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Nadph Diaphorase Histochemical Presence in Some Mammals' Penis

Vincenzo Tessitore, Maria Buscemi, Giuseppe Bonaventura and Aldo Gerbino
Dipartimento di Medicina Sperimentale (DI.ME.S)-Sezione di Istologia ed Embriologia, Arcangelo Pasqualino di Marineo, Facoltà di Medicina e Chirurgia, Università degli Studi di Palermo, Italy

Abstract: Intrabdominal and extrabdominal portion of penis (rat, guinea pig, hamster and rabbit) have been used for histochemical activity Nadph-diaphorase. Pharmacological and electrophysiological studies, both *in vivo* and *in vitro*, make us to belive that, in man, dog, rabbit and rat, the relaxation of vascular smooth muscle of the cavernous body of penis, that is responsible for erection, is produced by the activation of a Non-Adrenergic, Non-Cholinergic (NANC) nervous pathway whose mediator is a new neurotrasmitter: Nitric Oxide (NO). Our observations provide the morphohistochemical base for the localization of NADPH-diaphorase in the different constitutive structures of the penis in some mammalian species. NADPH-diaphorase is a histoenzymatic revealing method that has been used to identify the structural components responsible for NO synthesis.

Key words: Penis, rodent, nadph-diaphorase, NO

INTRODUCTION

Recent pharmacological and electrophysiological studies, both *in vivo* and *in vitro*, make us to belive that, in man, dog, rabbit and rat, the relaxation of vascular smooth muscle of the cavernous body of penis, that is responsible for erection, is produced by the activation of a Non-Adrenergic, Non-Cholinergic (NANC) nervous pathway (Ignarro *et al*., 1990; Costa *et al*., 1991; Burnett *et al*., 1992; Bush, *et al*., 1992; Trigo-Rocha *et al*., 1993) whose mediator is a new Neurotrasmitter: Nitric Oxide (NO). Nitric oxide has been considered for a long time a simple noxious gas, but nowadays literature identifyes NO as an extraordinary intracellular messanger. It is expressed by a number of cell types, it is able to modulate a wide range of functional responses of primary importance for many systems and organs: Central and nervous system, cardiovascular system, gastroenteric and respiratory apparatus (Bredt and Snyder, 1992; Costa *et al*., 1991; De Giorgio *et al*., 1993; Faussone and Bacci, 1993; Fischer *et al*., 1993; Tessitore *et al*., 1994 a, b, c, d; 1998).

The synthesis of this molecule from L-arginine is regulated by two enzymatic isoforms of Nitric Oxide Synthase (NOS): A costitutive isoform (cNOS) that is activated in physiological conditions and releases small controlled amount of NO with benefic effects and an inducible isoform (iNOS) that is induced in pathological conditions (during infections) by some cytokines and bacterial endotoxins and is responsible for the production of excessive and harmful amount of no (Bredt and Snyder, 1992; Moncada *et al*., 1991).

This production can be inhibited by arginine analogues that are used in many experimental conditions because they represents a good methodological strategy to study the roles of NO in the different parenchyma.

We have carried on a research project that addresses the histochemical distribution of NO in the different parenchyma of some mammals (rat, guinea pig, gerbil, hamster, ferret, rabbit, ox, man; Tessitore *et al*., 1994 a, b, c, d), we report histochemical data about the presence of this chemical messenger in the penis of some mammals (rodent), first organ examinated between the organs that form the urogenital apparatus, that subsequently will be extensively studied for NO from the immunohistochemical point of view.

MATERIALS AND METHODS

Nadph-diaphorase histoenzymatic revealing method has been used to identify the structural components responsible for NO synthesis. NADPH-diaphorase has been used because it has been demonstrated that NADPH diaphorase co-localized and is metabolically linked with NOS and both neuronal NOS and NADPH diaphorase have the same localization in brain and peripheral tissues (Dawson *et al*., 1991).

Transversal and longitudinal cryostat sections of intrabdominal and extrabdominal portion of penis (rat, guinea pig, hamster and rabbit) have been used. Sections have been incubated for 15-30 min 37°C in PBS buffer pH 7.4 containing 1.2 mM beta-Nadph and 0.3 mM

Correspondig Author: Vincenzo Tessitore, Dipartimento di Medicina Sperimentale-Sezione di Istologia, Facoltà di Medicina e Chirurgia, Università di Palermo-Via del Vespro n. 129, 90127 Palermo, Italia

NBT, 0.1 mM Tris HCl pH 7.2. Afterwards sections have been rinsed in PBS buffer and mounted in glycerol for microscopical examination.

RESULTS

In spite of the significant differences between the microscopic architecture of penis in the different rodent species examinated, we did not detect substantial histotopochemical differences in NADPH-diaphorase reactivity of the different structural components.

In the pelvic plexus, located in the pelvic cavity of the periprostatic region, made of neurons whose nervous fibres project to the erectile tissue, an intense NADPH-diaphorase reactivity has been detected (+++) in a numerous population of ganglion neurons and in the axons of the costitutive fibres bundles of the cavernous nerve (Fig. 1a, b).

At the base of the penis, between the striated muscles bundles that wrap the bulb of erectile structures an intense reactivity is detectable in secretory and excretory epithelial cells of the bulbo-urethral gland where reactive nervous terminals can be seen (Fig. 2 and 3). In longitudinal section, proceeding from the base towards the prossimal segment, the cavernous bodies seems to be flanked by nerves; ramifications of the cavernous arteries can be seen, their NADPH-diaphorase reactivity does not appear to be limited to the endothelial lamina but to the avventitial wall and also to the nervous small nerves that infiltrate it (Fig. 5).

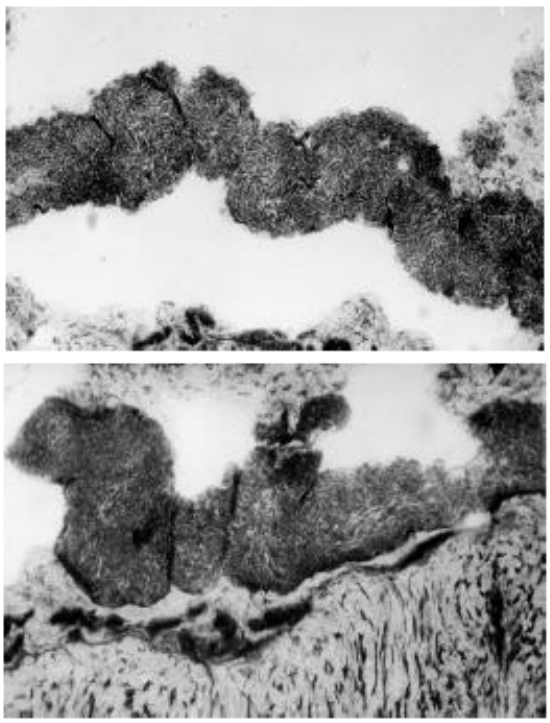

Fig. 1a, b: Hamster penis: NADPH-diaphorase reactivity in nervous fibers (630X)

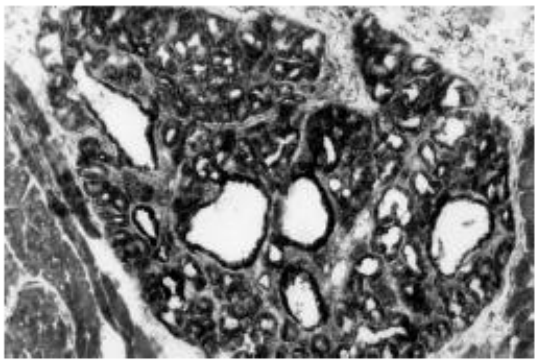

Fig. 2: Guinea pig penis: NADPH-diaphorase. Intense reactivity in secretory cells and in the excretory ducts of bulbo-urethral gland (200X)

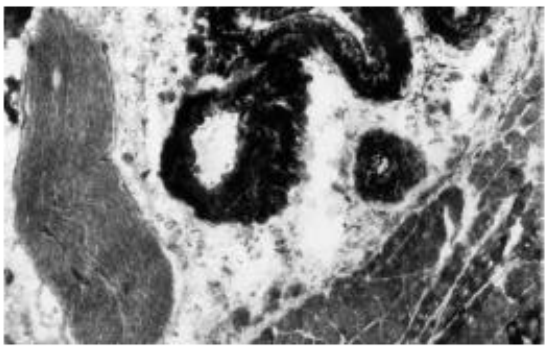

Fig. 3: Hamster penis: NADPH-diaphorase reactivity in bulbo-urethral gland, in nervous and smooth muscle fibers (630X)

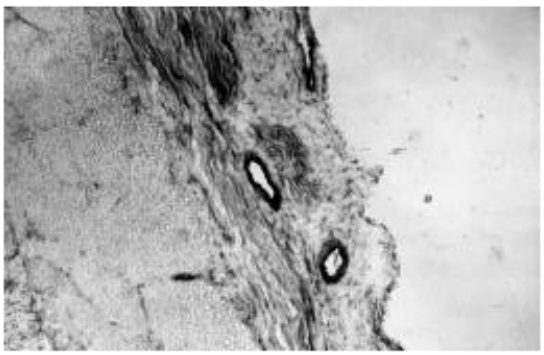

Fig. 4: Rat penis: NADPH-diaphorase strong reactivity in the vascular layer of smooth muscle cells (200X)

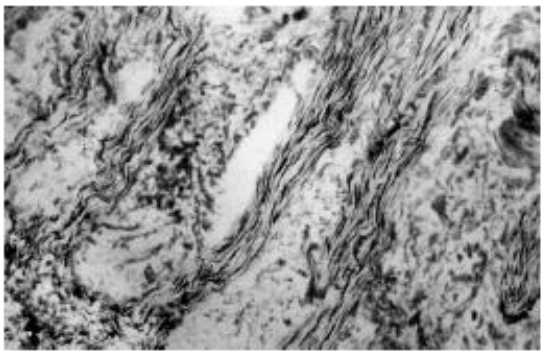

Fig. 5: Rabbit penis: NADPH-diaphorase reactivity in muscular cavernous bodies (200X)

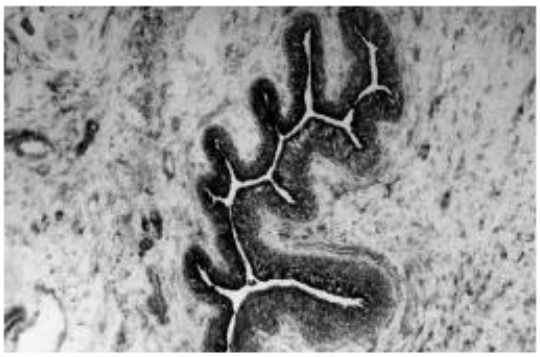

Fig. 6: Rat penis: NADPH-diaphorase reactivity in the urethral epithelium (200X)

An intense reactivity we also detect in the large muscular net that forms the cavernous bodies (Fig. 6). In the urethral epithelium is clearly visible a strong reactivity (Fig. 7).

CONCLUSION

Previous *in vitro* biochemical studies led to the identification of a constitutive NO synthase in the cavernous bodies of rabbit. Other electrophisiological data suggest that NO has a key role in the control of cavernous body relaxation in man, rat and rabbit.

Our observations provide the morphohistochemical base for the localization of NADPH-diaphorase in the different constitutive structures of the penis in some mammalian species, thus giving the opportunity of interpret and compare the NO-role of these regions from the histochemical point of view.

The selective distribution od NADPH-diaphorase reactivity in the different structural components of the penis, both neural and not-neural, indicates the existence of multiple cell types that synthetise NO, as we already demonstrated in other organs (Tessitore *et al*., 1994 a, b, c, d; 1998) and thus suggests the possibility of a role in the differentiation process (neural and paraneural) for NO in the penis.

The existence of NADPH-diaphorase reactivity in the neuronal soma, in the nervous fibers of pelvic plexus, in the walls of cavernous vessels and in the muscular cells' net of the cavernous bodies, make us to belive that NO really represents the physiological neurotransmitter of the NANC relaxation of the cavernous bodies in the examined mammals and thus it could be the most important chemical messenger for the erection.

The functional consequence of this data in the clinical-therapeutic field of sexual dysfunction in man has generated and still generates great interest. Thus NOS inhibitors (L-NAME) could be responsible for the some pathologies such as priaprism. The recent habit to use of NO-donors (Linsidomina, Sildenafil) could represent a new therapeutic strategy in the treatment of the different types of erectile dysfunction aimed to correct deficit in the biosynthesis of this molecule.

The existence of NADPH-diaphorase reactivity in the epithelial structures of the penis (the urethral epithelium and the enclosed glands) suggests that the NO, synthesized by a constitutive NOS of these type of cells, could have new paraneural roles through the transduction of nitric-oxyde-dependent modulating signals that are involved in the regulation of the functions of these epithelial cells (urinary continence and miction).

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