The effect of N-methyl-D-aspartate receptor antagonist (memantine) on esophageal and gastric smooth muscle: functional investigation in a rat hydrocephalus model

Arzu Özyer Bektaş, Melih Tugay, Sevinç Tugay, Semil Selcen Göçmez, Volkan Etüş, Tijen Utkan

Object: The purpose of this study was to investigate the effect of N-methyl-D-aspartate (NMDA) receptor antagonist on esophageal and gastric smooth muscle reactivity in a rat hydrocephalus model.

Material and Methods: Hydrocephalus was induced in rats by injection of kaolin into the cisterna magna. Two weeks after the procedure, memantine (20 mg/kg per day, 2 weeks) was given to rats with hydrocephalus in the memantine group (MG). The rest of the rats with hydrocephalus received serum physiologic (hydrocephalus group, HG). The control group (nonhydrocephalic rats, CG) was sham operated. The fourth group consisted of nonhydrocephalic rats with treated memantine (memantine control group, MC). Contractile (KCl, carbachol) and relaxant (isoprenaline, papaverine) esophageal and gastric smooth muscle reactivity were determined by in vitro muscle technique.

Results: No significant difference was found in the KCl (nonreceptor-mediated)-induced esophageal smooth muscle reactivity among the groups. Carbachol (receptor-mediated)-induced smooth muscle reactivity significantly decreased in HG compared to other groups. The isoprenaline (receptor-mediated)-induced smooth muscle reactivity significantly decreased in HG compared to other groups. No significant difference was found in smooth muscle reactivity to papaverine (nonreceptor-mediated) among the groups. Gastric smooth muscle reactivity to KCl significantly increased in HG compared to other groups. Also, KCl-induced smooth muscle reactivity significantly increased in MG compared to CG and MC. Carbachol-induced smooth muscle reactivity significantly decreased in HG compared to MG, CG, and MC. No significant difference was observed in isoprenaline- and papaverine-induced smooth muscle reactivity among the groups.

Conclusion: Our findings suggest that memantine may influence esophageal and gastric smooth muscle reactivity in hydrocephalus.

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It is thought that glutamate (the brain’s primary excitatory neurotransmitter) plays an important role in neuronal cell death, which is common to all neurodegenerative diseases. Glutamate may be effective in the dorsal vagal complex and involved in the vagal reflex [1-4]. Vagal reflex plays an integral role in the regulation of gastrointestinal motor functions [5-6]. N-Methyl-D-aspartate (NMDA) receptors in the dorsal vagal complex can be selectively activated to evoke vagally mediated increased gastric motor function [7]. Memantine as an NMDA receptor antagonist can prevent the pathologic stimulation by blocking the action of glutamate. In this background, we suggest that memantine may have an effect on smooth muscle reactivity and could interfere with intestinal motility. Children with hydrocephalus who vomit have abnormal gastric motility as often as gastroesophageal reflux (GER). We recently showed that hydrocephalus associated with impaired esophageal and gastric smooth muscle reactivity may play a role in the development of GER [8,9]. If memantine had such an effect, its administration might restore impaired foregut smooth muscle reactivity. In this study, we evaluated memantine effects on impaired foregut smooth muscle reactivity in a rat hydrocephalus model.

1. Material and methods

Sprague-Dawley rats (8 weeks old, N = 54) (Experimental Medical Research Center, Kocaeli University, Kocaeli, Turkey) were used. The procedures were approved by the Animal Care and Use Committee of Kocaeli Medical Faculty. Animals were kept in 12:12-hour light-dark cycle with ad libitum food and water access. All the rats were operated on under ketamine (10 mg/kg im) anesthesia. Hydrocephalus was induced in 33 animals using the model described by McAllister et al [10]. Two weeks after the surgery, magnetic resonance imaging was used to assess ventricle size, and then the rats with hydrocephalus were divided into 2 groups. Thirteen of the 20 rats received memantine by intraperitoneal (ip) injection (20 mg/kg per day) for 2 weeks in the memantine group (MG). Seven rats died during memantine treatment. Only 12 of the 13 rats received serum physiologic (ip) for 2 weeks in the hydrocephalus group (HG). One rat died during treatment. Twenty-one animals were used for 2 control groups. A 27-gauge needle was inserted percutaneously into the cisterna magna in the control animals. Twenty-two animals from the first control group (CG, nonhydrocephalic rats) received a sham injection consisting of needle insertion only. Only 1 animal died before organ bath study. The second control group (MC, memantine control group) composed of nonhydrocephalic rats (n = 9) received a sham injection and memantine (20 mg/kg per day ip) for 2 weeks. At the end of the 2 weeks, rats were decapitated. 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 [11]. Total esophagectomy and gastrectomy were performed. Esophageal and gastric fundus strips were prepared in a manner consistent with the method described previously by Baxter et al [12] and Vane [13], respectively, for organ bath study. Esophageal and gastric fundus strips were placed and mounted in standard 20-mL organ bath filled with Tyrode’s solution (mmol/L): NaCl, 136.0; KCl, 2.7; CaCl$_2$, 1.8; MgCl$_2$, 1.05; NaH$_2$PO$_4$, 0.42; NaHCO$_3$, 11.9; glucose, 5.5. The solution was oxygenated (95% O$_2$-5% CO$_2$) and heated at 37 ± 0.5°C continuously during study. The strips were placed in a reservoir and tied to stainless steel hooks at one end of the organ bath, and 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 0.5 g for esophagus and 1 g for gastric fundus. The strip activities were recorded on an online computer via a 4-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems, Inc, Santa Barbara, CA) using the software BSL PRO v 3.6.7 (BIOPAC Systems) which also analyzes the data. The tissues were equilibrated for at least 60 minutes. Bath fluid was replaced every 15 minutes. Agonist compounds were added directly to the organ bath. At the end of 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).

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 (60 minutes) period, strips were contracted with 80 mmol/L KCl. Concentration-response curves were produced by exposing strips to carbachol ($10^{-5}$-$10^{-4}$ 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, strips were equilibrated in organ bath for at least 60 minutes. At the end of this period, strips were contracted by addition of a sub-maximal concentration of carbachol ($3\times10^{-7}$-$10^{-6}$ mol/L) to the bathing solution to obtain the relaxation-response curve for isoprenaline ($10^{-9}$-$10^{-4}$ mol/L). Upon establishment of a stable contraction, a cumulative concentration-effect curve for relaxation to isoprenaline was constructed. After the isoprenaline relaxation response was obtained, the strips were washed with fresh solution and allowed to recover for 60 minutes before contracting with carbachol and the same procedure was repeated to study papaverine ($10^{-7}$-$10^{-3}$ mol/L) relaxation response.
1.1. Analysis of data

All results of the experimental data were expressed as means ± SEM. Calculated ventricular ratios of the groups were statistically compared using 1-factor analysis of variance (ANOVA). Some of the results were excluded owing to nonresponse of tissue strips to agonists or technical failure during study (noncontractile response before the relaxation study, tissue breakdown, losing resting tension, etc). The contractile force was expressed as grams of developed tension per gram of tissue wet weight. The relaxant effects of agonists were expressed as a percentage of the precontraction to carbachol. Maximum response ($E_{\text{max}}$) and $pD_2$ value (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 ANOVA test. Probabilities of less than 5% ($P < .05$) were considered significant.

2. Results

Nine animals (MG, $n = 7$; CG, $n = 1$; HG, $n = 1$) died before the organ bath experiments. 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 rats with hydrocephalus ($0.81 \pm 0.068$ mm) compared to controls ($0.22 \pm 0.03$ mm) (ANOVA, $P < .01$).

2.1. Esophageal strip responses

Totally, 6 organ bath studies were excluded from the KCl results owing to technical failures (MG, $n = 1$; CG, $n = 2$; MC, $n = 1$) or nonresponsive (MG, $n = 1$; HG, $n = 1$) smooth muscle strips. KCl-induced contractile response was slightly increased in HG compared to CG, MC, and MG (Table 1). Totally, 14 organ bath studies were excluded from the carbachol results owing to technical failures or nonresponsive smooth muscle strips. Carbachol-induced contractile response was not observed in esophageal smooth muscle strips (MG, $n = 1$; CG, $n = 2$; MC, $n = 1$; and HG, $n = 2$, respectively). Carbachol-induced contractile response was significantly decreased in HG compared to MC, CG, and MG. This response was similar in CG, MC, and MG (Fig. 1, Table 1).

Because contraction response was required for the relaxation study, 6 noncontractile esophageal smooth muscle strips (MG, $n = 1$; CG, $n = 2$; MC, $n = 1$; and HG, $n = 2$) were not available for the relaxation study. Five esophageal smooth muscle strips (MG, $n = 2$; HG, $n = 2$; CG, $n = 1$) were excluded from the study owing to technical failure. Isoprenaline-induced relaxation response was significantly decreased

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Actions of different compounds on the contractions (g/g) and relaxations (% of carbachol) in rat esophagus strips</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (g/g contraction)</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>16.13 ± 3.77</td>
</tr>
<tr>
<td>Carbachol</td>
<td>16.68 ± 4.1</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>95 ± 4.42</td>
</tr>
<tr>
<td>Papaverine</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

$pD_2$ (% relaxation)

| Carbachol | 6.31 ± 0.2 | 6 | 6.52 ± 0.18 | 8 | 4.79 ± 0.18 | 8 | 5.29 ± 0.25 | 9 |
| Isoprenaline | 6.67 ± 0.14 | 8 | 6.25 ± 0.13 | 8 | 5.66 ± 0.17 | 8 | 6.96 ± 0.13 | 10 |
| Papaverine | 4.89 ± 0.14 | 7 | 4.65 ± 0.15 | 9 | 5.77 ± 0.12 | 9 | 4.55 ± 0.16 | 8 |

$n$ indicates number of strips.

* $P < .05$, different from control groups (CG and MC).

# $P < .05$, different from MG.

* *P < .05, compared with the control groups (CG and MC).

# *P < .05, compared with MG.
in HG compared to CG, MC, and MG (Fig. 2, Table 1). Isoprenaline-induced relaxation response was not different between CG, MC, and MG.

Five noncontractile esophageal smooth muscle strips (MG, n = 1; CG, n = 2; and HG, n = 2) were not available for the relaxation study. Seven strips were excluded from the study owing to technical failure (MG, n = 2) and nonresponsive strips (MG, n = 2; CG, n = 2; HG, n = 1). Relaxation response to papaverine was not significantly changed between the groups (Table 1).

### 2.2. Gastric fundus strip responses

Two organ bath studies were excluded from the KCl results owing to technical failures. In addition, there were 3 nonresponsive gastric smooth muscle strips to KCl in MG (n = 1), CG (n = 1), and HG (n = 1). KCl-induced contractile response was significantly increased in HG compared to other groups. Also, significantly increased response to KCl was observed in MG compared to CG and MC. However, $E_{\text{max}}$ value was relatively small in MG compared to that of HG (Table 2). KCl-induced contractile response was similar between CG and MC.

Twenty-eight strips were included in the carbachol results. There were 6 nonresponsive gastric smooth muscle strips to carbachol in MG (n = 2), CG (n = 1), and HG (n = 3), respectively. Eleven of the strips were not available for the study owing to technical errors (MG, n = 4; HG, n = 2; CG, n = 4; MC, n = 1). Contractile response to cholinergic agonist carbachol was significantly reduced in HG compared to CG, MC, and MG in a dose-dependent manner (Fig. 3). Corresponding $pD_2$ and $E_{\text{max}}$ values are summarized in Table 2.

In precontracted strips (n = 28), isoprenaline and papaverine produced concentration-dependent relaxations. No relaxation response to isoprenaline was observed in MG response was significantly increased in HG compared to other groups.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>CG</th>
<th>MC</th>
<th>n</th>
<th>HG</th>
<th>n</th>
<th>MG</th>
<th>n</th>
<th>n</th>
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<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>KCl</td>
<td>31.59 ± 8.11</td>
<td>29.65 ± 9.08</td>
<td>9</td>
<td>67.24 ± 12</td>
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<td>45.51 ± 7.61</td>
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<tr>
<td>Carbachol</td>
<td>165.25 ± 10</td>
<td>160.4 ± 9</td>
<td>6</td>
<td>119 ± 10.5</td>
<td>7</td>
<td>147 ± 9.8</td>
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<tr>
<td>Isoprenaline</td>
<td>99.05 ± 10.7</td>
<td>93.1 ± 2.5</td>
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<td>97.24 ± 4.8</td>
<td>8</td>
<td>99.37 ± 3.78</td>
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<tr>
<td>Papaverine</td>
<td>100 ± 0</td>
<td>99.8 ± 0.2</td>
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<td>100 ± 0</td>
<td>9</td>
<td>100 ± 0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>$pD_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>6.17 ± 0.25</td>
<td>6.15 ± 0.17</td>
<td>6</td>
<td>4.49 ± 0.18</td>
<td>7</td>
<td>5.18 ± 0.25</td>
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<tr>
<td>Isoprenaline</td>
<td>7.22 ± 0.14</td>
<td>6.88 ± 0.25</td>
<td>8</td>
<td>6.92 ± 0.17</td>
<td>8</td>
<td>6.72 ± 0.13</td>
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</tr>
<tr>
<td>Papaverine</td>
<td>5.30 ± 0.1</td>
<td>5.25 ± 0.15</td>
<td>8</td>
<td>5.15 ± 0.12</td>
<td>9</td>
<td>4.96 ± 0.16</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

n indicates number of strips.

* $P < .05$, different from control groups (CG and MC).

# $P < .05$, different from MG.
3. Discussion

Vomiting children with hydrocephalus usually have GER [14-17]. Most patients with GER may exhibit a variety of motor foregut dysmotility which may be related to abnormal modulation of the enteric nervous system by the central nervous system or to involvement of the enteric nervous system by the same process affecting the brain. Our previous observations on animal models showed impaired foregut smooth muscle reactivity in hydrocephalus and GER [18,19]. Glutamate mediates the vago-vagal reflex which plays a major role in the regulation of gastrointestinal function [7,20]. Memantine regulates glutamate by information processing, storage, and retrieval. Memantine appears to protect the brain’s nerve cells against glutamate, which is a messenger released in excess amounts in cell damages in Alzheimer’s disease or other neurologic disorders [21]. In this background, we investigated the role of memantine on upper gastrointestinal smooth muscle reactivity to determine whether any healing occurred after hydrocephalus. In the present study, our observations showed that memantine caused improvements in impaired foregut smooth muscle reactivity in rats with hydrocephalus. Memantine treatment in hydrocephalus may work by restoration of physiologic signal transmission and/or by prevention of nerve cell degeneration.

The present study showed that receptor-mediated contractile (carbachol) and relaxant (isoprenaline) response of esophageal smooth muscle reactivity was decreased in HG compared to other groups. However, no smooth muscle reactivity changes were observed in MG compared to CG. These data provided very important evidence that memantine had positive effect on impaired esophageal smooth muscle reactivity when hydrocephalus develops. Probably, post receptor activation or intracellular signal transduction was affected by memantine. We have not observed any relaxant response induced by receptor-mediated or nonreceptor-mediated mechanisms.

We found opposite responses of esophageal and gastric smooth muscle to carbachol contraction. Several factors such as age and different types of tissues caused such changes in smooth muscle reactivity in a rat hydrocephalus model previously [8,9]. This probably depends on different receptor sensitivities, contractile mechanisms, or other factors.

According to our study, esophageal and gastric smooth muscle reactivity was influenced by memantine. These findings are suggestive of improvement in structural and/or functional changes in foregut smooth muscle reactivity in hydrocephalic state induced by memantine. More research is required to elucidate the functional significance of these observations.

References


