Alterations in the mechanical properties of bladder smooth muscle in hydrocephalus rat model

Melih Tugay⁎, Sevinç Tugayb, Volkan Etușc, Yusufhan Yazırd, Tijen Utkane
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Department of Pediatric Surgery, Medical Faculty of Kocaeli University, Kocaeli 41380, Turkey
Department of Pediatrics, Acibadem Hospital, Kocaeli 41380, Turkey
Department of Neurosurgery, Medical Faculty of Kocaeli University, Kocaeli 41380, Turkey
Department of Histology and Embryology, Medical Faculty of Kocaeli University, Kocaeli 41380, Turkey
Department of Pharmacology, Medical Faculty of Kocaeli University, Kocaeli 41380, Turkey

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Hydrocephalus;
Bladder dysfunction;
Carbachol

Abstract
Objectives: It is now well established that hydrocephalus is associated with impaired bladder function. The aim of this study was to determine the effects of hydrocephalus on bladder smooth muscle (BSM) reactivity in the rat model.

Materials and Methods: Hydrocephalus was induced in 7-day-old rats by injection of kaolin into the cisterna magna (AH group). Control group rats underwent a sham operation. After 10 days, rats were decapitated. Each bladder was excised and BSM strips placed in an organ bath where contractile and relaxant responses were studied.

Results: Contractile response of BSM to KCl decreased in the AH group. Increased response to muscarinic agonist carbachol was observed in the AH group. The relaxant response to adrenergic agonist isoprenaline was significantly decreased in the AH group, whereas non-receptor-dependent agonist papaverine was unchanged in 2 groups.

Conclusion: Bladder smooth muscle reactivity is affected by the formation of hydrocephalus essentially by both receptor-dependent and non–receptor-dependent mechanisms. This pathway may be a novel target for the pharmacologic treatment of bladder dysfunction secondary to hydrocephalus.

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Hydrocephalus is a common neurologic condition characterized by pathologic dilation of the cerebral ventricles [1]. Bleeding and infection represent the major causes for communicating hydrocephalus. Also, complex congenital pathologies and intra- or interventricular obstructions may cause hydrocephalus [2]. Over half of all hydrocephalus are congenital mostly because of neural tube defects. Children with neural tube defects often have vesicourethral dysfunction manifesting upper urinary tract deterioration [3]. The association between bladder dysfunction and hydrocephalus from a variety of causes is well recognized, but its mechanism is unknown. Surgery of the malformation or increase in hydrocephalus may contribute to neuro-urologic worsening; also, secondary cord tethering and syrinx or hydromyelia

⁎ Corresponding author. Koç University Cerrahisi AD. Kat.2 Umuttepe Kocaeli 41380, Turkey. Fax: +90 262 3037003.
E-mail address: tugaym@hotmail.com (M. Tugay).
may be additional factors coming into play later in life. It is important that the increase in numbers of these patients be considered for surgical treatment because of chronic renal failure. Therefore, medical treatment of bladder dysfunction is extremely important for protection of upper urinary system. From this point, we hypothesized that not only neural tube defects and/or other pathologies but also hydrocephalus, which has a deleterious effect on brain, may play a role in bladder dysfunction secondary to impaired bladder smooth muscle (BSM) reactivity in this complex anomaly. For this reason, hydrocephalus rat model is surgically created without involving medulla spinalis. Bladder smooth muscle reactivity was studied using in vitro organ bath study to understand the pathogenesis of bladder dysfunction.

1. Materials and methods

Sprague–Dawley rats (7-day-old, n = 24) obtained from Experimental Medical Research Center (Kocaeli University, Kocaeli, Turkey) were used for the study. All experiments were made according to the rules of the Animals Ethics Committee of the Kocaeli University Faculty of Medicine. Two or 3 rats were kept in standard cages after the procedure. A day-night cycle applied, and there was free access to water and food. Two groups were created: a control group (n = 9) and a group with hydrocephalus (AH group) (n = 15). At zero day, a hydrocephalus model was created under ketamine (10 mg/kg) anesthesia [4]. This procedure was followed by median craniocervical incision under magnification; superficial and deep cervical muscles were separated off the midline, and the atlanto-occipital membrane was identified. The membrane was penetrated at a right angle by a sterile injector of 27G and the cisterna magna was introduced. The contaminating fluid out from the injection site; then, 0.05 mL of sterile kaolin (kaolin hydrated aluminum silicate K-7375, Sigma Chemical Co, St Louis, Mo) suspension (250 mg/mL in 0.9% NaCl) was slowly injected into the cisterna magna. Then, the surgical wound layers were closed. Control animals received a sham injection consisting of needle insertion only.

1.1. Histologic study

Ten days after the surgery, the animals were killed by decapitation. The brains were immersed in 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (4°C, pH 7.4). The degree of ventricular dilatation was determined from the size of the ventricles observed in coronal sections passing through the center of the anterior commissure. The ratio for ventricular dilatation (the ratio of the widest span of the frontal horns to the maximum width of the brain) was calculated [5].

1.2. Organ bath study

All bladder strips were subjected to the same stimulation protocol. Bladder was removed entirely during surgery. After removing adhering fat and connective tissue, the bladder was opened and divided into longitudinal strips and weighed [6]. The bladder strips were mounted between small clips for the measurement of isometric tension in a double-jacketed organ bath containing 20 mL physiologic saline solution of the following composition (mmol/L): NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.18; NaHCO3, 24.88; glucose, 5.55 (in ultrapure water) [7]. The solution was aerated with 95% O2 and 5% CO2 to obtain a pH of 7.4 at 37°C. The resting tension on the tissues was maintained at 1 g during which the solution was replaced at 15-minute intervals before adding compounds. This tension was shown in preliminary experiments to be optimal for this setting, and strip length was adjusted several times until the optimal length was obtained. Each strip was connected to a force-displacement transducer (FDT 10-A, May IOB5S 99, COMMAT Iletisim Co, Ankara, Turkey) for measuring isometric force, which was continuously displayed and recorded online on a computer via a 4-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc, Santa Barbara, Calif) using appropriate software (BSL PRO v 3.6.7, BIOPAC Systems Inc). The compounds were added to the chamber in half-log concentration increments and cumulative concentration responses obtained. The compounds were dissolved so that for every concentration the volume added to the chamber was 50 μL. At the completion of each experiment, weight of muscle strips was measured.

At the end of the equilibration period, strips were stimulated with 80 mmol/L KCl. For the measurement of the contractile response to the muscarinic agonist carbachol (Cch) (10−10 to 3.10−4 mol/L), cumulative concentration-response curves were constructed in a stepwise manner after the response to the previous concentration reached plateau before adding the next one. After completion of Cch concentration-response curves, tissues were washed for another 30 minutes and precontracted with a submaximal concentration of Cch (3.10−6 mol/L). After the contraction reached plateau, cumulative concentration-response curves to β-adrenergic agonist isoprenaline (10−11 to 10−4 mol/L) and papaverine (10−6 to 10−4 mol/L), respectively, were obtained.

1.3. Data analysis

All data were expressed as the mean ± SE of different experiments. The contractile responses to Cch were calculated as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of isoprenaline were expressed as a percentage of the contraction induced by Cch. Contractile tensions and relaxation responses were normalized using the wet weight of the BSM strip or by using the percentage contraction induced by Cch, respectively. To evaluate the effects of agonists, maximum
response (E_{\text{max}}) and EC_{50} values were calculated. EC_{50} values were calculated from each concentration response curve. These values were used as measure of sensitivity of the tissues for each agonist. The significance of differences was calculated by Student’s t test, and \( P < .05 \) was considered to indicate significance.

1.4. Compounds

The following compounds were all obtained from Sigma Chemical Co: Cch chloride and isoprenaline (isoproterenol hydrochloride). All compounds were dissolved in distilled water and freshly prepared on the day of the experiments. In the high K⁺ solution, NaCl was exchanged for equimolar amounts of KCl. Fresh solutions were prepared on the day of the experiments.

2. Results

The mean ratio of the widest span of the frontal horns to the maximum width of the brain in coronal sections passing through the center of the anterior commissure was signifi-

![Fig. 1](image1.png)  
**Fig. 1** Comparison of contractile responses to KCl in control and AH group BSM strips. Values are expressed as tension (mg/mg). Points represent mean ± SEM of strips. *\( P < .01 \) compared to control group (Student’s t test).

![Fig. 2](image2.png)  
**Fig. 2** Comparison of contractile responses to Cch in control and AH group BSM strips. Values are expressed as tension (mg/mg). Points represent mean ± SEM of strips. *\( P < .01 \) compared to control group (Student’s t test).

![Fig. 3](image3.png)  
**Fig. 3** Comparison of relaxant responses to isoprenaline in control and AH group BSM strips. Values are expressed as tension (% of Cch). Points represent mean ± SEM of strips. *\( P < .01 \) compared to control group (Student’s t test).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Control group</th>
<th>AH group</th>
</tr>
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<tbody>
<tr>
<td>E_{\text{max}}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>133.74 ± 25</td>
<td>47.46 ± 5*</td>
</tr>
<tr>
<td>Cch</td>
<td>1.57 ± 0.02</td>
<td>2.44 ± 0.01*</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>27.05 ± 4</td>
<td>58 ± 4*</td>
</tr>
<tr>
<td>Papaverine</td>
<td>99.47 ± 0.04</td>
<td>98.85 ± 0.23</td>
</tr>
<tr>
<td>(% of Cch)</td>
<td>(% of Cch)</td>
<td></td>
</tr>
<tr>
<td>EC_{50}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cch</td>
<td>8.1 (10^{-8}) ± 0.14</td>
<td>7.2 (10^{-8}) ± 0.25</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>2.2 (10^{-8}) ± 0.41</td>
<td>3.9 (10^{-9}) ± 0.65*</td>
</tr>
<tr>
<td>Papaverine</td>
<td>1.85 (10^{-6}) ± 0.04</td>
<td>1.84 (10^{-6}) ± 0.21*</td>
</tr>
</tbody>
</table>

* \( P < .05 \) compared to the control BSM strips (Student’s t test).

Table 1: Actions of different compounds on the contractions (mg/mg) and relaxations (% of Cch) on the BSM strips.
significantly reduced compared to the control group. The EC\textsubscript{50} values and maximal response to isoprenaline were also significantly changed in the AH group (Table 1). The non-receptor-dependent agonist papaverine induced complete relaxation response in both groups with similar dose-response curve (Table 1).

3. Discussion

Hydrocephalus associated with the long-term neuro-urologic dysfunctions is well known in different clinical pathologies such as spina bifida and intraventricular hemorrhage [8,9]. Although the basis of this association is unclear, an animal model of hydrocephalus is very useful for the etiology of the bladder dysfunction. We used kaolin as a mechanical plug to create obstructive-type hydrocephalus [4].

Intraventricular hemorrhage in preterm infant remains a significant problem, particularly when it is associated with hydrocephalus leading to long-term impaired neurologic and bladder functions. Spinal dysraphism is the most common cause of neurogenic bladder in newborns and associated with hydrocephalus [10,11]. Urodynamic findings in these patients include uninhibited bladder contractions, bladder areflexia, decreased compliance, and detrusor-sphincter dysynergia [12]. Surgery of the back and presence of hydrocephalus may contribute to neuro-urologic symptoms, secondary to urodynamic abnormalities of the bladder and renal parenchymal damage coming into play later in life. The detrusor is the main muscle component of the urinary bladder wall. Its ability to contract over a large length interval and to relax determines the bladder function during filling and micturition. These processes are regulated by several external nervous and hormonal control systems, and the detrusor contains multiple receptors and signaling pathways. Functional changes of the detrusor can be found in several clinically important conditions, such as spina bifida, hydrocephalus, and congenital bladder outlet obstruction. Animal models have many histopathologic similarities to humans and can be used. Previously, we demonstrated impaired foregut smooth muscle reactivity in the same model [13]. In the present study, we have compared the pressure transients generated in isolated bladders derived from a group of healthy rats with those from a group of rats with hydrocephalus. The urinary bladder has 2 important functions: storage of urine and emptying. Storage of urine occurs at low pressure, which implies that the bladder relaxes during the filling phase. Mainly, BSM reactivity changes result from impaired contractile or relaxant system in the bladder. The physiologically most important contractile system in the urinary bladder are muscarinic receptors (receptor-mediated Cch), whereas the most important relaxant system are β-adrenoceptors (receptor-mediated isoprenaline) [14,15]. For this reason, we studied contractile and relaxant responses in BSM of the hydrocephalus model. Our observations showed that the properties of spontaneous contractility are significantly changed in AH group bladder for both contractile and relaxant responses. Impaired BSM reactivity that was demonstrated by this study might result to disturbances of the storage and emptying functions of the bladder may cause at least in part lower urinary tract symptoms.

We found decreased contraction response to KCl, which acts through voltage-gated calcium channels in the AH group (Table 1, Fig. 1). In a BSM where the Ca\textsuperscript{2+}-induced activation of myosin light-chain kinase and the myosin light-chain phosphatase activity are the main pathways for contraction and relaxation, a relation between the Ca\textsuperscript{2+}, the extent of myosin light-chain phosphorylation, and force would exist [16,17]. These changes might be attributable to altered Ca\textsuperscript{2+} influx, calcium storage, or secondary mechanisms during contraction.

The amplitude of muscarinic agonist-induced contractions generated in the AH group was larger than that in the control group (Table 1, Fig. 2). Cch, acting on both M\textsubscript{2} and M\textsubscript{3} receptors, which have an altered, ejeet on BSM in hydrocephalus. The reason for the increased BSM strip contractility to Cch is not clear. Possible explanations for this may include a gradual increase in local neurotransmitter and release in excitatory nerve endings, changes of structure and sensitivity of receptors, and alteration of regulatory and contractile apparatus [18]. Our findings showed that BSM strips taken from AH group presented a depressed contractile response to the direct depolarizing action of KCl. Although the reason for this is not readily apparent, KCl-induced contractions rely solely on the influx of extracellular Ca\textsuperscript{2+} through Ca\textsuperscript{2+} channels to initiate contraction. However, Ca\textsuperscript{2+}-induced contractions, which use both intracellular as well as extracellular Ca\textsuperscript{2+} stores to activate contraction, showed increased contractile response in the AH group. Thus, it is plausible that the function of Ca\textsuperscript{2+} channels involved in Ca\textsuperscript{2+} influx as well as receptor mechanisms may be particularly compromised in BSM strips after the formation of hydrocephalus.

A possible alteration of β-adrenoceptor function is another likely candidate for an involvement in bladder dysfunction in hydrocephalus. Isoprenaline was used for β\textsubscript{2}-adrenoceptor-mediated BSM relaxation in rats. Isoprenaline seemed to have an altered effect on BSM relaxation in the AH group. This finding is highly suggestive of functional changes in the local adrenergic system of neurogenic bladders. Cyclic adenosine monophosphate is the prototypical second messenger of β-adrenergic receptors [19,20]. Signal transduction mechanisms such as isoprenaline-induced cyclic adenosine monophosphate formation and G proteins as well as receptor sensitivity may play a role in impaired relaxation response in the AH group. Therefore, we propose that β-adrenoceptor dysfunction may contribute to the pathophysiology of such conditions.

To identify unique properties of the BSM, possibly involved in hydrocephalus, is a challenge to the research...
field. Many factors, such as central and peripheral nervous control and the contribution of other components of the BSM, may influence reactivity. In conclusion, bladder dysfunction has been attributed to, at least in part, impaired BSM reactivity in hydrocephalus.

References