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THE INTEGRATIVE FUNCTION OF VAGAL NERVES IN URINARY BLADDER ACTIVITY IN RATS WITH AND WITHOUT INTRAVESICAL NOXIOUS STIMULATION

Abstract: *The integrative function of vagal nerves in urinary bladder activity in rats with and without intravesical noxious stimulation.*

Introduction & Objectives: There is no evidence that vagal nerve innervate the urinary bladder. The aim of the study was to evaluate the effect of left vagal nerve dissection (LVND) on urinary bladder activity in normal rats and after intravesical noxious stimulation (INS).

Material & Methods: Experiment was performed on 30 rats. All animals were divided into 4 groups — I: control rats (n = 12), II: rats after LVND without (INS) (n = 6), III: rats after INS without LVND (n = 6), IV: rats after LVND with INS (n = 6). Cystometry was performed under urethane anaesthesia. The INS was induced by continuously infusion of hypertonic saline (2080 mOsm/l) at a rate of 0.046 ml/min.

Results: LVND induced an increase of basal-BP (+92%), threshold-TP (+21%), and maximal voiding pressure-MVP (+28%), as well as intercontraction intervals-ICI (+84%), functional bladder capacity-fBC (+87%), and compliance (+81%). INS induced detrusor overactivity (DO) characterised by a decrease of ICI (–51%) and fBC (–50%), and also an increase of BP (+118%), detrusor overactivity index-DOI (+413%) and motility index-MI (+33%). LVND diminished the severity of DO characterised by an increase of ICI (+86%), fBC (+84%), compliance (+62%), as well as a decrease of DOI (–50%) and MI (–18%).

Conclusions: The modulation of vagal nerve activity affect the urinary bladder function in naïve conditions, as well as in case of INS (hyperosmolar). These data implies the integrative action of visceral vagal nerve innervation in urinary bladder function. However, further evaluations are strongly required in order to understand this action in various conditions.

Key words: vagal nerve, urinary bladder, overactive bladder, rat, cystometry, noxious stimuli

INTRODUCTION

Overactive bladder (OAB) is common among patients with functional dyspepsia, even if they do not suffer from irritable bowel syndrome (IBS). The severity of lower urinary tract symptoms (LUTS) in patients with OAB is greater in participants with dyspeptic symptoms than without them [1]. Ustinova *et al.* [2] study showed that irritation of rat colon sensitizes urinary bladder afferents to noxious mechanical and chemical stimuli. Thus, the overlap of chronic pelvic pain (CPP) may be a consequence of pelvic afferent cross-sensitization [2]. Viscero-visceral interaction *via* the convergence of the peripheral stimuli into the central nervous system have been described. Such interaction seems to occur due to the visceral inputs convergence at the spinal and supraspinal level [3]. Qin *et al.* [3] showed that responses of superficial and deeper lumbosacral spinal neurons to convergent inputs from urinary bladder and colon suggested that these neurons might contribute to the cross-talk that occurs between visceral organs. The integrative action of vagal nerves in cardiovascular, respiratory and gastrointestinal systems has been described. The vagal branches contain about 22 000 nerve fibres (11 000 in each subdiaphragmatic vagal trunk) within 16 000 nerve fibres are afferent and 6000 efferent. About 85% of the preganglionic neurons project through the gastric branches, 11% through celiac branches and 4% through the hepatic branch of the subdiaphragmatic vagal nerve [4]. There is still no evidence that vagal afferent fibers and celiac ganglia innervate the urinary bladder. However, Berthoud *et al.* [5] revealed that efferent fibres of vagal nerve may affect the bladder activity *via* regulation by afferent inputs from viscera integrating in the celiac ganglia.

AIM

The aim of the study was to evaluate the effect of complete left vagal nerve dissection at cervical level on urinary bladder activity in normal rats and after intravesical noxious (*hyperosmolar*) stimulation.

MATERIAL AND METHODS

Animals

Experiment was performed on 30 adult female Wistar rats (weight: 200–250 g). Rats were housed individually per cage. The animal room was maintained at a constant temperature of 23°C, humidity and a 12:12h alternating light--dark cycle. They were fed with animal's food (Labofeed; Kcynia, Poland) with any restraint to water. The study has been approved by the Animals Ethical

Committee of Jagiellonian University (Cracow, Poland) — no. 19/2011 and 132/2012.

Anaesthesia

Bladder catheter implantation and urodynamic studies were performed under anaesthesia with intraperitonealy injection of 1.2 g/kg urethane (Sigma-Aldrich, St. Louis, USA) [6, 7].

Intravesical noxious stimulation

The urinary bladder noxious stimulation was induced by continuously intravesically infusion of hypertonic saline solution (2080 mOsm/l) at a rate of 0.046 ml/min., as previously described [7].

Vagal nerve dissection

The complete dissection was performed under urethane anaesthesia. The left vagal nerve was uncovered on the animal neck from surrounding tissues (muscles, parotid gland, etc.), and gently isolated from the cervical vessels (external and internal artery), and cut with 5 mm fragment of this nerve removal from operative area.

Bladder catheter implantation

Under urethane anaesthesia, the abdomen was opened through a midline incision and the bladder end of the polyethylene catheter (outer diameter: 0.97 mm/ inner diameter: 0.58 mm; BALT, Poland) was passed through a 1 mm incision at the apex of the bladder dome and secured in place by silk ligature 4–0, as previously described [6–8].

Urodynamic studies

Cystometry was performed under urethane anaesthesia after a 1h recovery period from the surgical procedure. Room temperature saline solution at different concentration (308 mOsm/l — group I and II; 2080 mOsm/l — group III and IV) was infused at a rate of 0.046 ml/min. continuously into the bladder. The free end of the implanted catheter was connected via T-stopcock to a pressure transducer (UFI, MorroBay, CA, USA) and injection pump (Unipan340A, Poland). Cystometry was recorded using ML110-BridgeAmp (ADInstruments, Australia)

hardware and PowerLab/8SP (ADInstruments, Castle Hill, Australia) software, as previously described [9]. The measurements in each animal represent the average of five bladder storage and voiding phases, after obtaining repetitive voiding. The following cystometrogram's (CMGs) parameters were recorded: BP — basal pressure [cmH$_2$0], PT — threshold pressure [cmH$_2$0], MVP — maximal voiding pressure [cmH$_2$0], ICI — intercontraction interval [min.], Compliance [ml/cmH$_2$0], fBC — functional bladder capacity [ml]. Motility index (MI) [cmH$_2$O x s/min.] defined as the enclosed area between the sampled data and their minimum on the selected interval was calculated in 10-minutes intervals. Moreover, DI — detrusor index [cmH$_2$0/ml] in group I and II, as well as DOI — detrusor overactivity index [cmH$_2$0/ml] in other groups, depicted as quotient of the sum of amplitudes of all detrusor contractions during filling phase and functional bladder capacity was evaluated [6–8].

Study protocol

All animals were randomly divided into four groups: group I — control rats (n = 12), group II — rats after complete left vagal nerve dissection without intravesical noxious stimulation (n = 6), group III — rats after intravesical noxious stimulation without left vagal nerve dissection (n = 6), group IV — rats after complete left vagal nerve dissection with intravesical noxious stimulation (n = 6). Cystometry was performed 1h after surgical procedure in all groups.

Statistical analysis

The results are expressed as mean and standard deviation (± SD). The data was compared using the Student t-test. Statistical significance was set at p ≤ 0.05 for all tests.

RESULTS

Effect of complete left vagal nerve dissection on urinary bladder motor activity in rats (group I and II). Cystometric curves of vagotomised rats were different as compared to healthy (non-vagotomised) animals. The complete left vagal nerve dissection induced a significant increase of basal pressure (*approx.* +92%), threshold pressure (*approx.* +21%), maximal voiding pressure (*approx.* +28%), intercontraction intervals (*approx.* +84%), functional bladder capacity (*approx.* +87%), and compliance (*approx.* +81%). No statistical differences of detrusor index and motility index were obtained (Tab. 1, Fig. 1, Fig. 2, Fig. 5).

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Cystometric evaluations of non-vagotomised rats (group I), vagotomised rats without intravesical noxious stimulation (group II), non-vagotomised rats with intravesical noxious stimulation (group III), vagotomised rats with intravesical noxious stimulation (group IV)

CMG parameters	Group I Non-vagotomised rats	Group II Vagotomised rats without intravesical noxious stimulation	Group III Non-vagotomised rats with intravesical noxious stimulation	Group IV Vagotomised rats with intravesical noxious stimulation	p value
BP [cmH_2O]	1.41 ± 0.60	2.70 ± 0.32*	3.07 ± 0.16**	2.61 ± 0.24	* 0.002 ** 0.015
PT [cmH_2O]	5.70 ± 1.22	6.90 ± 1.00*	6.12 ± 0.26	6.00 ± 0.15	* 0.001
MVP [cmH_2O]	27.40 ± 4.90	35.0 ± 3.80*	25.90 ± 4.90	23.8 ± 4.12	* 0.032
ICI [min.]	5.28 ± 1.55	9.73 ± 0.80*	2.59 ± 0.26**	4.81 ± 0.32#	* 0.001 ** 0.001 # 0.001
Compliance [ml/ cmH_2O]	0.059 ± 0.019	0.107 ± 0.031*	0.040 ± 0.008	0.065 ± 0.014#	* 0.001 # 0.001
fBC [ml]	0.240 ± 0.070	0.448 ± 0.042*	0.120 ± 0.012**	0.221 ± 0.022#	* 0.001 ** 0.001 # 0.001
DI/DOI [cmH_2O/ml]	121.9 ± 33.0	110.3 ± 18.0	625.8 ± 101.4**	310.4 ± 34.6#	** 0.001 # 0.001
MI [cmH_2O x s/min.]	185.4 ± 45.9	178.2 ± 27.4	245.2 ± 61.5**	201.8 ± 23.0#	** 0.009 # 0.001

*   statistically significant differences between Group I and II ($p < 0.05$)
**   statistically significant differences between Group I and III ($p < 0.05$)
#   statistically significant differences between Group III and IV ($p < 0.05$)
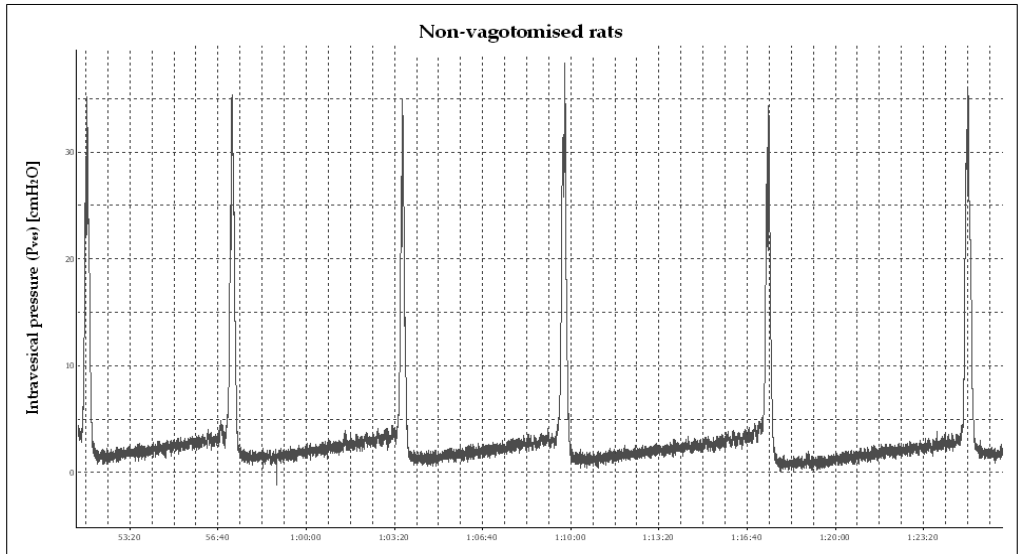
Fig. 1. Cystometrogram trace in non-vagotomised rats. The figure shows 35-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (–5)–40 cmH$_2$O range
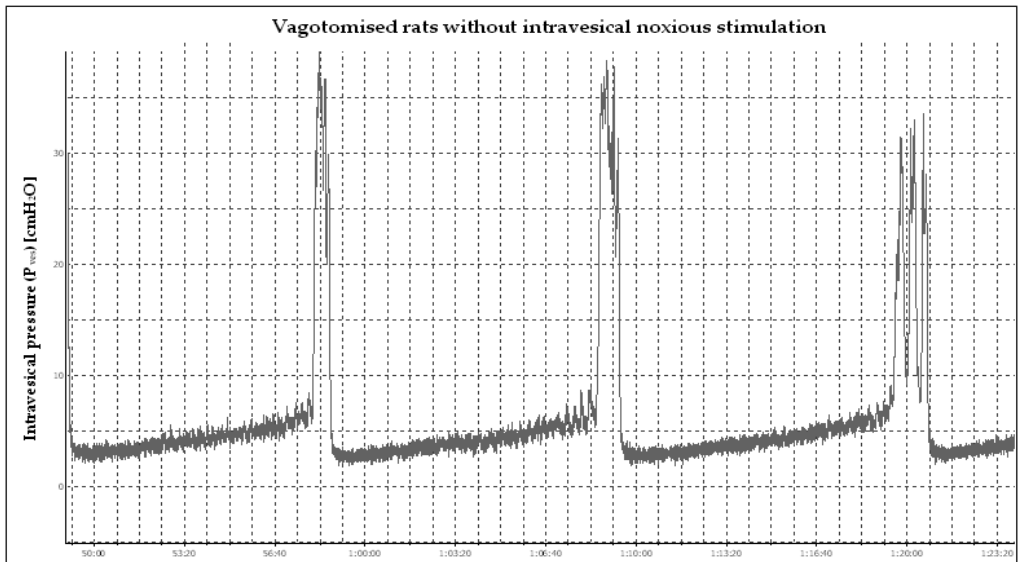

Fig. 2. Cystometrogram trace in vagotomised rats without intravesical noxious stimulation. The figure shows 35-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (–5)–40 cmH$_2$O range

Effect of intravesical noxious stimulation on urinary bladder motor activity in non-vagotomised rats (group I and III). Intravesical noxious stimulation using hyperosmolar (2080 mOsm/l) saline solution induced detrusor overactivity (DO). All hyperosmolar DO rats did not exhibit macroscopical signs of bladder inflammation, i.e. redness, oedema as well as wall thickening, mucosal erosions, ulcerations, petechial haemorrhages on the serosal surface. Cystometric evaluations revealed a significant decrease of intercontraction intervals (*approx.* –51%) and functional bladder capacity (*approx.* –50%). Additionally, an increase of basal pressure (*approx.* +118%), detrusor overactivity index (*approx.* +413%) and motility index (*approx.* +33%) were observed (Tab. 1, Fig. 1, Fig. 3, Fig. 5). No statistical differences of threshold, maximal voiding pressure and compliance were obtained.
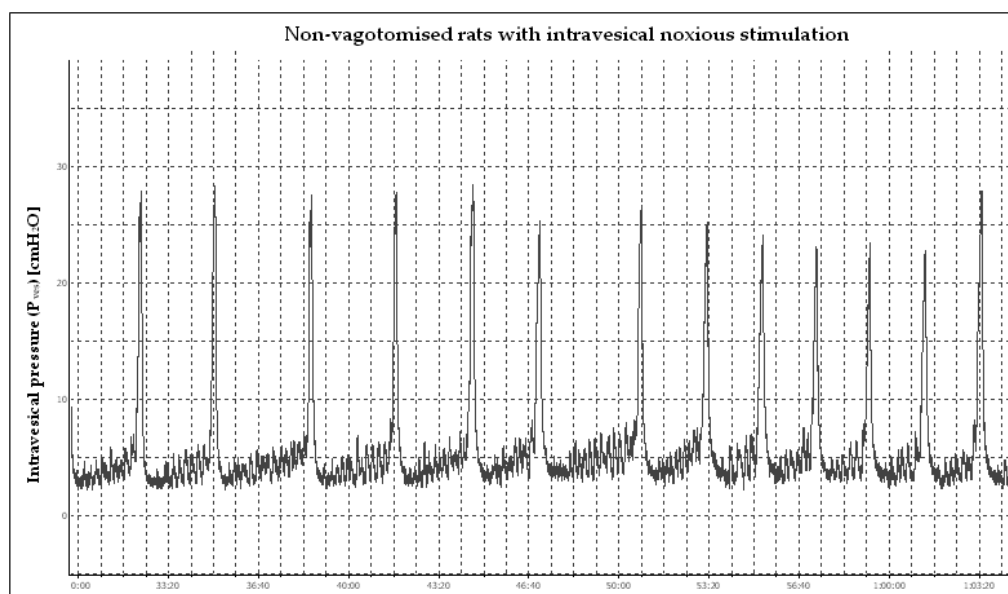

Fig. 3. Cystometrogram trace in non-vagotomised rats with intravesical noxious stimulation. The figure shows 35-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (–5)–40 cmH$_2$O range

Effect of complete left vagal nerve dissection on urinary bladder motor activity in rats with intravesical noxious stimulation (group III and IV). The complete left vagal nerve dissection diminished the severity of detrusor overactivity. The increase of intercontraction intervals (*approx.* +86%), functional bladder capacity (*approx.* +84%), compliance (*approx.* +62%) were observed. Additionally, detrusor overactivity index (*approx.* –50%) and motility index (*approx.* –18%) decreased significantly. The difference of basal, threshold and maximal voiding pressure were not statistically significant (Tab. 1, Fig. 4, Fig. 5).
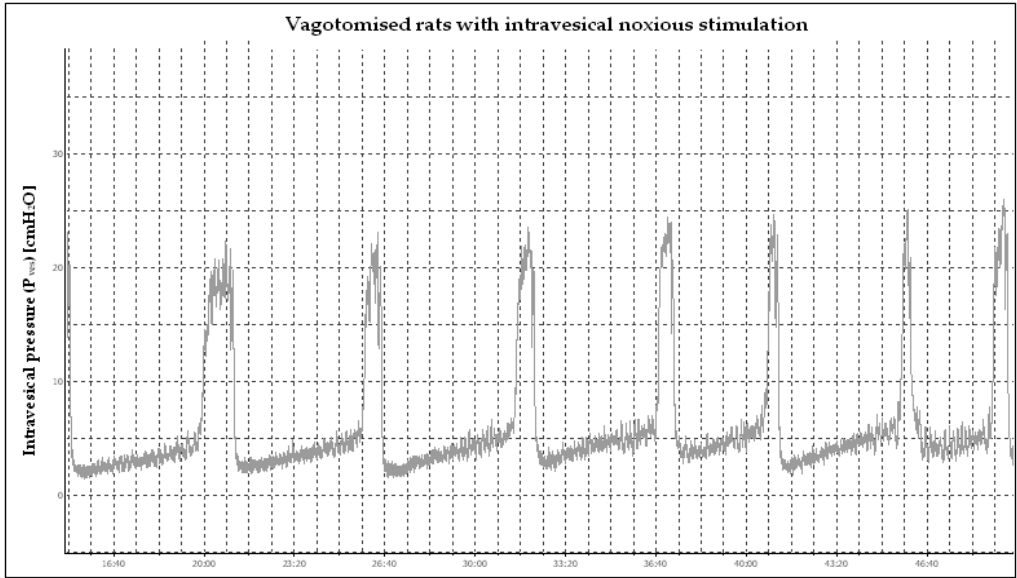
Fig. 4. Cystometrogram trace in vagotomised rats with intravesical noxious stimulation. The figure shows 35-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (–5)–40 cmH$_2$O range
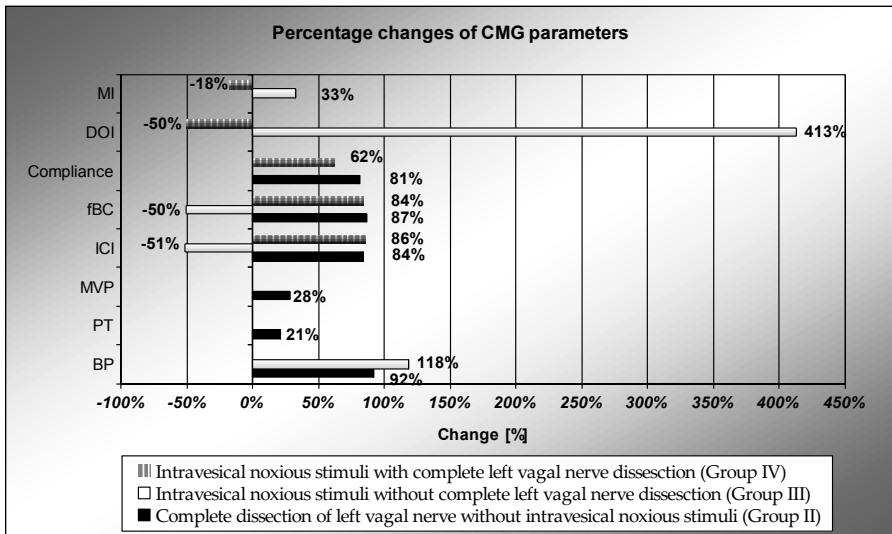

Fig. 5. Percentage changes of cystometric parameters (*mean values*) of vagotomised rats without intravesical noxious stimulation (group II) and non-vagotomised rats with intravesical noxious stimulation (group III) versus non-vagotomised rats (group I), as well as vagotomised rats with intravesical noxious stimulation (group IV) versus non-vagotomised rats with intravesical noxious stimulation (group III)

DISCUSSION

Visceral organs are innervated by vagal and spinal visceral afferent neurons that encode physical and chemical events in the visceral organs and convey this information to spinal cord and lower brain stem. They are the interface between the visceral organs and the central nervous system. Pelvic organs have a dual spinal visceral afferent innervation. The sacral component is essential for the regulation of urine storage and voiding, as well as for the generation of non-painful and most painful sensations associated with pelvic organs. The thoracolumbar visceral afferents seem to be important for the generation of the pain [10]. The urinary bladder innervation originates from hypogastric and pelvic nerves which consist of autonomic branches (*sympathetic and parasympathetic*), as well as sensory branches. Postganglionic axons in hypogastric nerve originate from the superior mesenteric and celiac ganglia, which are innervated by vagal nerve [5, 11]. Vagal afferent fibres innervate the oesophagus *via* cervical and thoracic branches, and also entire gastrointestinal tract *via* abdominal branches. Recently, Kaddumi *et al.* [12] showed that distal oesophagus distension and electrical stimulation of the vagal nerve significantly increased the micturition frequency in rats. However, bilateral cervical vagotomy eliminated the effect of distal oesophagus distension and electrical stimulation of vagal nerve on micturition cycles. It suggests that modulation of vagal nerve and celiac ganglia activity may directly influence on urinary bladder function. Although, there is still no evidence that vagal nerve modulation affect urinary bladder motor activity in urodynamic studies.

Our experiment revealed the correlation between vagal nerve innervation and urinary bladder function in naïve conditions, as well as in case of noxious stimulation. In comparison with control animals, the vagal nerve dissection induced an increase of intravesical pressure characterized by elevated levels of basal (+92%), threshold (+21%) and maximal voiding pressure (+28%). Moreover, the intercontraction intervals (+84%), functional bladder capacity (+87%) increased. Additionally, the urinary bladder in vagotomised rats had better compliance (+81%), as compared to naïve animals. No statistical differences of detrusor index and motility index were obtained. Intravesical noxious stimulation induced detrusor overactivity characterised by a decrease of intercontraction intervals (–51%) and functional bladder capacity (–50%), and also an increase of basal pressure (+118%), detrusor overactivity index (+413%) and motility index (+33%). No statistical differences of threshold, maximal voiding pressure and compliance were obtained. Contrary, vagal nerve dissection diminished the severity of detrusor overactivity characterised by an increase of intercontraction intervals (+86%), functional bladder capacity (+84%), compliance (+62%), as well as a decrease of detrusor overactivity index (–50%) and motility index (–18%).

The influence of urinary bladder distension (UBD) on cardiac function has been described in humans. Bradycardia was observed in patients with acute UBD, which was resistant to atropine, but which resolved immediately the acute bladder distension was treated [13]. Moreover, Hassan *et al.* [14] showed that distension of the urinary bladder in anaesthetised dogs lead to a decrease in vagal efferent fibres activity, which responded to carotid baro- and chemoreceptors stimulation. The vagal afferent inputs from mechano-, chemo-, temperature-, and osmoreceptors seems to play an excellent peripheral afferent complex which enable appropriate autonomic, endocrine, and behavioural responses *via* central reflex complex. The role of vagal nerve in nociceptive transmission, as characterised by affective-emotional reactions (*e.g. increased blood pressure and tachycardia*), typically associated with the pain perception, has been described [15]. Previous studies showed that perineurally and systemically capsaicin-induced denervation of vagal and celiac afferent fibres prevented from degenerative effects of cold restraint stress on the rat urinary bladder [16]. Vagal afferent are able to exert excitatory, and inhibitory modulation of spinal nociceptive transmission *via* bulbo-spinal pathways [17]. Moreover, Kaddumi *et al.* [18] observed that chemical irritation with 2% acetic acid of the urinary bladder induced an increase of medullary reticular formation neurons responsive to abdominal branches of vagal nerve stimulation. Additionally, the pelvic nerve (PN) — responsive medullary reticular formation (MRF) neurons that respond to urinary bladder distension show higher convergent inputs from vagal abdominal branches than non-responsive neurons to distension. Therefore, the described high convergence of pelvic and visceral organs inputs with the inputs form vagal nerve stimulation indicates a dual innervation of the pelvic and visceral organs [19]. Both urinary bladder and distal colon exhibit alternating contractions in spinal cord intact and chronic spinalized rats. This indicates that the spinal neural circuits associated with both organs systems inhibit each other reciprocally. Activation of sacral afferents from the urinary bladder inhibits colon activity and activation of colon afferents inhibits urinary bladder motor activity. This reciprocal inhibition of the colon and urinary bladder may be generated by alternating effect of spinal interneurons [20]. On the other hand, Jancso *et al.* [21] postulated that urinary bladder may be innervated by myelinated fibres in the vagal nerve. The selective stimulation of left vagal nerve by multi-electrode nerve cuff in dogs revealed a decrease of intravesical pressure during stimulation period [22]. Moreover, the graded stimulation of visceral afferents from urinary bladder by capsaicin led to a graded depression of bradykinin-induced plasma extravasation, and subdiaphragmatic vagotomy potentated depression produced by intravesical stimuli [23]. Also, Chen *et al.* [24] revealed that vagal afferent fibres modulate visceral pain in rats. The low-intensity electrical vagal stimulation which activates afferent Aδ fibres reduces visceral pain.

CONCLUSIONS

The modulation of vagal nerve activity affect the urinary bladder function in naïve conditions, as well as in case of noxious (*hyperosmolar*) stimulation. These data implies the integrative action of visceral vagal nerve innervation in urinary bladder function. However, further investigation are strongly required in order to understand such interactions between vagal nerves complex and urinary bladder in various conditions.

CONFLICT OF INTERESTS STATEMENT

None declared.

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