Resistance of Urethral Sphincter of Female Rats

The Effect of a Nitric Oxide Synthase Inhibitor on Urethral Sphincter Resistance of Female Rats under Normal Conditions and with Chemically Induced Cystitis

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ABSTRACT

Objective: The aim of this work was to investigate urethral sphincter resistance in female rats under normal conditions as well as with chemically induced cystitis.

Materials and Methods: Female rats were anesthetized with urethane (1.2 g/kg, subcutaneously) and submitted to a gradual increase of the intravesical pressure with saline. The effect of increased cumulative doses of N^G-L-Arginine methyl ester and L-Arginine, injected via intrathecal and intracerebroventricular was evaluated in these animals.

Results: We verified that the injection through intrathecal of N^G-L-Arginine methyl ester and L-Arginine did not affect the normal rats. But rats treated with a single dose of cyclophosphamide (150 mg/kg, intraperitoneally), 20-22 hours following the experiment, and in normal or treated rats when injections of N^G-L-Arginine methyl ester (or L-Arginine) were applied in increased cumulative doses via i.c.v. there was a significant increase (decrease) of the magnitude of the dose-dependent responses of the external urethral sphincter, respectively.

Conclusions: Our results suggest that nitric oxide participates in the EUS function, and is regulated by the supraspinal area. Furthermore, the results also indicate that the participation of nitric oxide in the lumbosacral area only occurs in cystitis conditions.

INFORMAÇÕES

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INTRODUCTION

The storage and elimination of urine require coordinated function between activities of the smooth muscles of the bladder and the external urethral sphincter (EUS). This activity is integrated in regulating centers found in the supraspinal nuclei, in the spinal cord nuclei and in the peripheral ganglia. These centers regulatethree groups of peripheral fibers: sympathetic fibers that release noradrenaline in the smooth muscle causing contraction in the bladder neck and in the EUS, as well as relaxationof the detrusor; parasympathetic fibers that cause nitrergic inhibitory reaction in the urethral smooth muscle and contractile reaction in the bladder muscle; and lumbosacral somatic fibers, which cause contractile action in the striated muscle of the EUS[1]. In this way, the relationship between action in both the bladder and urethra is reciprocal during urine filling/storage or voiding/micturition, i.e. during the storage of urine the bladder tonus is reduced, while the EUS tonus is elevated. However, at micturition, the smooth muscle of the bladder contractsand the striated external urethral sphincter relaxes[1].

In neuroanatomy studies with rats, various authors have shown that neurons from the micturition pontine center (PMC) ventrolateral area have direct projection on the Onuf's nuclei. It has been verified that the electrical or chemical stimulation of these nuclei evokes EUS contractions[2-5]. Furthermore, the striated muscle of the EUS is innervated by the pudendum nerve that arises in the dorsolateral nuclei (DL) of the dorsal horn (Onuf's nucleus)[6] and its long dendrites project dorsolaterally towards the *intermediumlateraliscell* group (IML), ventromedially towards the central canal and towards the medial parts of the lamina VIII and dorsal commissural L_5-S_1 of the spinal cord[7,8].

Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d), an indicator of the presence of neuronal nitric oxide synthase (nNOS), has been detected in sympathetic and parasympathetic preganglionic neurons, in laminae V-VI, and parasympathetic postganglionic neurons that innervate the urethra and bladder[9-14]. This fact suggests that the urethral smooth muscle is regulated by sympathetic and parasympathetic preganglionic neurons. Using retrograde axonal tracing studies with *pseudorabiae* virus in the urethral smooth muscle, labeled cells were identified in the sacral parasympathetic nuclei, in the dorsal commissure, around the central canal of the spinal cord and in theintermediumlateralis area of the L_6-S_1 segments[6,15,16].

The EUS muscles are innervated by somatic cholinergic fibers and non-adrenergic and non-cholinergic fibers associated to neurotransmitter/neuromo-dulator nitric oxide (NO) which induces relaxation of both the smooth and striated muscles of the urethra[17,18]. Pharmacologically, it has been reported that the N-Nitro-L-Arginine (L-NOARG), a nitric oxide synthase (NOS) inhibitor, reduced the magnitude and duration of the urethral reflex during micturition. This effect was decreased or inhibited after L-Arginine (L-ARG) administration, suggesting the involvement of NO[18,19]. In addition, the NO as a mediator of relaxation of the smooth muscle and of the urethral striated muscle has been reported in different experimental animals[20-22].

Considering thatboth spinal cord (lumbosacral) and supraspinal nuclei have active participation in the EUS function, the purpose of the present research was to determine if NO is involved at spinal cord and supraspinal levels in the EUS resistance in both normal and pathological female ratswhich underwent augmented intravesical pressure.

METHODS

Ethical approval

The protocol applied in this experiment was approved by the Committee of Ethics of the Ribeirão Preto Medical School– University of S. Paulo. This current study was developed in the Laboratory of Neurourology - Division of Urology,Ribeirão Preto MedicalSchool - University of S. Paulo. All efforts were made to minimize suffering and reduce the number of animals used in this experimental protocol.The animals were maintained under standard laboratory conditions (at a temperature of 22±2 ºC, in a 12:12 h, light/dark cycle), with free access to food pellets and tap water.

Experimental procedure

Twenty female Wistar rats, weighing 230-260 g, were anesthetized with urethane (1.2 g/kg, subcutaneously). After a midline incision in the lower abdomen (~1.0 cm), the bladder was exposed and a polyethylenecatheter(PE-50; id=0.58 mm, od: 0.96 mm)containing a small collar created with a fire-flared tip was inserted into the bladderdomeand tied with 4-0 silk for bladder filling and pressure recording simultaneously.Thereupon the animal was placed on a special table for this kind of procedure in rats (B. Braun Melsungen AG, type 876030) which permitted a bipedal position (similar to the human position), without provoking muscular stretching. The catheter inserted into the bladder was connected via a three-way stopcock to a pressure transducer (WPI BPcable,Instruments Inc., Akron, OH, USA) to monitor bladder pressure during cystometry. Areceptacle containing 0.9% saline (37 ºC) should be moved slowly along a shaft in perpendicular position which will slowly infuse the bladder by gravity, thus gradually determining the volume/pressure. When the first fluid dropwas expelled, via urethra (leak point pressure), evoked by distension of the bladder, the pressure valuecorresponding to maximum resistance of the EUSwas defined. The correspondent recording was repeated three times at each step of the experiment. The recordeddata were collected and stored in the computer using the Windaqsoftware (DATAQ-DI-720 Instruments Inc., Akron, OH, USA). When the experiments were finished, the animals were euthanatized by exsanguination.

Implantation of the Intrathecal Catheter

For intrathecal (i.t.)catheter implantation, the animals were anesthetized with ethyl ether inhalation; after that a surgical

REVISTA UROMINAS - 2318-0021

incision (~1.0 cm) was made in the lower lumbar region of the vertebral column, preserving the muscular layer. A special needle was inserted in the intervertebral space between L_5-L_6. The correct position of the needle in the subarachnoid space was confirmed when the animal showed a tail-flick reflex. Subsequently, a polyethylene-10catheter (PE-10; od = 0.61 mm, id = 0.28 mm) with 10% heparin was inserted through the needle, and its tip was placed in the adjacent site of the spinal cord (L_6 through S_1); the catheter was tied with 4-0 silk to the subcutaneous tissue. The needle was removed and the external tip of the catheter was sealed by electrocautery. This catheter was placedtemporarily in the subcutaneous space. The skin was then running sutured with 6-0 mononylon thread. The experiment recordings of bladder pressure were performed 4-6 days after implantation of the i.t. catheter. The surgical evaluation of the accurate position of the catheter tip in the spinal cord of the animals was confirmed with dye (methylene blue) administration via i.t. immediately after the experimental recording.

Implantation of the Intracerebroventricular Cannula

For intracerebroventricular (i.c.v.) cannula implantation, the animals were anesthetized with 2.5% tribromoethanol (1.0 µL/100 g of the body weight, intraperitoneally). Additional doses of the anesthetic were administered when necessary. The animals were placed in the stereotaxic frame (David Kopf Instruments, USA) for the implantation of the cannula in the right lateral ventricle (coordinates of 1.0 mm caudal to bregma, 1.6 mm lateral from midline, 3.2 mm ventral from dura) in accordance with Paxinos& Watson Atlas[23]. The cannula was fixed into the skull with a small screw placed as an anchor. An occlusive metallic mandrill was placed in the cannula to avoid cerebroventricular fluid infection or drainage. Procaine and potassicbenzilpenicillin (8.000 UI, i.m.) was administered immediately after the surgery ended. These animals were maintained in special boxes in the facility with a synchronized light/dark cycle of 12/12 hour, at temperatures of 24 ± 2.0 ºC, with free access to food and water. The experimental recordings of bladder pressure were performed 4-6 days after the i.c.v. cannula implantation.

Surgical evaluation of the accurate position of the catheter tip in animals was confirmed with dye (methylene blue) administration viai.c.v. immediately after the experimental recording.

Intrathecal and Intracerebroventricular Injections

The drugs were injected directly into the subarachnoid space (L_6-S_1) or into the right lateral ventricle witha volume of 5.0 µL, followed by flushing of 5.0 µL of physiological saline, using a Hamilton'smicrosyringe (10.0 µL) attached to the i.t. catheter or to the i.c.v.cannula, by means of a PE-10 catheter.

Induction of Cystitis

Cystitis was induced by cyclophosphamide (CYP) (150 mg/kg, intraperitoneally). This drug is metabolized to acroleinand excreted in the urine, inducing bladder a hypersensitivity process. The experiments were performed after 45-50 hours of drug administration.

Drug Administration

To evaluate the NO involvement on the EUS of female rats in normal conditions and with cystitis, (a) NG-Nitro-L-Arginine Methyl Ester (L-NAME), a potent non-selective NOS inhibitor, was administered via i.t. or i.c.v., using cumulative doses of 1.0, 3.0 and 10.0 mmol; (b) L-Arginine (L-ARG) drug, a NO precursor, was also administered via i.t. or i.c.v., using cumulative doses of 5.0 and 10.0 mmol. Drugs were dissolved in Milli-Q water. Stock solutions were made and kept at -20 ºC. Subsequent dilutions were made with 0,9% saline. Drug administration was performed slowly (5.0 µL/min), at intervals of 25-30 min. Catheter (PE-10) volume attached to the Hamilton's microsyringe was approximately 7 µL.

Four groups were created: G1 (control, n=6); G2 (rats treated with CYP, n=4); in both groups (G1 and G2) the drugs were administered via intrathecal; G3 (control, n=4); G4 (rats treated with CYP, n=6): in both groups (G3 and G4) the drugs were administered viaintracerebroventricular.

Drugs

NG-Nitro-L-Arginine Methyl Ester, L-Arginine and Urethane (Lab. Tocris, Ellisville, MO, USA), Cyclophosphamide and Tribromoethanol (Lab. Sigma Chemical, Co. St. Louis, MO, USA), Ethyl Ether (Lab. CAQ, SP, Brazil), Procaine and Potassic Benzilpenicillin (Lab. EMS Indústria,SP, Brazil)were drugs used in the current study.

Methods of Analysis

All data values were expressed as mean plus or minus standard error of the mean, and results were calculated at a percentage of the maximal effect (mean of three recordings) obtained after the drug application.The significance between groups was determined using Student's t test. Values of $P<0.05$ were taken to indicate statistical significance. Graphs were generated and parameters computed using the GraphPad Prism-5 program.

RESULTS

We observed that rats treated with CYP showed a thick, tumescent, friable, hemorrhagic bladder wall, and the weight of the bladder was apparently greater than that of untreated rats. There was no significant difference in body weight of these animals before and after catheter (i.t.) or cannula (i.c.v.) implantation. After 60 min of anesthetic administration, the animals were placed in an orthostatic position on the Braun Melsungen table; warm physiological saline solution (37 ºC) placed in a small bottle started to slowly infuse the bladder. The first sign of fluid loss that occurred via urethra was considered as leak point pressure.

The effect of NOS activator and inhibitor on the EUS after intrathecal drug administration.

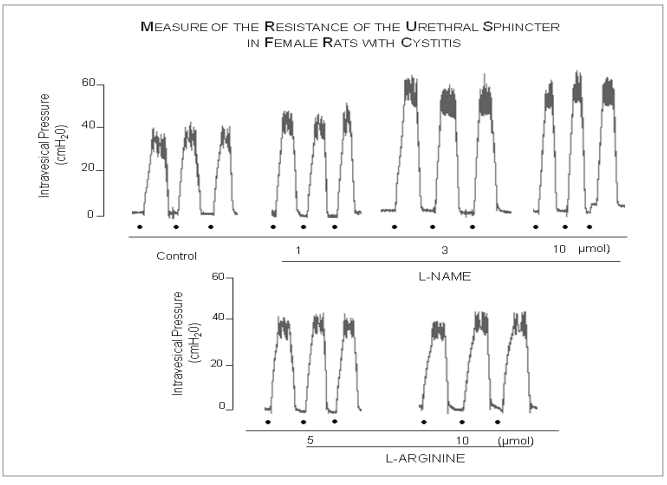
During the experiment, significant changes in the EUS were not observed after the administration of cumulative doses of L-NAME 1.0, 3.0 and 10.0 mmol, or L-ARG 5.0 and 10.0mmolin normal animals. However, the animals treated with CYP showed a dose-dependent curve after 15 min of L-NAME administration The intravesical pressure was gradually increased in the presence of L-NAME; and this increased pressure(induced by L-NAME)was significantly reverted after the administration of 5.0 and 10.0 mmol of L-ARG.

The effect of NOS activator and inhibitor on the external urethral after intracerebroventricular drug administration

L-NAME cumulative graduated doses - 1.0, 3.0 and 10.0mmol - and L-ARG5.0 and 10.0mmol - were administered via i.c.v. in animals of both groups (normal and treated with CYP). Dose-dependent responses were observed in normal rats andlikewise in rats treated with CYP; and thegradual effect of the pressure induced by L-NAME wasslowly reverted after the administration of 5.0 and 10.0 mmol of L-ARG.

FIGURE 1

Graphic representation of the intravesical pressure that expresses urethral sphincter resistance values of a female rat after CYP administration, following the injection of L-NAME and L-ARG via i.t, with the addition of cumulative doses which increase and decrease the urethral sphincter resistance, respectively. Three recordings were performed during the experimental evaluation, 25-30 min after the administration of each drug dose. The top of the recording corresponds to the maximum intravesical pressure of the leak point of the fluid via urethra. Represents the beginning of fluid infusion into the bladder and the correspondent increase of the intravesical pressure.



Fonte: Arquivo Pessoal.

DISCUSSION

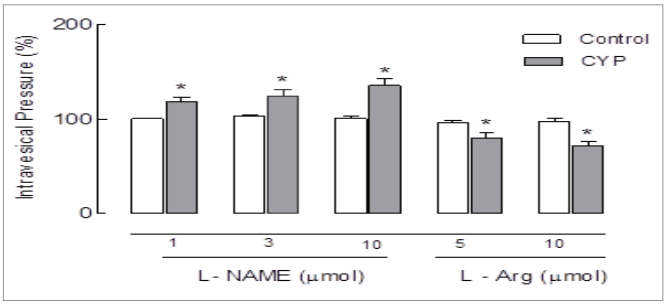
In the present study, an experimental model was used to measure EUS maximal resistance under volume/pressure (leak point pressure - LPP), evaluating the effect of the NO antagonist in the lumbosacral spinal cord (L_6-S_1) and supraspinal levels.

Experimental evaluations of the parasympathetic and nitrergic system participation in EUS functionhave been published[18,24]. It is indicative that the parasympathetic nervous system has relevant action in urethra relaxation mediated by NO. Through microdialysis it has been shown that the administration of nNOS, a selective inhibitor(also known as the neuronal isoform), reduces the acetylcholine (ACh) function released in different areas of the central nervous system (hypothalamus and locus cœruleus), and that the administration of a NO donor such as diethylamineNONOate (DEA/NO) and S-nitroso-N-acethylpenicillamine (SNAP) facilitates the neurotransmitter release[24,25]. From these findings it is possible to consider that the acetylcholine released in the urethra smooth muscle could be reduced by the addition of L-NAME or L-NOARG inhibitors, consequently inhibiting EUS relaxation. This effect can be reverted by the administration of L-ARG (a metabolic precursor in the formation of L-citrulline and NO). In vivoresults presented here, verified in the female rat urethra under the effect of intravesical pressure/volume increase, are convincing evidence that the relaxation of the urethral sphincter involves nitric oxide. These data are consistent with immunohistochemistry and NADPH-diaphorasehistochemistry assays, displaying that the EUS is densely innervated by nNOS containing nerve fibers, including sarcolema of striated fibers[18,26].

REVISTA UROMINAS - 2318-0021

FIGURE 2

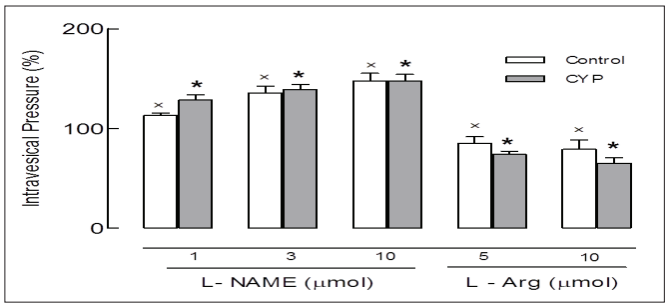
Graphic evaluation of normal female rats and rats with cystitis induced by CYP (150 mg/kg, i.p.). The drug effects are expressed by mean ± SEM (%) in rats which underwent the L-NAME and L-ARG administration via i.t., at 25-30 min intervals, at maximum intravesical pressure. All animals were disposed on the special table in bipedal position. Values correspond to maximum intravesical pressure at the moment when there was loss via urethra of saline infused slowly in the bladder (leak point pressure). The asterisk (filled bar) indicates P<0.05.



Fonte: Arquivo Pessoal.

FIGURE 3

Graphic evaluation of normal female rats and rats with cystitis induced by CYP (150 mg/kg, i.p.) The effect of the drugs is expressed by mean ± SEM (%) in rats which underwent the L-NAME and L-ARG administration via i.c.v., at 25-30 min intervals, at maximum intravesical pressure. All animals were disposed on the special table in bipedal position. in bipedal position. Values correspond to the maximum intravesical pressure at the moment when there was loss via urethra of saline infused slowly in the bladder (leak point pressure) . The X (open bar) and asterisk (filled bar) indicate P<0.05.



Fonte: Arquivo Pessoal.

Surprisingly, the administration of L-NAME and L-ARG microinjections via i.t. in the untreated rat group showed no effect on the EUS during the experiment until 25-30 min after the last dose administration of L-ARG. This observation suggests three hypotheses: first, under normal conditions, very few cells found in the L_6-S_1 are immunoreactivetowards NOS, i.e., they have little effect on the pathway that mediates the EUS function, suggesting that NO is not involved in the normal micturition reflex; or secondly, the NOS inhibitor dose that was administered in normal rats is not representative since the NO is not stocked in synaptic vesicles but synthesized immediately when stimulated as has been observed through immunohistochemical assays[12,26-28];finally, a third possibility is that the NO acts as a neuromodulator but not a neurotransmitter between the afferent ends and preganglionic neurons[27].

A significant quantity of NOS was not identified in the normal rat lumbosacral spinal cord (L_6-S_1) in immunohistochemicalassays. However, the NOS quantity in this region was significantly increased when these animals were treated with bladder irritant agents or suffered spinal injury[14,25]. It is consistent that these data explain the absence of activation by the drugs (L-NAME and L-ARG) injected via i.t. used in the current experiment on EUS in normal rats. However,that activationwas present in rats treated with CYP.

It has beenreported that bladder capacity and maximal bladder pressure at micturition was elevated in genetically modified mice when compared with normal mice[29]. These observations were similar to data noted in rats and mice treated by aminoguanidine, a selective inhibitor of iNOS[30]. It is indicative that L-NAME, an NOS inhibitor, can be effective in an identical way, inhibiting the EUS relaxation mediated by NO and, consequently, can cause an increase in bladder capacity and compliance associated with ahypertonic EUS.

The density of the nNOS isoform is expressive in the paraventriculararea[31], unlike what occurs in the lumbosacral segment (L_6-S_1)1^0.It is consistent that responses observed in normal rats and in rats treated with CYP, after the administration of L-ARG and its analogue L-NAME, via i.c.v., are due to the large concentration of NOS in the supraspinal nervous system. Furthermore, the rapid activation and inhibition of EUS resistance observed in this experiment, soon after the L-NAME and L-ARG administration into the lateral ventricle, suggests that the EUS is susceptible to the influence of these drugs. This possibility is consistent with experimental observation after injection of [^{14}C]-Nω-Nitro-L-Arginine (L-NA),L-[^{14}C]-Citrulline, N^{ω}-Monomethyl-L-Arginine(LMMA) and 7-Nitro-Indazole(L-NIO). These drugs were immediately detected in adjacent tissues;they decreased rapidly, in parallel

TABLE 1 - Urethral resistance in female Wistar rats after administration of L-NAME and L-ARG.

		L-NAME			L-ARG	
		1.0 μL	**3.0 μL**	**10.0 μL**	**5.0 μL**	**10.0 μL**
i.t.	**Normal**	100.5 ± 0.8	103.2 ± 1.3	105.5 ± 2.1	95.9 ± 2.6	74.0 ± 5.2
	CYP	118.9 ± 4.1	123.7 ± 7.3	135.4 ± 7.0	80.3 ± 5.7	71.0 ± 4.2
i.c.v.	**Normal**	113.3 ± 2.5	135.5 ± 6.9	147.8 ± 7.5	85.1 ± 6.8	79.1 ± 9.3
	CYP	128.4 ± 5.3	138.7 ± 5.2	147.8 ± 5.2	74.0 ± 3.2	65.3 ± 5.5

L-NAME = N^G- nitro L-arginine methyl ester; L-ARG = L-arginine i.t.= intrathecal; i.c.v = intracerebroventricular / CYP = cyclophosphamide

with the inhibition of brain NOS[31].Thence, it is reasonable to infer that the immediate response verified in this study soon after the L-NAME and L-ARG administration via i.c.v. in normal rats or rats treated with CYPindicates participation of the periaqueductal graymatterin the EUS action mechanismvia PMC.In addition, it must be considered that the quick onset of maximal inhibition seen in brain regions after i.c.v. L-NAME administration in the brain, even those areas distal to the site of administration, arerapidly exposed to the peak concentration of the inhibitor[31].

It mustbe alsoconsidered that during recordings of the intravesical pressure, leaking of saline solution through the urethra did not occur because of detrusor muscle contractions but becauseleaking of the saline solution by the urethra was the result of intravesical pressure greater than the EUS resistance. During the experiment, bladder hyperreflexia signs were not recorded in the resting or stimulus condition, in eithernormal rats orrats with cystitis.

In summary, the results of the present study showed that L-NAME and L-ARG administration via i.t. did not cause effects on the EUS in normal rats; but this same drug administration via i.t. caused dose-dependent effects in female rats treated with CYP, increasing or decreasing the EUS resistance pressure, respectively. The L-NAME and L-ARGadministration via i.c.v. caused a dose-dependent effect in untreated rats and in rats treated with CYP. These data indicate that EUS relaxation both in untreated rats and in rats treated with CYP is mediated by NO at the supraspinal level, but that this relaxation is also mediated by NO at the lumbosacral spinal cord level, but only in pathological conditions.

REFERENCES

1. Fowler CJ, Griffiths D, de Groat WC: The neural control of micturition. Nat Rev Neurosci. 2008;9(6):453-66.
2. Kruse MN, Noto H, Roppolo JR, de Groat WC: Pontine control of the urinary bladder and external urethra sphincter in the rat. Brain Res. 1990; 532(1-2):182-90.
3. Mallory BS, Roppolo JR, de Groat WC: Pharmacological modulation of the pontine micturition center. Brain Res. 1991;546(2):310-20.
4. Noto H, Roppolo JR, Steers WD, de Groat WC: Electrophysiological analysis of the ascending and descending components of the micturition reflex pathways in the rat. Brain Res. 1991;549(1):95-105.
5. Ding YQ, Takada M, Tokuno H, Mizuno N: Direct projections from dorsolateral pontinetegmentum to pudendalmotoneurons innervation the external urethral sphincter muscle in the rat. J Comp Neurol. 1 995;357(2):318-30.
6. McKenna K, Nadelhaft I: The organization of the pudendal nerve in the male and female rat. J Comp Neurol. 1986;248(4):532-49.
7. Nadelhaft I, Vera PL: Neurons in rat brain and spinal cord labeled after pseudorabies virus injected into the external urethral sphincter. J Comp Neurol. 1996;375(3):502-17.
8. Marson L: Identification of central system neurons that innervate the bladder body, bladder base, or external urethral sphincter of female rats: a transneuronal tracing study using pseudorabies virus. J Comp Neurol. 1997;389(4):584-602.
9. Mumtaz FH, Khan MA, Thompson CS, Morgan RJ, Mikhailidis DP: Nitric oxide in the lower urinary tract: physiological and pathological implications. BJU Intern. 2000;85(5):611-3.
10. Dun NJ, Dun SL, Forstmann U, Tseng LF: Nitric oxide synthase immunoreactivity in rat spinal cord. NeurosciLett. 1992;147(2):217-20.

REVISTA UROMINAS - 2318-0021

11. Valtschanoff JG, Weinberg RJ, Rustioni A:NADPH diaphorase in the spinal cord of rats. NeurosciLett. 1992;321(2):209-22.

12. Vizzard MA, Erdman SL, de Groat WC: Localization of NADPH-diaphorase in bladder afferent and postganglionic efferent neurons of the rat. J AutonNerv Syst.1993;44(1):85-90.

13. Saito S et al.: Rat spinal cord neurons contain nitric oxide synthase. Neuroscience 1994;59(2); 447-56.

14. Vizzard MA et al.: Localization of NADPH diaphorase in the lumbosacral spinal cord and dorsal root ganglia of the cat. J Comp Neurol. 1994;339(1): 62-75.

15. Nadelhaft I, Vera PL: Separate urinary bladder and external urethral sphincter neurons in the central nervous system of the rat: simultaneous labeling with two immunohistochemically distinguishable pseudorabies virus. Brain Res. 2001;903(1-2):33-44.

16. Vizzard MA, Erickson VL, Card JP, Roppolo JR, de Groat WC: Trans-neuronal labeling of neurons in the adult rat brainstem and spinal cord after injection of pseudorabies virus into the urethra. J Comp Neurol. 1995;355(4):629-40.

17. Ho MH, Bhatia NN, Khorram O: Physiologic role of nitric oxide synthase in female lower urinary tract. CurrOpinObstet Gynecol. 2004;16(5):423-9.

18. González-Soriano J, Martin-Palacios S, Rodriguez-Veiga, E, Triguero D, Costa G, Garcia-Pascual A: Nitric oxide synthase in the external urethral sphincter of the sheep: immuno-histochemical and functional study. J Urol. 2003;169(5):1901-6.

19. Bennett BC, Kruse MN, Roppolo JR, Flood HD, Fraser M, de Groat WC: Neural control of urethral outlet activity in vivo: role of nitric oxide. J Urol. 1995;153(6):2004-9.

20. Kakizaki H, de Groat WC: Reorganization of somato-urethral reflexes following spinal cord injury in the rat. J Urol. 1997;158(4): 1562-7.

21. Bridgewater M, McNeil HF, Brading AF: Regulation of tone in pig urethral smooth muscle. J Urol. 1993;150(1):223-8.

22. Persson K, Andersson KE: Non-adrenergic non-cholinergic relaxation and levels of cyclic nucleotides in rabbit lower urinary tract. Eur J Pharmacol. 1994;268(2):159-67.

23. Paxinos G, Watson C: The rat brain in stereotaxic coordinates. Fifth Edition. London; Elsevier Academic Press; 2005. Figure 33-40.

24. Dokita S, Morgan WR, Wheeler MA, Yoshida M Latifpour J, Weiss R: NG-Nitro-Arginine inhibits non-adrenergic non-cholinergic relaxation in rabbit urethral smooth muscle. Life Sciences 1991;48(25):2429-36.

25. Prast H, Tran MH, Fischer H, Philippu A: Nitric oxide-induced release of acetylcholine in the nucleus accumbens: role of cyclic GMP, glutamate, and GABA. J Neurochem. 1998;71(1):266-73.

26. Vizzard MA, Erdman SL, Forstermann U, de Groat WC: Differential distribution of nitric oxide synthase in neural pathways to the urogenital organs (urethra, penis, urinary bladder) of the rat. Brain Res. 1994;646(2):279-91.

27. Vizzard MA, Erdman SL, de Groat WC: Localization of NADPH diaphorase afferent and postganglionic efferent neurons of the rat. J AutonNerv Syst.1993;44(1):85-90.

28. Lagos P, Ballejo G: Role of spinal nitric oxide synthase-dependent process in the initiation of the micturition hyperreflexia associated with cyclophosphamide-induced cystitis. Neuroscience2004;125(3):663-70.

29. Sutherland RS, Kogan BA, Piechota HJ, Bredt DS: Vesicourethral function in mice with generic disruption of neuronal nitric oxide synthase. J Urol. 1997;157(3):1109-16.

30. Felsen D, et al.: Inducible nitric oxide synthase promotes patho-physiological consequences of experimental bladder outlet obstruction. J Urol. 2003;169(4):1569-72.

31. Salter M, Duffy C, Garthwaite J, Strijbos PJLM: Substantial regional and hemispheric differences inbrain nitric oxide synthase (NOS) inhibition following intracerebroventricular administration of N-omega-nitro-L-arginine (L-NA) and methyl ester (L-NAME). Neuropharmacology. 1995;34(6);639-49.