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Effect of Distal Esophageal Irritation on the Changes of Cystometry Parameters to Esophagus and Colon Distentions in Rats

Ezidin G. Kaddumi

Department of Basic Medical Sciences, Faculty of Medicine, Al-Balqa Applied
University, Al-Salt, Jordan

PROOFS AND CORRESPONDENCE TO:

Dr. Ezidin G. Kaddumi

Department of Basic Medical Sciences

Faculty of Medicine

Al-Balqa Applied University

Al-Salt 19117, Jordan

Tel: Office +962 5 3491111Ext. 3574

Mobile +962 795463808

E-Mail: kaddumi@bau.edu.jo

ABSTRACT

The coexistence of different visceral pathologies on patients suffering from irritable bowel syndrome, interstitial cystitis, and other pathologies, necessitates the study of these pathologies under complicated conditions. In the present study, cystometry recordings were used to investigate the effect of distal esophageal chemical irritation (DEI) on the urinary bladder interaction with distal colon distention (DCD), distal esophageal distention (DED), and electrical stimulation of abdominal branches of vagus nerve (abd-vagus). DEI significantly decreased the intercontraction time (ICT) via decreasing the voiding time (VT). DEI, also, significantly decreased the pressure amplitude (P-amplitude) by decreasing the maximum pressure (MP). Following DEI, DED was able to significantly decrease the ICT by decreasing the storage time (ST). Whereas, 3 ml DCD significantly increased the ICT by increasing the ST. On the other hand, following DEI, abd-vagus didn't have any significant effect on ICT. However, abd-vagus significantly increased the P-amplitude by increasing the MP. The results of this study demonstrate that urinary bladder function and interaction of bladder with other viscera can be affected by chemical irritation of distal esophagus.

Keywords: urinary bladder, cystometry, esophagus, distal colon, vagus nerve

INTRODUCTION

Although the interactions between visceral organs are considered a necessity for their, physiologically, coordinated processes, such as; micturition, defecation and ejaculation (Dong and Swanson 2006; Rouzade-Dominguez et al. 2003). It also forms the base for functional disturbance in certain visceral pathologies (Whorwell et al. 1986). In our previous studies, nociceptive mechanical distention of distal colon, which is in the same pelvic territory, was able to inhibit the activity of urinary bladder (Kaddumi 2016a). Whereas, the nociceptive mechanical distention of the distal esophagus, which is in the vagal territory, was able to excite the activity of urinary bladder (Kaddumi et al. 2012). On the other hand, chemical irritation of distal colon significantly affected the bladder function by increasing the bladder frequency. It, also, complicated the effect of mechanical distal colon and esophageal stimuli on the urinary bladder function (Kaddumi 2016b). However, the effect of esophageal inflammation on the urinary bladder function or its interaction with other viscera have not been studied yet.

The possible effect of esophageal chemical irritation on bladder processes could happen through supraspinal centers, that coordinate bladder function. That effect could be mediated through the vagal afferents, which is known to innervate the esophagus (Sengupta 2000). Previous electrophysiological studies proved that inputs from urinary bladder and vagus nerve converge into certain supraspinal centers (Kaddumi and Hubscher 2006). On the other hand, vagal

nerve afferents innervating esophagus are responsive to chemical irritation (Page et al. 2002; Sengupta 2000).

In this study, the effect of acute distal esophageal chemical irritation on the urinary bladder function was investigated. The effect of esophageal irritation on the urinary bladder interaction with other viscera was assessed, as well.

MATERIALS AND METHODS

Eight male Wistar rats (300-350g) were used in this study. Animals were purchased from the animal house of the Jordan University of Science and Technology and housed under standard conditions in the animal house at The Hashemite University. All experimental methods were approved by the Hashemite University Institutional Board and Animal Ethical Committee, which meet the requirements of the National Institute of Health (NIH, USA) guide for the use and care of laboratory animals.

At the day of experiment, each rat was anesthetized with 1.2 g/kg of 50% urethane (Sigma, St. Louis, Missouri, USA) in water. Half of the anesthetic solution was administered intraperitoneally, and the other half was given subcutaneously (Meyer and Fish 2008). Carotid artery was cannulated for pressure monitoring. Also, jugular vein was cannulated for anesthetic supplementation. Anesthetic level was evaluated regularly throughout the

experiment by assessing the withdrawal reflexes. Accordingly, anesthetic supplements were given as necessary to keep the animal just at the subconscious level of anesthesia.

In preparation for cystometry, urinary bladder and ureters were exposed via an abdominal incision. A 20-gauge needle, that is connected to a programmable pump (AL-1000; World Precision Instrument, Sarasota, FL), was inserted through the urinary bladder's dome. Normal saline was pumped into the urinary bladder at a rate of 0.25 ml/min. Urinary bladder temperature and hydration were preserved throughout the experiment using cotton pellets soaked in warm normal saline. The needle was connected to a pressure transducer, which in turn connected into a pressure monitor (BP-1; World Precision Instrument, Sarasota, FL). Data obtained from the pressure monitor were amplified and visualized on a computer using data acquisition system (National Instruments®, www.ni.com). Cystometrograms were recorded using a BioBench software program (National Instruments®, www.ni.com).

In the beginning of the experiment, cystometry recordings were done in each animal for 30 minutes without any stimulus. Following chemical irritation of distal esophagus, cystometry recordings were done for another 30 minutes. Then, cystometry recordings in each animal were done for ten minutes with each stimulus and ten minutes off stimulus (Kaddumi 2016b).

Chemical irritation of the distal esophagus was done in the anesthetized animals by infusing 0.5 ml of 2% acetic acid into the distal esophagus through a catheter (comprised of PE 60 tubing attached to a syringe). A cotton pallet was introduced with the catheter by fixing part of the pallet to the distal end of the catheter tub, while leaving the remaining free part in direct contact with the esophageal mucosa. This procedure insured the localization of the irritant within the distal esophagus.

Distal esophagus and distal colon distentions were done using 10 mm long balloon. The balloon was made from latex material attached at the end of a 25G X $\frac{3}{4}$ " catheter (Berkley et al. 1993). The esophagus balloon was connected to 1 ml syringe and inserted to the distal esophagus; about 7 cm from the upper incisors. Esophageal balloon was inflated by 0.5 ml (which produces noxious stimulus) normal saline during the stimulus (Qin et al. 2009).

The colon balloon was connected to a 3 ml balloon and inserted into the distal colon; about 10 cm from the anus. The colon balloon was taped to the base of the tale to prevent its movement. Distal colon balloon was inflated with three increasing increments of normal saline (1 ml, 2 ml, and then 3 ml) during distal colon distention stimuli. Last increment (3 ml) produced greater than or equal to 70 mm Hg pressure, which is reported to be a noxious stimulus (Ness et al. 1999; Rong et al. 2005).

The abdominal branches of the vagus nerve were electrically stimulated by digital stimulator (PG 4000 A; Cygnus Technology, Inc., Delaware Water Gap, PA) and isolated using isolated current source (SIU 91; Cygnus Technology, Inc., Delaware Water Gap, PA). The stimulation is done indirectly through the esophageal wall using a bipolar electrode that was introduced orally down to the esophagus. The stimulation was conducted at frequency of 1 train/sec with 100 msec train duration. Each train was composed of 14 pulses (at 70 pulses/sec). Each pulse intensity was set at 8 mA with 2 msec duration (Hubscher et al. 2004). This stimulus produces compound action potentials, which were recorded from the vagus nerve in the cervical region (Khasar et al. 1998).

Each animal received the same stimuli with same order. The timings for each stimuli and off stimuli were fixed in all animals. All cystometrograms with or without stimuli were recorded for offline analysis.

The following parameters were extracted, offline, from the cystometry recordings for statistical analysis: intercontraction time (the whole micturition cycle time) (ICT); the time of the voiding phase (VT); and the time of the storage phase (ST), and the intravesical pressure parameters, resting pressure (RP); pressure threshold (PT); maximum pressure (MP); and pressure amplitude (P-amplitude; the difference between MP and RP).

For statistical analysis, the measurements of cystometry parameters preceded the stimulus were considered as control for the measurements following the stimulus. For each variable, at least three consecutive cycles for control and stimulus in each animal were considered for statistical analysis, and

the same number of cycles for the control and stimulus were considered in each animal for this purpose.

Statistical analysis was performed using two tailed, unpaired student t-test (t). Results were considered significant when $P < 0.05$. All data are presented as mean $\pm$ standard error.

RESULTS

Chemical irritation of distal esophagus (DEI) significantly decreased the intercontraction time (ICT). DEI, also, significantly decreased the voiding time (VT). However, DEI had no effect on the storage time (ST). On the other hand, DEI significantly decreased the pressure amplitude (PA) and maximum pressure (MP) but had no significant effect neither on the resting pressure (RP) nor on the pressure threshold (PT). The effects of DEI on the cystometry parameters and an example of cystometry recording showing the effect of DEI urinary bladder function are presented in Figure 1.

Distal esophagus distention (DED), under DEI, was able to significantly decrease the ICT and ST of micturition cycle. However, DED had no significant effect on the VT. DED, also, had no significant effect on the intravesical pressure parameters (PA, RP, MP, and PT) during the micturition cycle. The effects of DED, under DEI, on the cystometry parameters are presented on Figure 2. Also,

an example of cystometry recording showing the effect of DEI on micturition cycle is presented on Figure 2.

On the other hand, electrical stimulation of the abdominal branches of vagus nerve (abd-vagus), under DEI, had no significant effect on the parameters of the micturition cycle except a significant increase in the PA and MP. The effects of abd-vagus on the cystometry parameters and an example of these effects as a cystometry recording are presented on Figure 3.

Regarding the effect of distal colon distention (DCD), under the DEI, 1 ml and 2 ml increments (innocuous stimuli) of DCD had no significant effect on the micturition cycle's parameters. However, 3 ml DCD (noxious stimulus) significantly increased the ICT and ST, without any significant effect on VT. Under DEI, 3 ml DCD significantly decreased the PA without any significant effect on the remaining pressure parameters (RP, MP, PT). The effects of 1 ml, 2 ml, and 3 ml DCD on cystometry parameters are presented on Figures 4 and 5.

DISCUSSION

The results of this study show that distal esophageal chemical irritation (DEI) can affect the urinary bladder function. It also affects the way that urinary bladder interacts with other viscera. Some human clinical studies demonstrated that patients with esophagitis will be, significantly, more prone to develop pathologies affecting other viscera. For example, about one third of all patients

with esophageal reflux affected by respiratory disorders, such as, asthma, cough or laryngeal problems (Jaspersen et al. 2003). In addition, a fellow up study, over three years period, on about 9000 patients diagnosed with esophagitis proved that significantly much more of those patients developed bladder pain syndrome compared to control (Kang et al. 2013).

In the present study, DEI significantly increased the micturition frequency. This increase in bladder frequency is mainly caused by the effect on voiding phase rather than the storage phase, where the voiding time decreased significantly following DEI. The increase of micturition frequency following DEI could be explained by the sensitization of the supraspinal neural centers controlling the micturition process. In a study using c-fos expression, it was proven that esophageal irritation by hydrochloric acid increased the neuronal activity in supraspinal centers, such as parabrachial nucleus and reticular nucleus of medulla (Shuai and Xie 2004). In turn, these centers can affect the urinary bladder function. Electrophysiological studies in rats demonstrated the role of parabrachial nucleus in controlling the bladder function (Kruse et al. 1990; Liu et al. 2007). Other electrophysiological studies also demonstrated that neurons in the medullary reticular formation receives convergent inputs from the urinary bladder and from vagal afferents, as well (Kaddumi and Hubscher 2006). The decrease in pressure amplitude following DEI that is contributed mainly through the decrease of the maximum pressure could be also explained by the sensitization of the micturition neuraxis.

In the present study, distal esophageal distention (DED) following DEI was able to further increase the micturition frequency significantly, however this effect of DED was mainly by affecting the storage phase without changing the voiding phase. The effect of DED is consistent with our previous results on non-irritated animals (Kaddumi et al. 2012). On the other hand, electrical stimulation of vagus nerve didn't have an effect on the micturition frequency, although it was able to do so in the intact non-irritated animals (Kaddumi 2016b), which indicates that DEI obscured the effect of vagal inputs in changing the micturition frequency. The discrepancy in the effect of esophageal distention and electrical stimulation of vagus nerve on bladder function under the DEI could be related to the neural circuitry and its subset of afferent fibers and neuronal centers handling the different inputs. So, at the time that esophagus distention may stimulate different set of neurons or centers than the ones sensitized by DEI, vagus stimulation may use the same set of neurons and centers as the DEI. Esophagus is dually innervated by spinal and vagal afferents. Different electrophysiological studies on animals demonstrated that both spinal (Euchner-Wamser et al. 1993; Garrison et al. 1992) and vagal (Page et al. 2002) afferents innervating esophagus can convey mechanical (distention) and/or chemical inputs. Further investigations are needed to specify the pathways responsible on the results of the present study.

The effect of distal colon distention (DCD) on bladder function following DEI reveled that only 3 ml colon distention was able to affect the bladder function. 3 ml DCD, which is considered a nociceptive stimulus, significantly decreased the micturition frequency via increasing the storage time rather than

voiding time. These results are consistent with results of previous studies (Kaddumi 2016a). The deference in neural circuitry mediating the effects of DEI and DCD may explain the remaining effect of DCD following DEI. The effect of colon distention on bladder function is mainly spinally mediated (Qin et al. 2005; Ustinova et al. 2006). Whereas, as we explained above, the effect of DEI is mainly mediated via supraspinal centers.

Although the present study was conducted on male rats, gender and hormonal treatment effects on the viscerovisceral interaction could be tested on future studies. Some human studies on esophagitis demonstrated a significant difference either in the local (Lynch et al. 2016) or distant (Jaspersen et al. 2003) manifestations between male and female patients.

In conclusion, the results of this study indicate that esophageal chemical irritation can affect urinary bladder function and at the same time it can complicate the viscerovisceral interaction between urinary bladder and other viscera. All these effects are mediated through different sets of neural circuitries. More studies are needed to reveal these neuronal circuits.

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FIGURE LEGENDS

Figure 1

Fig. 1 The effect of distal esophagus chemical irritation (DEI) on cystometry parameters. **a** a diagram showing the effects DEI on cystometry timing parameters. The irritation of distal esophagus significantly decreased the intercontraction time (ICT) and voiding time (VT) without any effect on the storage time (ST). *Significantly different (t, $P < 0.03$) from control; **Significantly different (t, $P < 0.04$); (n, 69). **b** a diagram showing the effects of DEI in cystometry pressure parameters. The irritation of distal esophagus significantly decreased the pressure amplitude (PA) and the maximum pressure (MP) without affecting the resting pressure (RP) or the pressure threshold (PT). *Significantly different (t, $P < 0.01$) from control; **Significantly different (t, $P < 0.04$); (n=69). **c** cystometry record showing the effect of distal esophagus irritation on urinary bladder function. Vertical bars represent standard error of the mean. Downward arrow indicates the beginning of the chemical irritation. Control, the micturition cycles preceding the esophageal irritation.

Figure 2

Fig. 2 The effect of distal esophagus distention (DED), following distal esophagus irritation, on cystometry parameters. **a** a diagram showing the effect of DED on cystometry timing parameters. The distention significantly decreased the intercontraction time (ICT) and storage time (ST) without any effect on the voiding time (VT). *Significantly different (t, $P < 0.02$) from control; **Significantly different (t, $P < 0.01$); (n=59). **b** a diagram showing the effect of DED on cystometry pressure parameters. The distention had no effect on pressure amplitude (PA), resting pressure (RP), maximum pressure (MP) or pressure threshold (PT); (n=59). **c** cystometry record showing the effect of distal esophagus distention on urinary bladder function. distal-esophagus, distal esophagus distention; the horizontal bar above the record indicates the timings and duration of distal esophagus distention. Vertical bars represent standard error of the mean. Control, the micturition cycles preceding the distal esophagus irritation.

Figure 3

Fig. 3 The effect of stimulating abdominal branches of vagus nerve (abd-vagus), following distal esophagus chemical irritation, on cystometry parameters. **a** a diagram showing the effect of abd-vagus on the cystometry timing parameters. The stimulation had no effect on intercontraction time (ICT), voiding time (VT), or the storage time (ST). (n, 46). **b** a diagram showing the effect of abd-vagus on cystometry pressure parameters. The stimulation significantly increased the pressure amplitude (PA) and the maximum pressure (MP) without affecting the resting pressure (RP) or the pressure threshold (PT). *Significantly different (t, $P < 0.002$), (n=46). **c** cystometry record showing the effect of abd-vagus stimulation on urinary bladder function. Vertical bars represent standard error of the mean. Downward arrow indicates the beginning of the stimulus. Control, the micturition cycles preceding the electrical stimulation of the abdominal branches of the vagus nerve.

Figure 4

Fig. 4 The effect of increasing increments of distal colon distention (DCD), following distal esophagus chemical irritation, on the cystometry timing parameters. **a & b** diagrams showing the effect of 1 ml and 2 ml DCD, respectively, on cystometry timing parameters. 1 ml and 2 ml distentions had no effect on intercontraction time (ICT), voiding time (VT), or the storage time (ST). **c** a diagram showing the effect of 3 ml DCD on cystometry timing parameters. 3 ml distention significantly increased the ICT and ST without affecting the VT. *Significantly different (t, $P < 0.04$) from control; **Significantly different (t, $P < 0.02$). **d** cystometry record showing the effect of 3 ml distal colon distention on urinary bladder function. Vertical bars represent standard error of the mean. Downward arrow indicates the beginning of the stimulus. Control, the micturition cycles preceding each increment of distal colon distention. (n= 32, 29, and 26 for 1 ml, 2 ml, and 3 ml distal colon distention, respectively)

Figure 5

Fig. 5 Diagrams showing the effect of increasing increments of distal colon distention on the cystometry pressure parameters. **a & b** 1 ml distal colon distention & 2 ml distal colon distention, respectively, had no effect on pressure amplitude (PA), resting pressure (RP), maximum pressure (MP) or pressure threshold (PT). **c** 3 ml distal colon distention significantly decreased the PA without affecting the RP, MP or PT. *Significantly different (t, $P < 0.02$). Vertical bars represent standard error of the mean. Control, the micturition cycles preceding each increment of distal colon distention. (n= 32, 29, and 26 for 1 ml, 2 ml, and 3 ml distal colon distention, respectively)

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380x243mm (72 x 72 DPI)

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383x257mm (72 x 72 DPI)

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Fig. 4 The effect of increasing increments of distal colon distention (DCD), following distal esophagus chemical irritation, on the cystometry timing parameters. a & b diagrams showing the effect of 1 ml and 2 ml DCD, respectively, on cystometry timing parameters. 1 ml and 2 ml distentions had no effect on intercontraction time (ICT), voiding time (VT), or the storage time (ST). c a diagram showing the effect of 3 ml DCD on cystometry timing parameters. 3 ml distention significantly increased the ICT and ST without affecting the VT. *Significantly different (t, $P < 0.04$) from control; **Significantly different (t, $P < 0.02$). d cystometry record showing the effect of 3 ml distal colon distention on urinary bladder function. Vertical bars represent standard error of the mean. Downward arrow indicates the beginning of the stimulus. Control, the micturition cycles preceding each increment of distal colon distention. (n= 32, 29, and 26 for 1 ml, 2 ml, and 3 ml distal colon distention, respectively).

381x280mm (72 x 72 DPI)

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378x284mm (72 x 72 DPI)