INTRODUCTION

The most important afferents for the micturition are myelinated Aδ-fibres and unmyelinated C-fibres, which are believed to be insensitive in naive conditions (1). Afferent C-fibres are responding to mechanical, thermal and chemical stimuli (2, 3). Its stimulation leads to micturition activation. This alternative C-fibres mediated spinal reflex, play a key role in pathogenesis of bladder overactivity development, increment of OAB symptoms severity, as well as in patients after spinal cord injuries (4). The pivotal backgrounds for OAB development are as follow: the C-fibres sensitisation (increment of sensitivity to various stimuli acting on urotelium) and local effector function of afferent C-fibres endings leading to neurogenic inflammation. Previous studies have suggested that different types of unmyelinated afferent C-fibres, such as capsaicin-sensitive and capsaicin-resistant mediate the voiding reflex in overactive bladder (OAB). Considering its polymodal features, we explored the urodynamic effect of primary afferent neurons modulation on detrusor activity in normal and OAB rats. Experiments were performed on 48 female rats. OAB was induced by intraperitoneal administration of cyclophosphamide. All the surgical procedures and urodynamic studies were performed under urethane anaesthesia. Cystometry was done after a 1 h recovery period from the surgical procedure. All animals were randomly divided into six groups: control, chronic OAB, chronic OAB after capsaicin or lidocaine instillation, control capsaicin or lidocaine instillation. The measurements represent the average of five bladder micturition cycles. We analyzed: basal, threshold, micturition voiding pressure; intercontraction interval; compliance; functional bladder capacity; motility index; detrusor overactivity index. We used chronic cyclophosphamide OAB model for further investigations. In healthy rats, intravesical instillation of capsaicin caused complete inhibition of detrusor contractility preventing from proper voiding function of the bladder. Contrary, lidocaine has no influence on micturition cycles in intact animals. Also, intravesical instillation of capsaicin and lidocaine reduced the severity of detrusor overactivity of OAB rats leading to improvement of cystometric parameters.

Keywords: afferent C-fibres, overactive bladder, cystometry, capsaicin, lidocaine, rats

MATERIAL AND METHODS

Animals

Experiments were performed on forty-eight adult female Wistar rats (weight: 195-275 g). Rats were housed individually per cage. The animal room was maintained at a constant temperature of 23°C, humidity and a 12:12 h alternating light-dark cycle. They were fed with animal’s food (Labofeed; Kcytna, Poland) with any restraint to water. The study has been approved by the Animals Ethical Committee of Jagiellonian University (Cracow, Poland).

Animal model of overactive bladder

Chronic chemical cystitis leading to bladder overactivity was induced by cyclophosphamide (CYP). CYP (Endoxan, Baxter Oncology, Germany) was administrated intraperitoneally in four doses of 75 mg/kg, every 3rd day for 7 days of experiment (6).

Anaesthesia

All the surgical procedures and urodynamic studies were performed under anaesthesia with intraperitoneal injection of 1.2 g/kg urethane (Sigma-Aldrich, St. Louis, USA) (6) and in case of capsaicin instillation with 0.4 g/kg.

Administration of drugs

The following drugs were used: capsaicin (Sigma-Aldrich, Germany) and 2% lidocaine (Polfa, Warsaw, Poland). Capsaicin (final concentration 1 mM) was dissolved in special solution composed of 0.9% saline (80%), absolute ethanol (10%) and Tween 80 (Sigma-Aldrich, Germany) (10%) (the volume
participation of individual components in solvent expressed in the per cent, is provided in square brackets). Under urethane anaesthesia, the bladder was catheterized through the urethra and emptied. Volume of 0.3 ml of 1 mM capsaicin or 2% lidocaine were injected through the catheter (group V, VI) or 0.2 ml (group III, IV) at a rate of 0.15 ml/min. Capsaicin and lidocaine were left contact with the mucosa for 15 and 30 minutes, respectively. The bladder was emptied again and flushed out using 0.5 ml 0.9% saline at a rate of 0.15 ml/min. (7-9).

Surgical procedure

Bladder catheter implantation: under urethane anaesthesia, the abdomen was opened through a midline incision and the bladder end of the polyethylene catheter (o.d. 0.97 mm/i.d. 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).

Urodynamic studies

Cystometry was performed under urethane anaesthesia after a 1 h recovery period from the surgical procedure. Room temperature saline solution 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-stopock 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 (10).

Study protocol

All animals were randomly divided into six groups: group I – control group (n=12), group II – rats with chronic OAB (n=12), group III – rats with chronic OAB after capsaicin instillation (n=6), group IV – rats with chronic OAB after lidocaine instillation (n=6), group V – healthy rats after capsaicin instillation (n=6), group VI - healthy rats after lidocaine instillation (n=6). Cystometry was performed 1 h after surgical procedure in all groups. The surgical procedure was performed after 24 h of the 4th CYP dose administration in group II, after 24 h of the 4th CYP dose administration and capsaicin instillation in group III, after 24 h of the 4th CYP dose administration within 30 min after lidocaine instillation in group IV, after 24 h of the capsacin instillation in group V, after 30 min of the lidocaine instillation in group VI, as previously described with slightly modification (6, 11).

The measurements in each animal represent the average of five bladder micturition cycles, after obtaining repetitive voiding. The following cystometrogram’s (CMGs) parameters were recorded: BP - basal pressure (cmH2O), PT – threshold pressure (cmH2O), MVP – micturition voiding pressure (cmH2O), ICI – intercontraction interval (min), Compliance (ml/cmH2O), fBC – functional bladder capacity (ml). Moreover we calculated MI – motility index (cmH2O x s/min) in 10-minutes intervals. MI is defined as the enclosed area between the sampled data and their minimum on the selected interval. In addiction we analysed DI – detrusor index (cmH2O/ml) in group I and DOI - detrusor overactivity index (cmH2O/ml) in other groups (12), depicted as quotient of the sum of amplitudes of all detrusor contractions during filling phase and functional bladder capacity (13).

Statistical analysis

The results are expressed as mean and standard deviation (±SD). Kruskal-Wallis test was used to compare between groups and “post hoc” multiple comparison tests for statistically significant results. Statistical significance was set at p≤0.05 for all tests.

RESULTS

Chronic cyclophosphamide-induced cystitis

The chronic CYP-treated rats exhibited macroscopical signs of bladder inflammation, i.e. redness, oedema and also wall thickening, mucosal erosions, ulcerations, petechial hemorrhages on the serosal surface. In some animals the urine contained blood. As shown in Figs. 1, 2 and Table 1, CMGs performed in chronic bladder cystitis were different from in control group. Chronic CYP administration leads to bladder overactivity. After chronic CYP administration we observed
significant decrease of micturition voiding pressure (21.5%), intercontraction intervals (59%), functional bladder capacity (59.2%) and compliance (49.1%). Also increase of basal pressure (136.4%), detrusor activity (215%) and motility index (40.6%) were observed. The threshold pressure was not significantly changed.

Effect of intravesical instillation of capsaicin and lidocaine on bladder activity in normal rats

Intravesical instillation of 1 mM capsaicin (CAP) produced complete inhibition of detrusor contractility preventing from proper voiding function of the bladder. On the basis of performed CMGs we observed complete disorganization of micturition cycles (Fig. 3). In storage phase of micturition cycles we observed increased spontaneous detrusor overactivity, evaluated as phasic detrusor contractions of low amplitude with accompanying increased intravesical pressure. In voiding phase these was no proper detrusor contractility (generation of maximal voiding pressure – MVP). As a consequence of the lack of periodically generated MVP and incomplete bladder emptying, constantly lasting urine retention occured. In case of critical bladder fulfill achievement (maximal cystometric capacity) we recorded constant, dripping flow of urine through the urethra. Because of, no proper micturition cycles, bladder activity was estimated using only motility index (Table 2).

Contrary, intravesical instillation of 2% lidocaine (LDK) has no influence on micturition cycles (Fig. 4). Compared to control group, we observed significant increase of intercontraction interval (47.2%), compliance (79.7%), functional bladder capacity (47%) and detrusor index (49.8%). The basal, threshold and maximal voiding pressure was not significantly changed. As well, there were no significant changes of motility index after CAP or LDK intravesical instillation, compared to control group (Table 2).

Effect of intravesical instillation of capsaicin and lidocaine on bladder activity in rats with chronic OAB.

Intravesical administration of 1 mM capsaicin, as well 2% lidocaine reduced the severity of detrusor overactivity, leading to the improvement of cystometric parameters (Figs. 5, 6, Table 3).

Compared to rats with chronic OAB we observed, statistical significant increase of intercontraction interval (172.3% and 126%, respectively), functional bladder capacity (173% and 125.2%, respectively), and compliance (80% and 120%, respectively). Also statistical significant decrease of detrusor

| Table 1. Cystometrogram’s parameters in normal and after chronic CYP administration rats. |
|----------------------------------------|---------------------------------|------------------|-------|
| Group I: CONTROL                      | Group II: chronic OAB          | P Value         |
| BP [cmH2O]                            | 1.40 ± 0.60                    | 3.31 ± 1.85     | p<0.001* |
| PT [cmH2O]                            | 5.68 ± 1.22                    | 6.88 ± 1.93     | NS    |
| MVP [cmH2O]                           | 27.41 ± 4.86                   | 21.67 ± 1.94    | p<0.001* |
| ICI [min.]                            | 5.278 ± 1.549                  | 2.149 ± 0.350   | p<0.001* |
| Compliance [ml/cmH2O]                 | 0.059 ± 0.019                  | 0.030 ± 0.007   | p=0.005* |
| BC [ml]                               | 0.243 ± 0.071                  | 0.099 ± 0.016   | p<0.001* |
| DI / DOI [cmH2O/ml]                   | 121.92 ± 32.98                 | 384.03 ± 181.68 | p<0.001* |
| MI [cmH2O x s/min.]                   | 185.64 ± 45.95                 | 261.00 ± 33.92  | p=0.004* |

*statistically significant differences between group I and group II (p≤0.05).
Fig. 3. Cystometrogram trace in healthy rats after capsaicin instillation. The figure shows 15-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (-5) – 40 cmH₂O range. Micturition: 

Fig. 4. Cystometrogram trace in healthy rats after lidocaine instillation. The figure shows 15-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (-5) – 40 cmH₂O range. Micturition: 

Table 2. Cystometrogram's parameters in normal and after intravesical instillation of capsaicin or lidocaine.

<table>
<thead>
<tr>
<th></th>
<th>Group I: CONTROL</th>
<th>Group V: CONTROL + CAPSAICIN</th>
<th>Group VI: CONTROL + LIDOCAINE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP [cmH₂O]</td>
<td>1,40 ± 0,60</td>
<td>---</td>
<td>1,70 ± 0,58</td>
<td>NS</td>
</tr>
<tr>
<td>PT [cmH₂O]</td>
<td>5,68 ± 1,22</td>
<td>---</td>
<td>5,33 ± 0,88</td>
<td>NS</td>
</tr>
<tr>
<td>MVP [cmH₂O]</td>
<td>27,41 ± 4,86</td>
<td>---</td>
<td>27,33 ± 0,70</td>
<td>NS</td>
</tr>
<tr>
<td>ICl [min.]</td>
<td>5,278 ± 1,549</td>
<td>---</td>
<td>7,770 ± 1,308</td>
<td>p=0,003*</td>
</tr>
<tr>
<td>Compliance [ml/cmH₂O]</td>
<td>0,059 ± 0,019</td>
<td>---</td>
<td>0,106 ± 0,029</td>
<td>p=0,001*</td>
</tr>
<tr>
<td>fBC [ml]</td>
<td>0,243 ± 0,071</td>
<td>---</td>
<td>0,357 ± 0,060</td>
<td>p=0,004*</td>
</tr>
<tr>
<td>DI / DOI [cmH₂O/ml]</td>
<td>121,92 ± 32,98</td>
<td>---</td>
<td>182,62 ± 38,75</td>
<td>p=0,003*</td>
</tr>
<tr>
<td>MI [cmH₂O x s/min.]</td>
<td>185,64 ± 45,95</td>
<td>203,75 ± 54,10</td>
<td>209,98 ± 42,86</td>
<td>Test K-W</td>
</tr>
</tbody>
</table>

* statistically significant differences between group I and group VI (p≤0,05).
Fig. 5. Cystometrogram trace in chronic OAB after capsaicin instillation. The figure shows 15-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (-5) – 40 cmH₂O range. Micturition: ↓

Fig. 6. Cystometrogram trace in chronic OAB after lidocaine instillation. The figure shows 15-minutes interval (horizontal axis). Vertical axis estimates intravesical pressure of (-5) – 40 cmH₂O range. Micturition: ↓

Table 3. Cystometrogram’s parameters in rats with chronic OAB and after intravesical instillation of capsaicin or lidocaine.

<table>
<thead>
<tr>
<th></th>
<th>Grupa II: chronic OAB</th>
<th>Grupa III: chronic OAB + CAPSAICIN</th>
<th>Grupa IV: chronic OAB + LIDOCAINE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP [cmH₂O]</td>
<td>3.31 ± 1.85</td>
<td>3.23 ± 0.31</td>
<td>2.02 ± 0.28</td>
<td>Test K-W</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.163</td>
</tr>
<tr>
<td>PT [cmH₂O]</td>
<td>6.88 ± 1.93</td>
<td>8.28 ± 0.770</td>
<td>5.42 ± 0.42</td>
<td>p=0.010*</td>
</tr>
<tr>
<td>MVP [cmH₂O]</td>
<td>21.67 ± 1.94</td>
<td>23.43 ± 1.33</td>
<td>28.93 ± 2.68</td>
<td>p=0.001**</td>
</tr>
<tr>
<td>ICI [min.]</td>
<td>2.149 ± 0.350</td>
<td>5.852 ± 0.886</td>
<td>4.853 ± 0.792</td>
<td>p=0.001***</td>
</tr>
<tr>
<td>Compliance [ml/cmH₂O]</td>
<td>0.030 ± 0.007</td>
<td>0.054 ± 0.010</td>
<td>0.066 ± 0.010</td>
<td>p=0.001***</td>
</tr>
<tr>
<td>fBC [ml]</td>
<td>0.099 ± 0.016</td>
<td>0.270 ± 0.040</td>
<td>0.223 ± 0.036</td>
<td>p=0.001***</td>
</tr>
<tr>
<td>DI / DOI [cmH₂O/ml]</td>
<td>384.03 ± 181.68</td>
<td>91.48 ± 15.13</td>
<td>137.93 ± 27.06</td>
<td>p=0.001****</td>
</tr>
<tr>
<td>MI [cmH₂O x s/min.]</td>
<td>261.00 ± 33.92</td>
<td>211.30 ± 15.57</td>
<td>205.08 ± 36.80</td>
<td>p=0.002****</td>
</tr>
</tbody>
</table>

* statistically significant differences between group III and group IV (p≤0.05).
** statistically significant differences between group II and group IV, as well as between group III and IV (p≤0.05).
*** statistically significant differences between all groups (p≤0.05).
**** statistically significant differences between group II and group III, IV (p≤0.05).
overactivity index (319.8% and 178.4%, respectively) and motility index (23.5% and 27.3%, respectively), were observed.

Surprisingly, the amplitude of maximal voiding pressure (MVP) was significant higher after lidocaine instillation, compared to control animals with chronic OAB (28.93±2.68 vs. 21.67±1.94 cm H2O, p=0.001). Contrary, capsaicin has no such effect, as lidocaine on MVP amplitude. The differences of basal and threshold pressure were not statistically significant.

**DISCUSSION**

Cyclophosphamide treatment causes mucosal inflammatory response as indicated by macroscopic and microscopic changes in bladder histology and the presence of inflammatory cell infiltrates. Also it has been stated, that chemical CYP-cystis initiates urinary bladder hyperactivity by sensitizing mechanosensitive afferents and/or recruitment of silent afferents which are unresponsive to mechanical stimuli in healthy conditions such as bladder distension (14). Similarly, mild gastric mucosa irritation sensitizes the abdominal vagal afferents, which conduct the sensory information in the C-fiber range, and alters the gastric pacemaker activity, which seems to contribute to the dyspeptic sensations (15). Additionally sensitization of abdominal afferents can be induced by mucosal lesions secondary to bacterial infection leading to clinical symptoms. This fact was reinforced by Zycinska et al. (16) study which revealed that *H. pylori* infection is associated with gastroduodenal mucosal lesions in patients with Wegener's granulomatosis who undergo a complex therapy with glucocorticosteroids, cyclophosphamide, and non-steroidal anti-inflammatory drugs.

After chronic CYP treatment we observed significant decrease of micturition voiding pressure, intercontraction interval, functional bladder capacity, compliance and also an increase of non-voiding contraction (NVCs) frequency. These results are close to that reported previously by several authors in spite of slight modification of experimental protocol (10, 17). Surprisingly, we observed an increase of basal pressure and no significant changes of threshold pressure. Contrary Giuliani et al. (18) observed an increase of basal and micturition voiding pressure for voiding and non-voiding contractions in CYP-treated rats under isoflurane anaesthesia. Moreover, Hu et al. (19) observed bladder hyperactivity with increase of basal, threshold and micturition voiding pressure in acute and intermediate CYP-treated rats under no anaesthesia. It seems that basal pressure changes are caused by the disturbances of detrusor muscle cells activity to stimuli leading to the blockage the admission of sodium ions into the neurons. However, recently discovered TRPA1 receptors (transient receptor potential ion channel of the ankyrin type A1) on bladder afferent C-fibres, probably, also contribute to micturition. Du et al. (28) evaluated that agonist of that TRPV1 bladder afferents participates in normal bladder function (21). Also other human and animal studies involving capsaicin-evoked desensitization have shown that capsaicin-sensitive neurons are important in bladder physiology, particularly in the development of bladder overactivity (22, 23). Our experiments reveal that the modulation of bladder C-fibers activity by neurotoxin – capsaicin leads to disorganization of micturition cycles in healthy rats, as well as to improvement of bladder function (storage and voiding phase of micturition) in rats with chronic model of overactive bladder. In rats with overactive bladder, C-fibres desensitization by capsaicin caused a significant increased of intercontraction interval, functional bladder capacity and compliance, as well as a decrease of detrusor overactivity index, motility index and non-voiding contraction (NVCs) frequency. These observations are close to that reported by Komiya et al. (7) who obtained the inhibition of rhythmic bladder contractions after capsaicin and resiniferatoxin instillation in normal and chronic spinal cord injury rats.

Exemplified studies revealed that stimulation of TRPV1 by vanilloids or other agents (bradykinin, protons), vanillol-sensitive nerve terminals may release neuropeptides like substance P (SP), calcitonin gene-related peptide (CGRP) and interleukins, generating responses in blood vessels, mast cells and lymphocytes causing neurogenic inflammation and in consequence leading to overactivity of bladder (4, 24). Capsaicin, as agonist of TRPV1 receptors, initially stimulates and then subsequently desensitized, temporarily, the afferent C-fibres causing incomplete suppression of bladder overactivity, which confirms the presence of capsaicin-resistant C-fibre afferents (25). Intravesical instillation of capsaicin in patients with OAB causes pain and discomfort. The background of decreased OAB severity caused by intravesical capsaicin in chronic OAB rats may be the result of the suppression of C-fibres and it weakens the inflammatory neurogenic response of C-fibres to stimuli. Furthermore, local intravesical anaesthesia of the bladder with lidocaine, before capsaicin administration, has been used to reduce pungent effects of capsaicin (26, 27).

Contrary to intravesical instillation of capsaicin, lidocaine has no impact on disorganization of micturition cycles in healthy rats. Similarly to capsaicin modulation, the intravesical administration of lidocaine attenuates the severity of detrusor overactivity, leading to the improvement of urodynamic parameters in rats with chronic overactive bladder. In overactive bladder rats, lidocaine caused a significant increase of intercontraction interval, compliance, functional bladder capacity and detrusor index, compared to control group. The higher value of detrusor overactivity index is the result of the intense detrusor overactivity of bladder (4, 24). Capsaicin, as agonist of TRPV1 receptors, initially stimulates and then subsequently desensitized, temporally, the afferent C-fibres causing incomplete suppression of bladder overactivity, which confirms the presence of capsaicin-resistant C-fibre afferents (25). Intravesical instillation of capsaicin in patients with OAB causes pain and discomfort. The background of decreased OAB severity caused by intravesical capsaicin in chronic OAB rats may be the result of the suppression of C-fibres and it weakens the inflammatory neurogenic response of C-fibres to stimuli. Furthermore, local intravesical anaesthesia of the bladder with lidocaine, before capsaicin administration, has been used to reduce pungent effects of capsaicin (26, 27).

Additionally, an increase of micturition voiding pressure after lidocaine instillation in rats with overactive bladder was recorded. It seems that there are two underlying mechanisms responsible for the increase of micturition voiding pressure in response to lidocaine, as compared to capsaicin. Primarily, there is no over-stimulation of detrusor muscle in response to neuropeptides release by C-fibres as on stimulation of capsaicin, causing partial exhaustion of detrusor. Additionally, after lidocaine the desensitization phase of C-fibres does not occur which does not lead to impairment of modulating activity of non-adrenergic, non-cholinergic autonomic nervous system (NANC) on activity of bladder efferent nerves.
TRPA1 leads to bladder overactivity in rats. TRPA1 is co-expressed with TRPV1 in sensory neurons within lower urinary tract (24). Lefler et al. study showed that lidocaine activates, sensitizes TRPV1 and TRPA1, as well as causes strong, acute desensitization of TRPV1 in rodent sensory neurons (29). This term might suggest that local anaesthetics, such as lidocaine can also act directly on bladder afferent C-fibres and modulate a micturition cycles.

CONCLUSION

Our results obtained, that chronic “chemical” CYP-induced cystitis leads to the overactivity of urinary bladder in rats. Additionally, the modulation of C-fibres activity by capsaicin and lidocaine reduces the severity of detrusor overactivity in rats with chronic overactive bladder, and improve its urodynamic estimation. This observations confirm the hypothesis, that in pathophysiology of overactive bladder the pivotal role play two types of unmyelinated bladder afferent C-fibres, both capsaicin-sensitive and capsaicin-resistant.

Conflict of interests statement: None declared.

REFERENCES


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Author’s address: Kajetan Juszczak M.D., Department of Pathophysiology, Jagiellonian University, Medical College, Czysta Street 18, 31-121 Cracow, Poland; Phone: +48126333947, Fax: +48126329056; E-mail: kajusz13@poczta.onet.pl