The effect of hydrocephalus on lower esophageal sphincter smooth muscle reactivity: experimental study

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Abstract

Purpose: The aim of this study was to assess the pharmacophysiological significance of the enteric nervous system for the mechanical responses of lower esophageal sphincter (LES) in infantile rats with kaolin-induced hydrocephalus.

Material and methods: Hydrocephalus was created in 7-day-old rats by injection of kaolin into the cisterna magna. After 10 days, rats were decapitated. Contractile (KCl, carbachol) and relaxant (isoprenaline, papaverine) responses were determined by using in vitro muscle technique in isolated LES smooth muscle strips.

Results: The receptor-mediated contractile and relaxant response to carbachol and isoprenaline in the LES smooth muscle was impaired in rats with hydrocephalus. There was no significant difference in the KCl and papaverine response in hydrocephalic and sham operated rats.

Conclusion: Our findings suggest that hydrocephalus may impair receptor-mediated contractile and relaxant activity of LES smooth muscle leading to gastroesophageal reflux.

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Normal gastroesophageal function is a complex mechanism that depends on effective esophageal motility, timely relaxation, and contractility of lower esophageal sphincter (LES) and the effectiveness of contractility in emptying of stomach [1]. More than one of these factors are often abnormal in the same child with symptomatic gastroesophageal reflux (GER) [2]. Hydrocephalus is associated with the development of GER in children [3]. The cause of GER in these children has not yet been defined, but acute elevation of intracranial pressure has been shown to result in LES pressure changes [4-6]. Mechanism of smooth muscle contraction and relaxation and its pharmacologic influence in various pathologic states are of incremental interest of investigators and clinicians. We recently showed that hydrocephalus associated with impaired esophageal and gastric smooth muscle reactivity which may play a role in development of GER [7]. To further evaluate the mechanism of this effect, we tailored the present study for investigating the role of hydrocephalus on smooth muscle reactivity of isolated LES muscle strips in vitro.

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1. Material and methods

1.1. Animal preparation

Sprague Dawley rats (7 days old, n = 24) obtained from Experimental Medical Research Center (Kocaeli University, Kocaeli, Turkey) were used in this study. The experiments were approved by the Kocaeli University Faculty of Medicine animal use committee. Two or three rats were housed in standard cages with a 12-hour day/night lighting schedule and free access to water and pelleted food. The rats were divided into 2 groups: hydrocephalus-induced group (AH group) consisted of 13 animals and control group (sham-operated animals) consisted of 11 animals. The surgical method which has been described by McAllister et al. [8] was used for induction of hydrocephalus in rats. Anesthesia was induced in the rats by intramuscular injection of ketamine (10 mg/kg). Each rat’s neck was shaved, and a median craniocervical incision was done. Under magnification, superficial and deep cervical muscles were separated off the midline, and the atlantooccipital membrane was identified. The membrane was penetrated at a right angle by a sterile injector of 27 gauge, and the cisterna magna was identified. The cerebrospinal fluid outflow from the injection site was observed; then 0.05 mL of sterile kaolin (Kaolin hydrated Aluminum Silicate K-7375, Sigma Chemical Co, USA) suspension (250 mg/mL in 0.9% NaCl) was slowly injected into the cisterna magna. Control animals received a sham injection consisting of needle insertion only. The surgical field was washed with antibiotic solution, and a tiny piece of gel foam was placed over the injection site on the membrane. Cervical muscles and skin were closed with separate sutures.

1.2. Histologic Study

The rats were decapitated 10 days after the operation. 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 in these sections, as described previously by Miyazawa et al [9].

From esophagus to stomach, upper gastrointestinal structures were resected, freshly opened, and macroscopically evaluated. A part of the specimens were fixed in 10% formaldehyde solution (24 hours). Serial transversal 5-μm-thick sections from paraffin blocks were stained with hematoxylin and eosin. A blind observer microscopically assessed the specimens histologically.

1.3. Isolated LES preparations and drug treatments

To isolate LES, the stomach was opened along the greater curvature through the esophagus to reveal the junction between esophagus and stomach. Esophagus immediately proximal to the gastroesophageal junction was dissected as a strip 2 mm in width, and mucosal lining was removed under loop magnification. Lower esophageal sphincter strips were placed and mounted on standard 20-mL organ bath filled with Tyrode solution of the following composition (mmol/L): NaCl, 136.0; KCl, 2.7; CaCl2, 1.8; MgCl2, 1.05; NaH2PO4H2O, 0.42; NaHCO3, 11.9; and glucose, 5.5. Solution was oxygenated (95% O2-5% CO2) and heated at 37°C continuously during study. Lower esophageal sphincter strips were placed in a reservoir and tied to stainless steel hooks at one end to the organ bath; the other end was connected to a force transducer (FDT 10-A, May IOBS 99, COMMAT Iletisim Co, Ankara, Turkey) under a resting tension of around 1 g. Lower esophageal sphincter strip activities were recorded on an online computer via a 4-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems, Inc, Santa Barbara, Calif) using the software BSL PRO v 3.6.7 (BIOPAC Systems, Inc), which also analyze the data. The tissues were equilibrated for at least 60 minutes. Bath fluid was replaced every 15 minutes. Agonist compounds were added directly to organ bath. At the end of the each experiment, tissues were lightly blotted and weighed.

The following compounds were used (Sigma Chemical St Louis, Mo): carbachol chloride (carbamylcholine chloride), isoprenaline (isoproterenol bitartrate), and papaverine (papaverine hydrochloride).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control E&lt;sub&gt;max&lt;/sub&gt; (mg/mg)</th>
<th>Control pD₂</th>
<th>AH Group E&lt;sub&gt;max&lt;/sub&gt; (mg/mg)</th>
<th>AH Group pD₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>17.56 ± 3.32</td>
<td>–</td>
<td>13.57 ± 3.4</td>
<td>–</td>
</tr>
<tr>
<td>Carbchol</td>
<td>2.55 ± 0.06</td>
<td>1.7·10⁻⁸</td>
<td>1.58 ± 0.025</td>
<td>1.9·10⁻⁷ ± 0.24 mol/L</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>29.7 ± 5 (%)</td>
<td>2.3·10⁻⁷</td>
<td>65 ± 7 (%)</td>
<td>4.4·10⁻⁴ ± 6.4 mol/L</td>
</tr>
<tr>
<td>Papaverine</td>
<td>99.77 ± 0.04 (%)</td>
<td>8.1·10⁻⁶</td>
<td>99 ± 0.65 (%)</td>
<td>8.23·10⁻⁶ ± 0.2 mol/L</td>
</tr>
</tbody>
</table>

CCH indicates carbachol.
Mean values ± SEM, obtained from different experiments.
In the high K\+ solution, NaCl was exchanged for equimolar amounts of KCl. Compounds were prepared daily in distilled water and kept in ice during the course of experiments.

After the stabilization period, strips were contracted with 80 mmol/L KCl. Concentration-response curves were produced by exposing strips to carbachol (10^{-10}-3\times10^{-4} mol/L) in a cumulative manner, each incremental concentration being added when the response to the previous concentration reached a maximum. After the carbachol dose-response study was completed, LES strips were equilibrated in organ bath for at least 60 minutes. At the end of this period, LES strips were contracted by addition of a submaximal concentration of carbachol (3\times10^{-7}-10^{-6} mol/L) to obtain relaxation response for isoprenaline (10^{-10}-10^{-3} mol/L). Upon establishment of a stable contraction, a cumulative concentration-effect curve for relaxation to isoprenaline was constructed. After the isoprenaline relaxation responses were obtained, the strips were washed with fresh solution and allowed to recover for 60 minutes before recontracting with carbachol, and same procedure was repeated to study papaverine (10^{-10}-10^{-4} mol/L) relaxation response.

1.4. Analysis of data

All results of the experimental data were expressed as means ± SEM. Calculated ventricular ratios of the control and AH groups were statistically compared using 1-factor analysis of variance. The contractile force was expressed as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of agonists were expressed as a percentage of the precontraction to carbachol. In order to evaluate the effects of agonists; maximum responses ($E_{\text{max}}$) and pD\(_2\) values (apparent agonist affinity constants) were calculated. Agonist pD\(_2\) value was calculated from each agonist dose-response curve by linear portion of the curve and taken as a measure of the sensitivity of the tissues to each agonist. Statistical comparison between groups was performed using Student $t$ test. Probabilities of less than 5\% ($P < .05$) were considered significant.

2. Results

Among the 13 rat pups that received kaolin injections, 3 of them without significant enlargement of the ventricles were excluded from the study.

No histopathologic changes secondary to GER were observed in LES, esophageal, and gastric specimens in both groups. 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 significantly increased in the AH group (0.71 ± 0.078 mm), compared to the control group (0.25 ± 0.04 mm) (analysis of variance, $P < .01$).
There was no significant differences in LES strip wet weights between the AH group (0.0536 ± 0.020 mg) and control group (0.074 ± 0.028 mg).

Table 1 shows a slight decrease in contractile response to KCl of the AH group, compared to the control group. But this response did not reach significant level.

In the in vitro study, LES contractility in response to the cholinergic agonist carbachol was reduced in the AH group compared to the control group in a dose-dependent manner (Fig. 1). Corresponding EC$_{50}$ and $E_{max}$ values are summarized in the Table 1. The relaxation response induced by isoprenaline significantly reduced in the AH group compared to the control group (Fig. 2, Table 1). In precontracted strips, papaverine produced concentration-dependent relaxations. In contrast to isoprenaline, the relaxation elicited by papaverine was similar in all groups, and there were no significant changes in the EC$_{50}$ or $E_{max}$ values (Fig. 3, Table 1).

3. Discussion

It has been reported that an increase number of transient LES relaxations was considered as a main pathology causing GER in symptomatic children [10,11]. The incompetent LES mechanism present in most children with GER combined with the esophageal and gastric dysmotility as a cause of symptoms. Particularly in those patients with neurological disorders, there appears to be a high prevalence of autonomic neuropathy in which esophagogastric transit and gastric emptying are frequently delayed, producing a complex foregut motility disorder. Excitatory cholinergic and inhibitory $\beta$-adrenergic receptors are known to play a role in LES peristalsis of the human and animals [12,13].

Our previous work on the same experimentally induced hydrocephalus model demonstrated that impaired esophageal and gastric smooth muscle reactivity may play a role in development of GER [7]. The data reported in the present article show that hydrocephalus may affect the response to exogenously applied carbachol and isoprenaline in the LES in vitro.

It is unclear whether abnormal peristaltic activities are the cause or consequence of peptic esophagitis in GER disease. Experimentally, it has been shown that acute esophagitis caused impaired esophageal and gastric smooth muscle reactivity [14,15]. Previously, it was reported that abnormalities in peristalsis and LES pressure in patients with esophagitis do not improve after healing of the inflammation, suggesting that manometric abnormalities in these patients may be a primary phenomenon [16-18]. This possibility is also supported by the observation of a high relapse rate of esophagitis after discontinuation of medication, suggesting continued acid exposure to the esophagus despite healing of the inflammation [19]. In this study, hydrocephalic rats had the decrease in LES smooth muscle reactivity despite having no esophagitis. This observation is in accordance with aforementioned studies and suggests that esophageal body motor abnormalities is not always associated with esophagitis and, therefore, could be a primary phenomenon. However, acid-resistant histologic properties of the rat esophagus might cause esophagitis not to develop during chronic stage.

Esophageal peristalsis is controlled by the combined effects of inhibitory and excitatory nerves. In vitro studies have shown that the excitatory contribution involves muscarinic receptors [20]. We tested muscarinic receptor-mediated contractile response induced by carbachol. In the AH group, contractile response to carbachol significantly decreased compared to the control group. The decreased contractile response to carbachol in the AH group may not be explained by nonselective mechanisms because contraction produced by K$^+$ was not significantly changed in the AH group. Lower esophageal sphincter contractile response to K$^+$ depolarization depends on Ca$^{2+}$, which activates the contractile response. Contraction of LES smooth muscle depends on distinct signal transduction pathways [21]. In LES smooth muscle, acetylcholine-induced contraction uses intracellular Ca$^{2+}$ release arising from metabolism of phosphatidylinositol, and a calmodulin-myosin light chain kinase–dependent pathway [22]. G proteins transduce ligand binding to a cell surface receptor into intracellular signals. It was reported that esophagitis produced a change in the muscarinic receptors; G proteins and phospholipases activated by stimulation with a maximally effective dose of acetylcholine [23]. Therefore, that similar mechanisms can produce alterations in LES contractile properties after the development of hydrocephalus requires further study.

Adrenergic $\beta$ receptors are important in LES physiology, and alterations may be involved in hydrocephalus-induced LES dysfunction. Three subtypes of adrenergic $\beta$ receptors are now recognized [24]. These receptors are nonselectively activated by isoprenaline in rat LES where they mediate relaxation [13]. Isoprenaline-induced relaxation was accompanied by an increase in cyclic nucleotides as second messengers mediating LES relaxation [25]. We demonstrated that development of hydrocephalus caused decrease in adrenoceptor-mediated isoproterenol-induced relaxation response in LES strips [7]. These changes correlated with our earlier data on the same model, which showed impaired esophageal and gastric smooth muscle mechanically properties. We also tested nonselective relaxation mechanism induced by papaverine. We found no difference in relaxation response to papaverine in rats with hydrocephalus, compared with controls. Therefore, the most likely explanation for the changes in relaxation response may be the relation with adrenergic $\beta$ receptors or second messenger’s level.

This is the first report detailing the expression of LES smooth muscle mechanical properties in an experimentally induced hydrocephalus animal model. Although, extrapolation from a rat model of hydrocephalus should be approached cautiously, the present data may have some important implications. The current findings are consistent with the findings of the previous study, which pointed out
that impaired foregut smooth muscle reactivity may cause increased propensity for dysmotility and GER in hydrocephalus [7]. However, further detailed studies are required for explaining the afferent and efferent neural pathways and neuromuscular mediators of LES.

References