispel et al. 1991; Burnett et al. 1992; Rajfer et al. 1992; Mills et al. 1992; Kirkeby et al. 1993). This functional evidence has been recently supported by anatomical studies that show that nerve fibers in the penis of rat (Burnett et al. 1992; Alm et al. 1993) and human (Burnett et al. 1993) are stained by an antibody to nitric oxide synthase (NOS), as are appropriately located neurons in the pelvic plexus (Burnett et al. 1992), the acknowledged source of the inhibitory innervation to the penis.

Although most studies generally confirm the presence of NOS-immunoreactive (NOS-IR) fibers to blood vessels and trabecular smooth muscles in the penis, the angioarchitecture of the penis is complex, and questions remain regarding the distribution of the innervation to the various regions and tissues of the penis. For example, investigations have not included the glans penis, although glans erection is an important component of sexual reflexes in some species (Sachs 1983). Differing functional responses of the corpora cavernosa and the corpus spongiosum (Carati et al. 1987) or penile vessels and cavernous smooth muscle (Bowman and Gillespie 1983; Hedlund and Andersson 1985) suggest that important regional variations exist in the pattern of innervation. A recent investigation has found that there is no NANC response in human penile circumflex veins (Kirkeby et al. 1993), although it is clearly present in human cavernous smooth muscle (Holmquist et al. 1991). Moreover, differences in the innervation of penile tissues are presumed to be reflected in the phenotypes of penile neurons in the pelvic plexus, although there is evidence that many of the penile neurons in this location stain for NADPH-diaphorase (NADPH-D) (Keast 1992; Dail et al. 1992; Ding et al. 1993; Schirar et al. 1994), a smaller proportion seems to stain with an antibody to NOS (Vizzard et al. 1994). Finally, it is not clear whether endothelial cells in the penis have the ability to release NO. Keast (1992) has reported faint NADPH-D staining of the endothelium of penile vessels and a stronger reaction in some endothelial cells lining the

Correspondence to: W.G. Dail

cavernosal spaces, but this has not been confirmed by Schirar et al. (1994) using the same technique and species. An antibody directed against rat cerebellar NOS cross-reacts with endothelial cells, including those of rat penile vessels (Burnett et al. 1992), but to date no study has used an antibody method for endothelial NOS (eNOS) to assess directly whether penile endothelial cells are capable of synthesizing NO.

As a part of a larger study into the neural regulation of erectile tissue, we have used NADPH-D histochemistry and antibody methods for neural NOS (nNOS) and eNOS to examine more closely the NO-synthesizing ability of rat penile erectile tissues and of penile neurons in the pelvic plexus. Observations with eNOS have been compared with those obtained by NADPH-D staining in view of the recent suggestions that eNOS may have a requirement for NADPH (Tracey et al. 1993; Gabbott and Bacon 1993).

Materials and methods

Twelve male rats (Sprague Dawley, 200-300 g) were used for the detection of NADPH-D and NOS immunoreactivity in the penis. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). The penis was removed and cut into smaller blocks of tissue that included the crura, shaft, and penile bulb, and the glans.

Five animals were used to characterize NADPH-D and NOS immunoreactivity of penile neurons in the major pelvic ganglia (MPG). Under sterile conditions, the base of the penis was exposed by a midline scrotal incision. Fluorogold (4–6 µl; Flurochrome, Englewood, Colo.) were injected into each penile crus after removal of a portion of the ischiocavernosus muscle. Five to seven days later, rats were anesthetized, and the pelvic ganglia were removed and placed in fixative and further processed to visualize NADPH-D fibers or NOS immunoreactivity. Different filter combinations on the microscope were used to grade penile ganglionic neurons based on their reaction for NADPH-D or NOS.

NADPH-histochemistry

All tissue was placed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.3) for 4 h. Following removal of fixative with several washes of cold buffer, tissue was placed in 20% sucrose in PB overnight. Tissue blocks were frozen and 16-µm-thick sections were collected on gelatin-coated slides and processed for NADPH-D using a modification (McNeill et al. 1992) of the method described by Hope and Vincent (1989). Sections were hy-

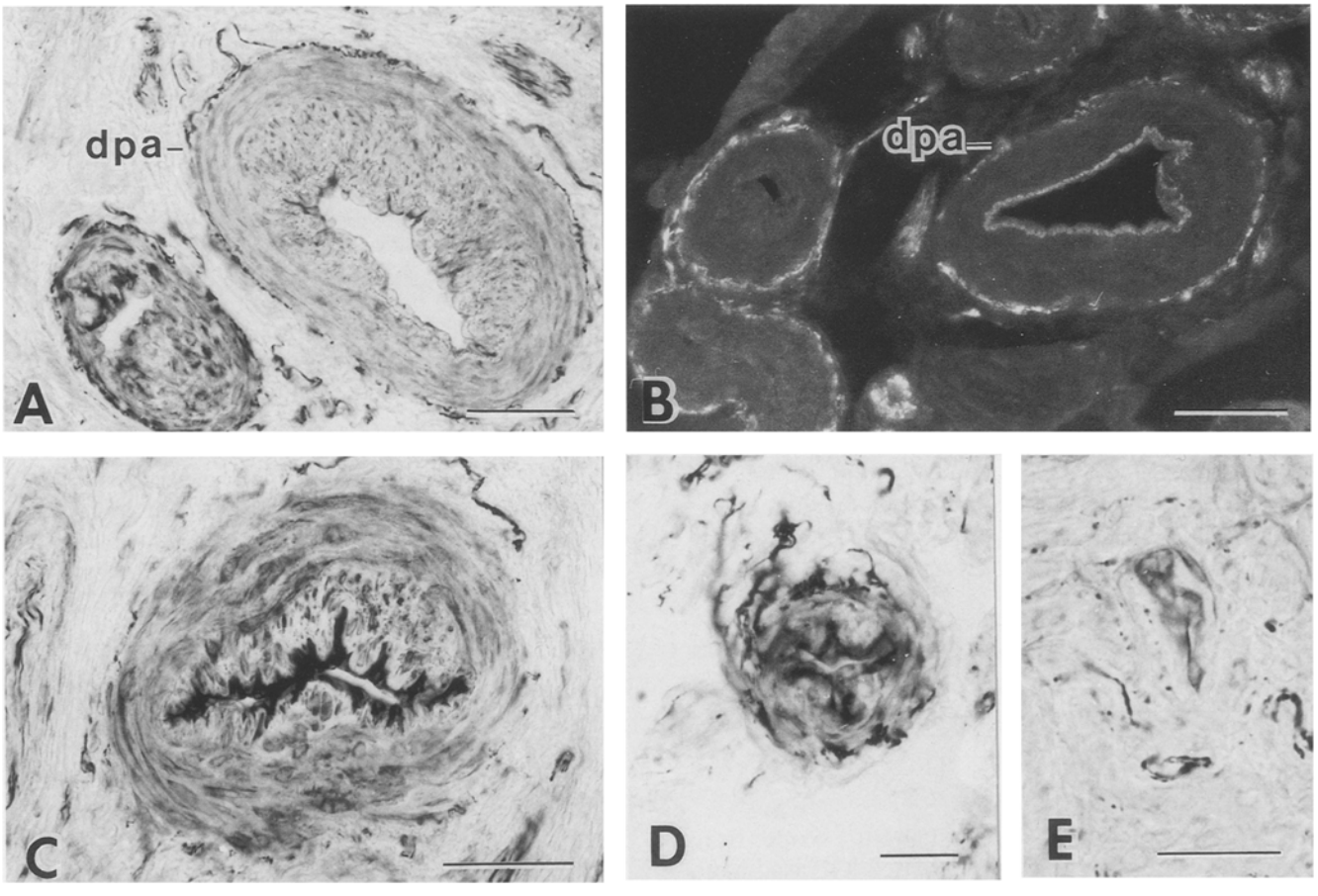

Fig. 1A–D. Photomicrographs of NADPH-D and NOS reactivity in rat penile vessels. **A** The deep penile artery (*dpa*), characterized by a prominent layer of inner longitudinal smooth muscle, is surrounded by a thin plexus of NADPH-D fibers. NADPH-D staining is also visible in smooth muscle cells, especially in the smaller vessel *left*. *Bar:* 60 µm; ×250. **B** NOS-IR fibers around the deep penile artery (*dpa*). The endothelium is unstained, but autofluorescence of the internal elastic membrane is clearly visible. *Bar:* 60 µm; ×250. **C** A tangential section of the deep penile artery illustrates the intense NADPH-D staining of the endothelium. *Bar:* 80 µm; ×210. **D** NADPH-D staining of the endothelium is apparent in this small innervated artery. *Bar:* 40 µm; ×260. **E** NADPH-D+ endothelial cells are characteristic of the smallest arteries, even when it is not clear whether the vessel has a smooth muscle coat. *Bar:* 40 µm; ×380

drated in 0.1 M PB and then incubated for 45 min in a solution containing 0.5 mg/ml β-NADPH, 0.25 mg/ml nitroblue tetrazolium, 0.3% Triton X-100 in PB (pH 7.3) at 37° C. All reagents were from Sigma (St. Louis, Mo.). Control sections included incubation in media in which the substrate was omitted and preincubation in the sulfhydryl inhibitor, 5,5'-dithio-bis-(2-nitrobenzoic acid) (Calbiochem, San Diego, Calif.).

nNOS immunohistochemistry

Tissues to be processed for NOS immunoreactivity were placed overnight in Zamboni's fixative (Zamboni and De Martino 1967), washed repeatedly in PB, placed for 2 h in 20% sucrose in PB, and frozen on cryostat chucks. Slide-mounted cryostat sections (16 μm thick) were preincubated in PB containing 0.3% Triton-X 100, and then incubated overnight at 4° C with rabbit antiserum to rat cerebellar NOS (Auspep Pty, Parkville, Australia) (1:500 dilution in 0.1 M PB with 0.3% Triton X-100). After being washed, sections were incubated with goat anti-rabbit fluorescein isothiocyanate (Sigma) (diluted 1:150) for 1 h. Control sections in which the primary antibody was substituted with normal rabbit serum were negative.

eNOS immunohistochemistry

Cryostat sections of penile tissues, fixed in Zamboni's as described above, were incubated overnight in the cold with an mouse monoclonal antibody directed against human eNOS (Transduction Laboratories, Lexington, Ky.). Immunocytochemical labeling was demonstrated by the avidin-biotin complex using the Elite ABC kit from Vector Laboratories (Burlingame, Calif.); 1 μg/ml antibody solution provided good differentiation of endothelial cells with low background. Control sections incubated without the primary antibody were negative.

Results

In general, NADPH-D was localized to nerve fibers at all smooth muscle locations in the penis. Where the deep penile artery penetrated the tunica albuginea to enter the cavernous tissue (and for some distance thereafter), it was accompanied by large branches of the cavernous nerve. The cavernous nerve contributed a thin plexus of NADPH-D and nNOS-IR fibers to the adventitia-media border of this vessel (Fig. 1A, B). A tangential section of the deep penile artery also revealed intense NADPH-D staining of the endothelium (Fig. 1C); this was not readily apparent in cross-sectional view (Fig. 1A). The more proximal branches of the deep penile artery acquired a more dense plexus of NADPH-D-positive (NADPH-D+) fibers, while retaining the usual features of the deep pe-

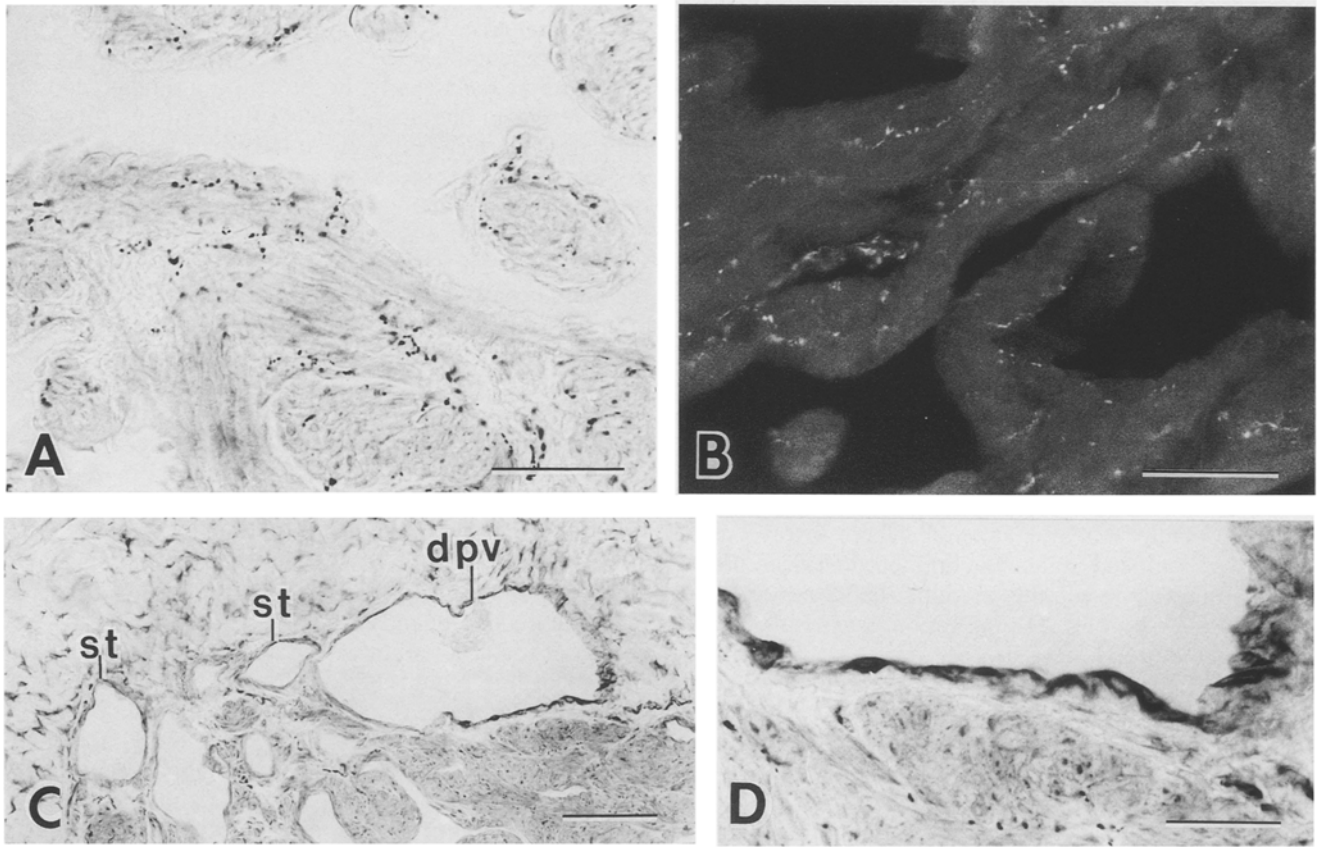

Fig. 2A–D. Staining for NADPH-D and for NOS in the penile crura. **A** NADPH-D+ fibers are distributed in the intrinsic smooth muscle in the penile crus. Note the lack of staining in the endothelial cells that line the cavernous spaces. *Bar:* 60 μm; ×290. **B** NOS immunoreactivity in the penile crus is comparable in density to the staining seen for NADPH-D. *Bar:* 60 μm; ×290. **C** Subtunical veins (*st*) near their union with the larger deep penile vein (*dpv*). These vessels are not innervated, but note the NADPH-D+ endothelium of the *dpv*. *Bar:* 120 μm; ×100. **D** An enlargement of a portion of **C** illustrating the NADPH-D+ endothelium of the dpv. *Bar:* 40 μm; ×360

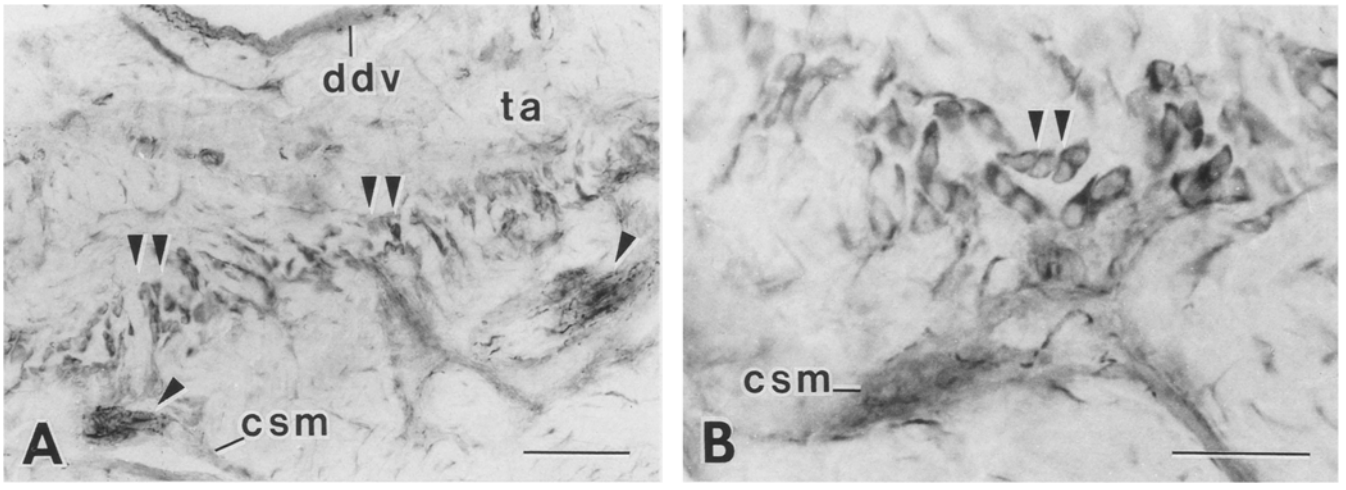

Fig. 3A, B. NADPH-D staining in the shaft of the rat penis. **A** Cavernous smooth muscle (*csm*), which lines the sinuses of the shaft, is innervated, as are branches of the deep penile arteries (*single arrowheads*) and the deep dorsal vein (*ddv*) of the penis. The boundary between the tunica albuginea (*ta*) and the erectile tissue in the shaft is marked by small cells (*double arrowheads*) that are moderately stained for NADPH-D. *Bar:* 120 μm; ×115. **B** Reactive cells (*double arrowheads*) below the tunic have prominent nuclei with angular cytoplasmic processes. *Bar*: 60 μm; ×290

nile vessel, viz., a prominent longitudinal smooth muscle layer and a distinct internal elastic membrane (Fig. 1A). All smaller branches, some of which could be seen emptying into the cavernous spaces, retained an innervation and an intensely stained endothelium (Fig. 1D, E). The wall of these latter vessels became indistinct; although they share some feature of helicine arteries, further analyses are necessary to determine whether epithelioid cells, a defining features of helicine vessels, are present. A similar distribution of fibers was seen with nNOS, but with less background (compare Figs. 1A, B). Endothelial staining was not apparent with the nNOS antibody.

In the penile crus, the intrinsic smooth muscle surrounding the cavernous spaces was richly supplied with nerve fibers. No differences in density or in the pattern of innervation were noted between histochemical and immunohistochemical methods (Fig. 2A, B). Unlike arterial endothelium, the endothelium of the cavernous spaces was unstained with the NADPH-D method (Fig. 2A). Moderate NADPH-D staining of the endothelium of the deep penile vein was apparent where it traversed the tunica albuginea. Some reaction product was also noted in some of the subtunical veins that coalesced to form this vessel (Fig. 2C, D), but this was generally less intense than that in arteries.

Fewer NADPH-D fibers were present in the distal penis (corpora cavernosa penis) where the walls of the cavernous sinuses were largely composed of connective tissue with only a thin lining of smooth muscle (Fig. 3A, B). As in the crura, arterial endothelial cells of the shaft were reactive for NADPH-D, but those lining the cavernous spaces (Fig. 3A, B) and the cavernous veins, the large channels draining the shaft, were unstained. Rows of small cells at the inner border of the tunica albuginea were also reactive for NADPH-D with an intensity between that of the nerve fibers and smooth muscle (Fig. 3A, B). The distribution of NADPH-D and nNOS-IR fibers of the dorsal structures of the penis, viz., the dorsal arteries, deep dorsal vein (Fig. 3A), and dorsal nerves, did not differ from previous descriptions, and thus will not be detailed.

Immunoreactivity for eNOS was examined in the penile crura and shaft only. In the crura, immunoreactivity was present in endothelial cells of all arteries, from the deep penile artery (Fig. 4A) to the small arteries emptying into cavernous spaces. Staining was absent in the endothelium lining the cavernous spaces; however, eNOS immunoreactivity was present in the endothelial cells of some subtunical veins in the penile crura (Fig. 4B). In the penile shaft, the endothelium of the dorsal artery and vein was reactive for eNOS, whereas the sinusoidal endothelium was unstained (Fig. 4C). In addition, the endothelium in some regions of the cavernous veins stained for eNOS (Fig. 4D).

In the corpus spongiosum, studies have been confined to the NADPH-D innervation of the urethral bulb (penile bulb) and the glans penis, as the penile portion of the urethra has been previously described (McNeill et al. 1992). The urethra of the penile bulb is surrounded by erectile tissue with a structure similar to that of the penile crura; consequently, the NADPH-D innervation is abundant (data not shown). Paraurethral glands are also present in this region and their acini are enclosed by a finely beaded plexus of NADPH-D fibers (Fig. 5).

The erectile tissue of the glans penis in the rat lies below an epithelium bearing penile spines (Fig. 6A). NADPH-D fibers are absent from the zone beneath the spinous epithelium, but a sparse innervation is found in the walls of the trabecular spaces. For the most part, innervation is confined to small arteries located in the trabecular wall (Fig. 6A, B). As in other regions of the penis, the endothelium of arteries is reactive for NADPH-D (Fig. 6B, C). Large nerve bundles run throughout the glans, but only a few of the fibers stain for NADPH-D.

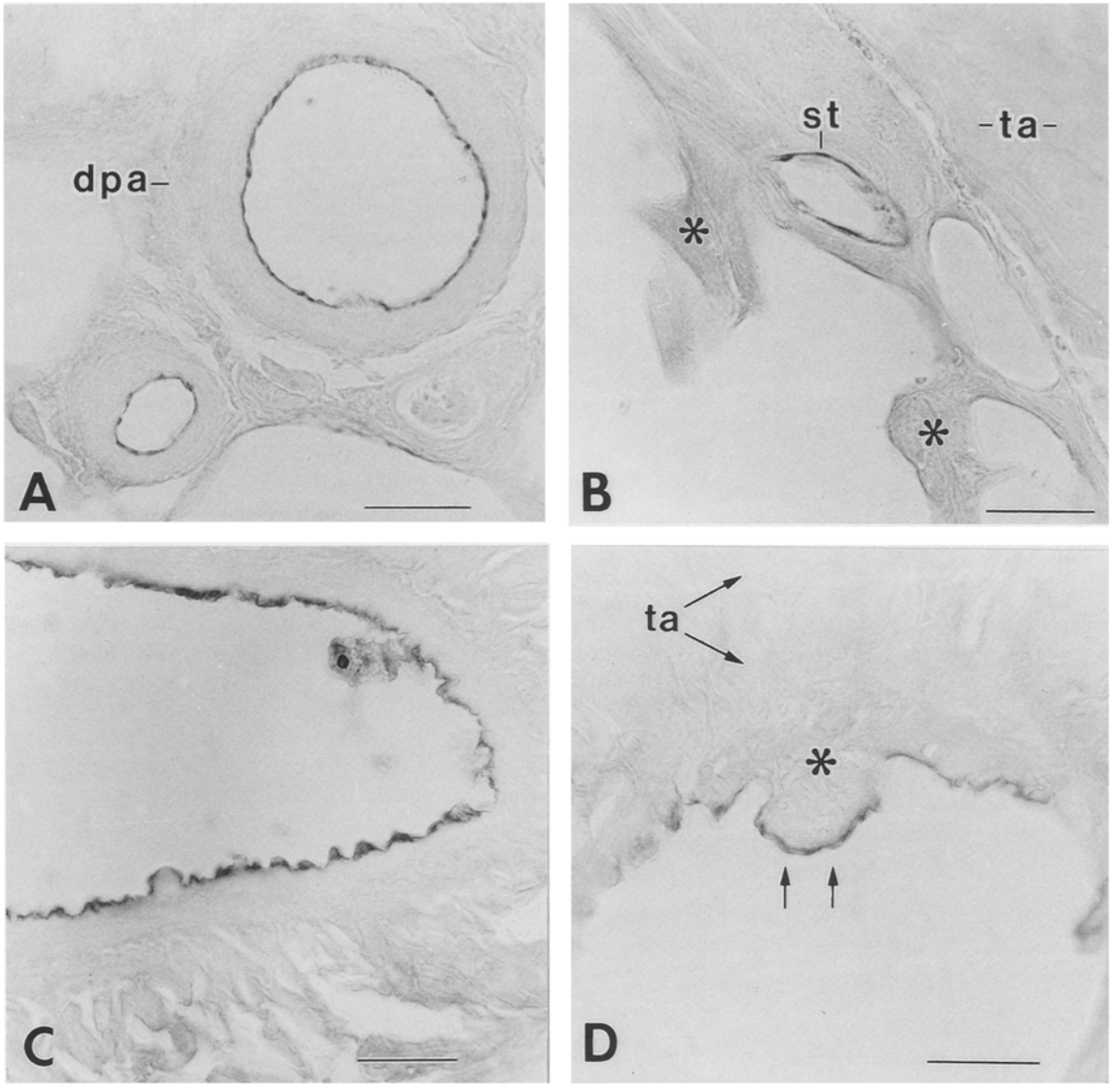

Fig. 4A–D. eNOS immunoreactivity in rat penile erectile tissue. **A** The endothelium of the deep penile artery (*dpa*) and one of its branches are intensely stained for eNOS. *Bar:* 60 µm, ×250 . **B** The endothelium of a subtunical vein (*st*) is stained for eNOS; however, there is no staining of the endothelium lining the cavernous smooth muscle (*). *ta* Tunica albuginea. *Bar:* 60 µm; ×250. **C** eNOS staining of the dorsal penile vein. *Bar:* 40 µm; ×350. **D** Cross-section of the cavernous vein in the penile shaft illustrating eNOS immunoreactivity of some of the endothelial cells (*arrows*). *ta* Tunica albuginea; * smooth muscle. *Bar:* 40 µm; ×400

Some of the large nerve bundles, which bulge into the vascular spaces or which are found within the trabecular walls, contain tortuous NADPH-D fibers (Fig. 6B, C).

All penile neurons labeled by injection of Fluorogold into the penile crura were intensely to moderately stained for NADPH-D (in three rats: 221 intense, 12 moderate, and 0 unstained ganglion cells) (Fig. 7A, B). In the MPG of two animals, 89% of penile neurons (220 of 246) were immunoreactive for nNOS (Fig. 7C, D).

Discussion

The antibody method for nNOS gives essentially similar results to those obtained with NADPH-D. Both techniques reveal that all smooth muscle regions of the penis are richly supplied with nerve fibers. Moreover, this innervation is derived from neurons in the pelvic plexus, most (if not all) of which seem capable of synthesizing NO. In contrast to the corpora cavernosa penis, only a few NADPH-D+ fibers lie adjacent to trabeculae in the glans penis and these either are related to small arteries or are present in largely unreactive nerve bundles. Finally, there is a good correspondence between NADPH-D staining and eNOS staining of endothelial cells in the penis. Distinct regional differences have also been noted: the endothelium of arteries and veins are reactive for NOS, whereas the endothelium of the cavernous spaces is unstained.

The present results provide additional evidence that penile neurons in the pelvic plexus comprise a uniform

population in regard to neuroactive substances, viz., practically all are immunoreactive for NOS and vasoactive intestinal polypeptide (Dail et al. 1983) and all demonstrate cholinergic characteristics (Dail and Hamill 1989). The latter phenotype is based on the strong reaction for acetylcholinesterase in penile ganglion cells and the presence of choline acetyltransferase in penile postganglionic nerve pathways (Dail et al. 1986; Dail and Hamill 1989). The results of NADPH-D staining of penile ganglion cells agree with our preliminary observations (Dail et al. 1992) and those Vizzard et al. (1994) and Schirar et al. (1994) who have also found that the great majority of penile neurons are stained with this method. We have also observed that a comparable percentage of penile neurons are stained by the nNOS antibody (some 89%), in contrast to Vizzard et al. (1994) who report a lower percentage (average of 21%). The reason for this lack of agreement is not clear; however, differences in antibody penetration may account for the disparate results (Vizzard et al. 1994).

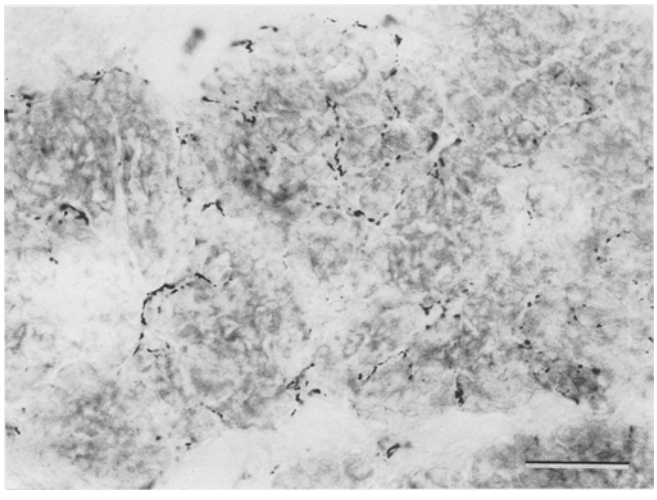

Fig. 5. NADPH-D+ fibers surround the acini of the paraurethral glands in the penile bulb of the rat. *Bar:* 60 μm; ×240

The widespread distribution of NOS fibers in arteries, intrinsic cavernous muscle, and most penile veins suggests that these tissues behave similarly; specifically, it is likely that all are relaxed by NO. For example, the NOS innervation of the deep dorsal vein described in the present study is consistent with the increase in blood flow in this vessel during erection (Andersson et al. 1984). In contrast, the paucity of smooth muscle in subtunical veins and in the deep penile vein, i.e., the penetrating vein at the hilum of the crura (see Fig. 2c), argues against nerve-mediated events and further supports the role of passive compression in restricting egress of blood from the corpora cavernosa penis (Fournier et al. 1987).

McNeill et al. (1992) have demonstrated an abundant NADPH-D plexus subjacent to the epithelium of the penile urethra and suggest that it represents sensory fibers in addition to the autonomic innervation of erectile tissue. We concur with these observations and further suggest that the paraurethral glands are another target tissue of NADPH-D neurons in the MPG (Santer and Symons 1993). The role of NADPH-D fibers in this tissue is not known, but presumably NO could regulate blood flow or glandular secretion and emptying.

Few NADPH-D fibers are associated with the trabecular walls of the glans penis, an observation in keeping with the abundance of elastic tissue in this region. The lack or scarcity of a motor innervation to the wall of the

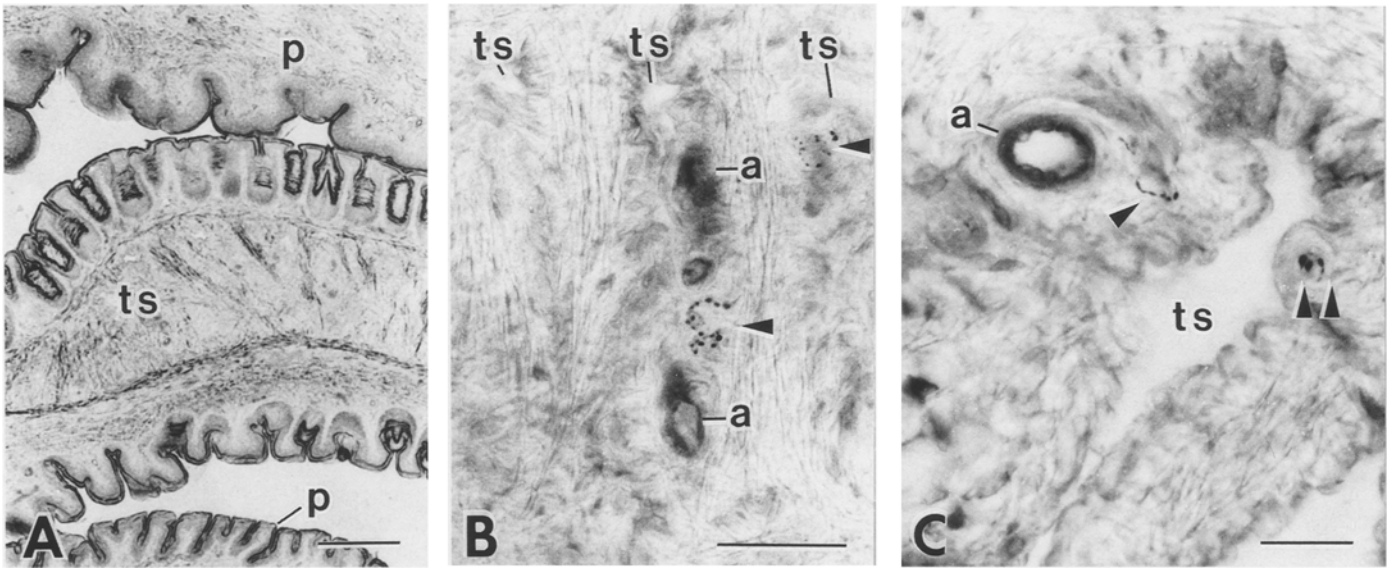

Fig. 6A–C. NADPH-D staining in the glans penis. **A** Longitudinal section of the glans penis showing the spiny portion of the glans penis surrounded by the prepuce (*p*). The elastic tissue that separates the trabecular spaces (*ts*) is evident at low magnification. *Bar:* 360 μm; ×140. **B** The NADPH-D staining of the vascular endothelium marks the presence of small arteries (*a*) in the walls of the trabecular spaces (*ts*). Nerve fibers are often present (*arrowheads*). *Bar:* 120 μm; ×300. **C** A small vessel (*a*), accompanied by nerve fibers (*single arrowhead*), and a NADPH-D+ fiber in a nerve bundle (*double arrowheads*) are present in the trabecular wall. *ts* Trabecular space. *Bar:* 60 μm; ×300

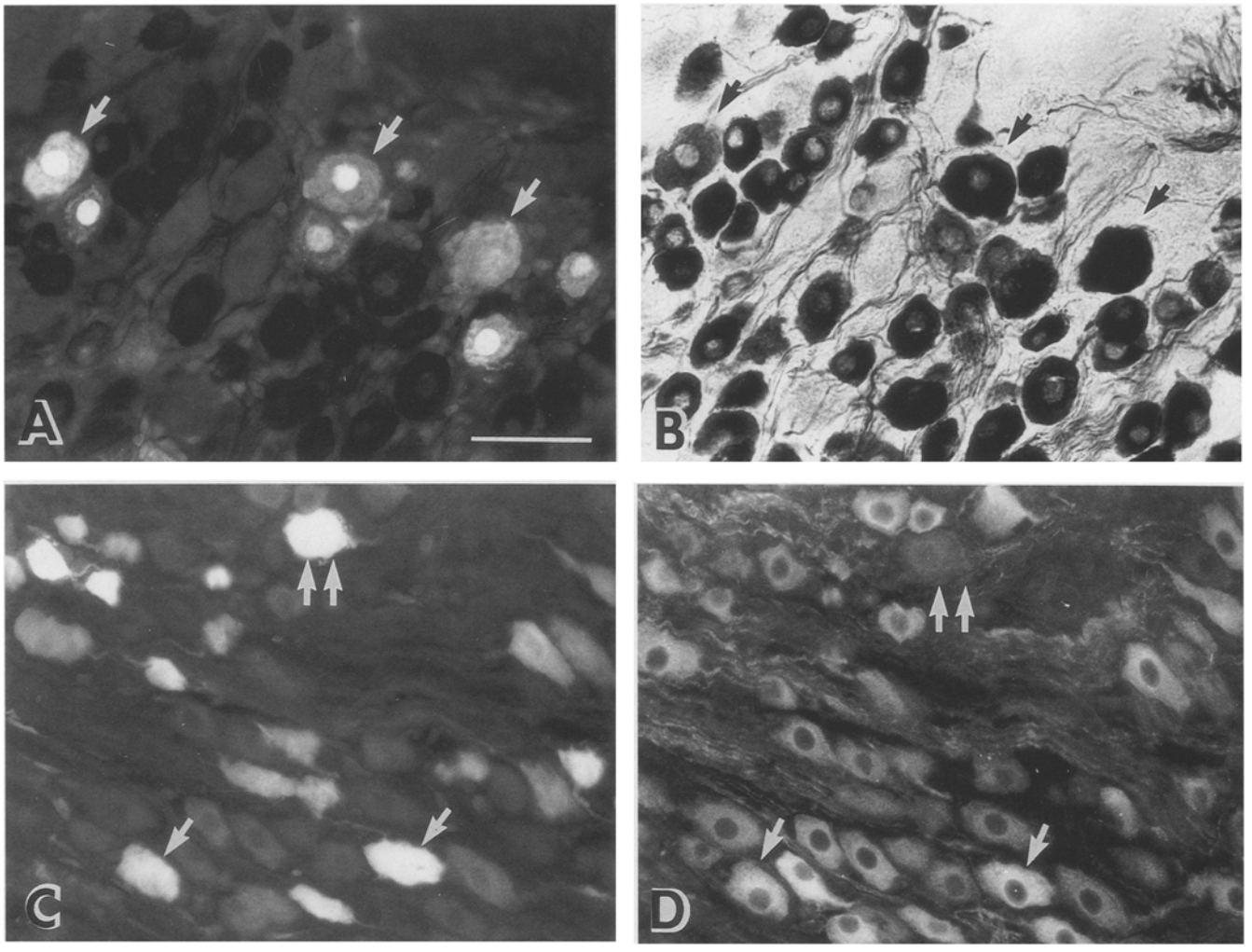

Fig. 7A–D. Paired photomicrographs of penile neurons in the major pelvic ganglion (MPG) stained for NADPH-D and nNOS. **A** *Arrows* mark some of the neurons that have been filled by injection of Fluorogold into the penile crura. **B** The same section stained for NADPH-D. All neurons are moderately to intensely stained for NADPH-D. **C** Fluorogold-filled ganglion cells. **D** The same section stained with an antibody to nNOS. The great majority of penile neurons stain for nNOS (*single arrows*), but some remain either unstained or weakly stained (*double arrow*). *Bar:* 40 μm; ×410 (all micrographs)

trabecular spaces in the glans argues in favor of a passive role for this tissue in erection. It follows that erection in the glans penis is probably the result of the extensive communication of its vascular sinuses with those of the more proximal penile bulb (Fernandez et al. 1991), with an additional source being provided by small arteries. The NADPH-D fibers around the trabecular spaces of the glans penis may be related to small blood vessels or perhaps are a component of the complex sensory innervation of this region of the penis. The processes of NADPH-D+ dorsal root ganglion cells are present in the dorsal penile nerves of the rat (McNeill et al. 1992); these may continue into deeper regions of the glans. Johnson and Halata (1991) have found small bundles of unmyelinated axons within some of the lamellated corpuscles in the glans penis of the rat and consider whether they represent an autonomic innervation similar to that of Pacinian corpuscles (Santini et al. 1971). Further studies are required however to identify the target tissues of NADPH-D fibers in the glans penis.

NADPH-D staining is particularly useful in signaling which endothelial cells are capable of producing NO. In all instances, eNOS immunoreactivity has verified results obtained with NADPH-D. Our results, which expand the observations of Keast (1992), indicate that the endothelium of all vessels is moderately to intensely stained by the NADPH-D method; moreover, the present study has further localized NADPH-D and eNOS staining to those vascular channels at the periphery of the cavernous spaces in the crura and to limited regions of the cavernous veins in the penile shaft. Previous studies (Fernandez et al. 1991) and the examination of serial sections suggest that these peripherally located channels in the penile crura are subtunical veins, rather than cavernous spaces.

In summary, the present data indicate that penile vessels of all calibers may be regulated by NOS vasodilator innervation and by NO released from their endothelial lining. It is presumed that the relaxation of cavernous muscle is solely under neural control, since there is no

evidence of NOS immunoreactivity in endothelial cells in this location. If true, investigations that have implicated cavernosal endothelial cells in the release of NO may have to be reconsidered.

Acknowledgements. The authors express their appreciation to Jana Bordegaray and Bernice Galloway for expert technical assistance. This work was supported by NIH grants RO11983-09 GM08222-03 to W.G.D.

References

Alm P, Larsson B, Ekblad E, Sundler F (1993) Immunohistochemical localization of peripheral nitric oxide synthase-containing nerves using antibodies raised against synthesized C- and N-terminal fragments of a cloned enzyme from rat brain. Acta Physiol Scand 148:421–429

Andersson P-O, Bloom SR, Mellander S (1984) Haemodynamics of pelvic nerve induced penile erection in the dog: possible mediation by vasoactive intestinal polypeptide. J Physiol (Lond) 350:209–224

Bowman A, Gillespie JS (1983) Neurogenic vasodilation in isolated bovine and canine arteries. J Physiol (Lond) 341:603–616

Burnett AL, Lowenstein CJ, Bredt DS, Chang TSK, Snyder SH (1992) Nitric oxide: a physiologic mediator of penile erection. Science 257:401–403

Burnett AL, Tillman SL, Chang TSK, Epstein JI, Lowenstein CJ, Bredt DS, Snyder SH, Walsh PC (1993) Immunohistochemical localization of nitric oxide synthase in the autonomic innervation of the human penis. J Urol 150:73–76

Carati CJ, Creed KE, Keogh EJ (1987) Autonomic control of penile erection in the dog. J Physiol (Lond) 384:25–538

Dail WG, Hamill RW (1989) Parasympathetic nerves in penile erectile tissue of the rat contain choline acetyltransferase. Brain Res 487:165–170

Dail WG, Moll MA, Weber K (1983) Localization of vasoactive intestinal polypeptide in penile erectile tissue and in the major pelvic ganglion of the rat. Neuroscience 10:1379–1386

Dail WG, Minorsky N, Moll MA, Manzanares K (1986) The hypogastric nerve pathway to penile erectile tissue: histochemical evidence supporting a vasodilator role. J Auton Nerv Syst 15:341–349

Dail WG, Galloway B, Bordegaray J, Walton G (1992) Functional and histochemical evidence for the involvement of nitric oxide in regulation of penile erectile tissue. Soc Neurosci 18:128

Ding Y, Wang Y, Qin B, Li J (1993) The major pelvic ganglion is the main source of nitric oxide synthase-containing nerve fibers in penile erectile tissue of the rat. Neurosci Lett 164:187–189

Fernandez E, Dail WG, Walton G, Martinez G (1991) The vasculature of the rat penis: a scanning electron microscopic and histologic study. Am J Anat 192:307–318

Fournier GR, Juenemann K-P, Lue TF, Tanagho EA (1987) Mechanisms of venous occlusion during canine penile erection: an anatomic demonstration. J Urol 137:163–167

Gabbott PLA, Bacon SJ (1993) Histochemical localization of NADPH-dependent diaphorase (nitric oxide synthase) activity in vascular endothelial cells in the rat brain. Neuroscience 57: 79–95

Hedlund H, Andersson K-E (1985) Comparison of the response to drugs acting on adrenoreceptors and muscarinic receptors in human isolated corpus cavernosum and cavernosum artery. J Auton Pharmacol 5:81–88

Holmquist F, Hedlund H, Andersson K-E (1991) L-N^G-nitro arginine inhibits non-adrenergic, non-cholinergic relaxation of human isolated corpus cavernosum. Acta Physiol Scand 141:441–442

Hope BT, Vincent SR (1989) Histochemical characterization of neuronal NADPH-diaphorase. J Histochem Cytochem 37:653–661

Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J (1990) Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biomed Biophys Res Commun 170:843–850

Johnson RD, Halata Z (1991) Topography and ultrastructure of sensory nerve endings in the glans penis of the rat. J Comp Neurol 312:299–310

Keast JR (1992) A possible neural source of nitric oxide in the rat penis. Neurosci Lett 143:69–73

Kim N, Azadzoi KM, Goldstein I, Saenz de 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

Kirkeby HJ, Svane D, Poulsen J, Tottrup A, Forman A, Andersson K-E (1993) Role of the L-arginine/nitric oxide pathway in relaxation of isolated human penile cavernous tissue and circumflex veins. Acta Physiol Scand 149:385–392

Knispel HH, Goessl C, Beckmann R (1991) Basal and acetylcholine-stimulated nitric oxide formation mediates relaxation of rabbit cavernous smooth muscle. J Urol 146:1429–1433

McNeill DL, Papka RE, Harris CH (1992) CGRP immunoreactivity and NADPH-diaphorase in afferent nerves of the rat penis. Peptides 13:1239–1246

Mills TM, Wiedmeier VT, Stopper VS (1992) Androgen maintenance of erectile function in the rat penis. Biol Reprod 46: 342–348

Rajfer J, Aronson WJ, Bush PA, Dorey FJ, Ignarro LJ (1992) Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N Engl J Med 326:90–94

Sachs BD (1983) Potency and fertility: hormonal and mechanical causes and effects of penile actions in rats. In: Balthazart J, Prove E, Gilles R (eds) Hormones and behavior in higher vertebrates. Springer, Berlin Heidelberg New York, pp 86–110

Santer RM, Symons D (1993) Distribution of NADPH-diaphorase activity in rat paravertebral, prevertebral and pelvic sympathetic ganglia. Cell Tissue Res 271:115–121

Santini M, Ibata Y, Pappas GD (1971) The fine structure of the sympathetic axons within the Pacinian corpuscle. Brain Res 33:279–289

Schirar A, Giuliano F, Rampin O, Rousseau J-P (1994) A large proportion of pelvic neurons innervating the corpora cavernosa of the rat penis exhibit NADPH-diaphorase activity. Cell Tissue Res 278:517–525

Tracey WR, Nakane M, Pollock JS, Forstermann U (1993) Nitric oxide synthases in neuronal cells, macrophages and endothelium are NADPH diaphorases, but represent only a fraction of total cellular NADPH diaphorase activity. Biochem Biophys Res Commun 195:1035–1040

Vizzard MA, Erdman SL, Forstermann U, Groat WC de (1994) Differential distribution of nitric oxide synthase in neural pathways to the urogenital organs (urethra, penis, urinary bladder) of the rat. Brain Res 646:279–291

Zamboni L, De Martino C (1967) Buffered picric-acid formaldehyde: a new rapid fixative for electron microscopy. J Cell Biol 35:148A