EFFECT OF ELEVATED INTRA-ABDOMINAL PRESSURE ON THE CONTRACTILE ACTIVITY AND REACTIVITY OF SMOOTH MUSCLE TISSUE FROM RAT GASTRO-INTESTINAL TRACT TO GALANTAMINE AND DROTAVERINE (NO-SPA)

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ABSTRACT

AIM: The aim of the present study was to determine the nature and intensity of changes in the contractile activity and reactivity of gastrointestinal smooth muscle tissue in conditions of increased intra-abdominal pressure.

METHODS: A method for recording isometric contractions of isolated smooth muscle preparations from gastric corpus, duodenum and sigmoid colon of rats was used.

RESULTS: Two groups of rats were used in the study – control animals and animals with elevated abdominal pressure. It was established that pressure of 25 mmHg for 60 min did not cause statistically significant change in the tone and parameters of the spontaneous contractions in all preparation types, as well as in their reactivity to drotaverine (no-spa). Statistically significant increase in the strength of the tonic effects of galantamine (1.10⁻⁶ – 1.10⁻³ mg/ml) was found in all types of smooth muscles preparations isolated from rats with increased abdominal pressure compared with preparations from the control rats.

CONCLUSIONS: The statistically significant increase in the galantamine-induced effects on smooth muscle preparations is associated with increase in the contractile effectiveness of acetylcholine. M-type cholinergic receptors are predominantly involved in the processes, probably sensibilized from processes activated by the increased intra-abdominal pressure.

Key words: abdominal compartment syndrome, increased intra-abdominal pressure, smooth muscles, gastrointestinal tract, cholinergic receptors

INTRODUCTION

Compartment syndrome is a condition in which increased pressure within fixed anatomical space impairs the functions of the tissues in this space. Abdominal compartment syndrome is defined as a consequence of intra-abdominal hypertension (IAH) higher than 20 mmHg with clinical evidence of multiorgan failure.¹ Intestines are considered as the most susceptible to elevated intra-abdominal pressure.² Their barrier function is impaired, which causes bacterial translocation from the intestinal lumen to the mesenterial lymph nodes, liver and spleen.³ The edema developing in the intestinal wall and the pathomorphological changes affect the intestinal motor function.⁵ Gastric and intestinal motor function is decreased⁶ and gastrointestinal tract (GI) evacuation is slowed down⁵, which is associated with higher expression of iNOS⁷. There is evidence that intra-abdominal hypertension exerts negative effect on the function of the intestinal cells of Cajal, reducing the spontaneous activity of the intestinal musculature.⁸ Rat models display decreased reactivity of isolated SM from ileum after field electrostimulation, as well as after treatment with acetylcholine.⁹

Elevated intra-abdominal pressure and abdominal compartment syndrome doubtlessly impair the motility and evacuation function of GI tract. At the same time, there are few studies revealing mechanisms associated with that gastrointestinal
dysfunction and its relation with following increase in the intra-abdominal volume and IAH (circulus vitiosus).

It is in this respect that the aim of the present study, i.e. to reveal the nature and intensity of the changes in the contractile activity and reactivity of gastrointestinal SM in conditions of increased intra-abdominal pressure, as well as some of the causative mechanisms, makes it up-to-date.

MATERIAL AND METHODS

MODEL OF ABDOMINAL COMPARTMENT SYNDROME

Adult male rats Wistar (n = 10; 280 – 310 g) were used in the model. A device containing insufflator of atmospheric air and manometer for pressure reading was used to produce the necessary intra-abdominal pressure. The device allowed quick increase in the intra-abdominal pressure without previous laparotomy of the animals, achievement of the required pressure of 25 mmHg, its maintenance for 60 min and easy decompression at the apposite time. The rats were anesthetized with 0.5 ml thiopental applied intraperitoneally and fixed in a supine position. During the experiment the body temperature of the rats was maintained at 36-37°C with a heat lamp.

The control group comprised 7 animals weighing from 290 to 310 g, which was not statistically different from the weight of the experimental animals. The rats underwent the above-described procedures except increasing intra-abdominal pressure.

All experimental animals were maintained under standard laboratory conditions: temperature 21-23°C, humidity 55 ± 10%, granulated standard food, water available ad libitum and 12/12-hour light-dark cycle.

The experiment was made in agreement with the Helsinki Declaration principles in the care and human attitude to experimental animals. The requirements of Order No 25/10. 06. 2003 of the Ministry of Health from 01.01.2004, published in the State Newspaper, issue 59/01.07.2003, amendment in the State newspaper, issue 73/19.08.2003 were followed strictly.

REGISTRATION OF CONTRACTILE ACTIVITY OF ISOLATED SMOOTH MUSCLES

The experiments were performed on smooth muscle (SM) preparations from gastric corpus, duodenum and sigmoid colon dissected from the two groups of rats. The animals were killed previously by decapitation under slight ether anesthesia.

Circular (gastric corpus) and longitudinal (sigmoid colon & duodenum) SM preparations 12–13 mm long n 1.0–1.1 mm wide were used. These were fixed stationary – at one end to a glass holder placed in a bath with tempered Krebs solution (37°C) and to a tension transducer Swema (Sweden) at the other. The initial mechanical tension of the SM preparations was 10 mN. The Krebs solution (pH = 7.4) was aerated with a gaseous mixture of 5% CO2 and 95% O2. SM tissues were treated with different substances applied by adding aliquots of their concentrated solutions in volume not exceeding 0.5% of the total Krebs solution volume in the tissue bath. Drug-induced effect were assessed according to the basal tone levels and data of spontaneous contractile activity registered after prior 60-minute adaptation of the preparations. During that period the Krebs solution was repeatedly refreshed and the contractile activity of the preparations was tested twice with 1.10^-6 mol/l acetylcholine.

The change of the spontaneous activity of the SM preparations and their reactivity in treatment with galantamine and drotaverine was studied. The drug choice was determined by their wide use in the clinical practice. The galantamine and drotaverine concentrations used to treat the SM preparations in the tissue bath were comparable with the doses used in the clinical practice.

The mechanical activity was registered with Microtechna amplifier (Czech Republic) and recorded with Linsseis recorder (Germany). The solution pH was measured with microcomputer pH-meter 6201 (Jenco Electronics, UK).

DRUGS, CHEMICALS, SOLUTIONS

The medical preparations used in the experiments were galantamine (Nivalin) from Sopharma, drotaverine (No-spa) from Chinion and acetylcholine from Dispersa Baeschlin (Germany). pH of the Krebs solution was 7.40. The solution components included (in mM): NaCl – 120, KCl – 5.9, CaCl2 – 2.5, MgCl2 – 1.2, NaH2PO4 – 1.2, NaHCO3 – 15.4, and glucose – 11.5. The preparation solution contained NaCl : KCl : CaCl2 in a ratio of 27.2 : 1.1 : 1.0. All substances used in the preparation of the solutions were products of Merck (Darmstadt, Germany).

STATISTICAL ANALYSIS

Each value was presented as mean ± SE. The effects of equimolar drug concentrations on SM preparations from rats with increased intra-abdominal pressure and from control animals were compared for any characteristic of the contractile activity (tone, fre-
frequency and amplitude) and for any type of SM tissue alone. The SM preparation tone was measured in mN. The strength of phase contractions (mN) was determined as mean value of at least 8 consecutive spontaneous contractions before and 10 min after treatment with corresponding drug concentration. The frequency (min\(^{-1}\)) was determined for a period of 5 min immediately before and 10 min after the drug treatment. The data were analysed with variance analysis (Student’s t-test) using INSTAT software. Differences were accepted as statistically significant at P < 0.05.

RESULTS

1. INFLUENCE OF THE INTRA-ABDOMINAL HYPERTENSION ON THE CONTRACTILE ACTIVITY OF ISOLATED SM PREPARATIONS

Increased intra-abdominal pressure to 25 mmHg for 60 minutes does not change statistically significantly (P > 0.05) the character and strength of the spontaneous phasic contractions of SM preparations from the stomach, duodenum and sigmoid colon of rat.

2. EFFECTS OF GALANTAMINE ON THE TONE OF SM PREPARATIONS

Galantamine influences the tone of SM preparations isolated from the GI tracts of both group animals. The effects are contractile in character and show increasing concentration-effect relationship in the concentration zone 1.10\(^{-6}\) – 1.10\(^{-3}\) mg/ml. The strength of the reactions is different for the different types SM preparations.

At most of the concentrations used the preparations from IAH rats show greater strength of galantamine-induced contractions than those of the control preparations (Table 1).

3. EFFECTS OF GALANTAMINE ON THE PARAMETERS OF THE SPONTANEOUS CONTRACTILE ACTIVITY OF SM PREPARATIONS

3.1. On the amplitude of the phasic contractions

It is only in a concentration of 1.10\(^{-3}\) mg/ml that galantamine causes statistically significant increase in the strength of the spontaneous contractions of SM preparations from gastric corpus and sigmoid colon. The drug has no effect on the amplitude of the phasic contractions of duodenum. The differences between the effects of equimolar concentrations of galantamine on each type of the SM preparations isolated from IAH and control rats are short of statistical significance.

3.2. On the frequency of phasic contractions

Within the examined concentration range galantamine does not influence the spontaneous contractions frequency of SM preparations from different regions of the GI tract of the control and IAH rats.

4. EFFECTS OF DROTAVERINE ON THE TONE OF SM PREPARATIONS

Drotaverine relaxes SM tissue from the GI tract. The relaxation power is concentration dependent (Table 2). It is greatest in preparations from the stomach and decreases in those from duodenum and sigmoid colon.

The differences in the effects of equimolar concentrations from the drug preparations obtained between each of the SM preparations from the control and IAH rats do not reach statistical significance (P > 0.05).

Table 1. Comparison of the strength of tonic contractions (mN) triggered by equimolar concentrations of galantamine on same type SM preparations from control and IAH rats;

<table>
<thead>
<tr>
<th>Galantamine mg/ml</th>
<th>Smooth muscle preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control rats</td>
</tr>
<tr>
<td></td>
<td>Gastric corpus (n = 7)</td>
</tr>
<tr>
<td>1.10(^{-6})</td>
<td>0.14 ± 0.25</td>
</tr>
<tr>
<td>1.10(^{-5})</td>
<td>0</td>
</tr>
<tr>
<td>1.10(^{-4})</td>
<td>1.04 ± 0.50</td>
</tr>
<tr>
<td>1.10(^{-3})</td>
<td>3.16 ± 0.41</td>
</tr>
</tbody>
</table>

* P < 0.05.
5. Effects of Drotaverine on the Parameters of the Spontaneous Contractile Activity of SM Preparations

5.1. On the amplitude of the phasic contractions

Drotaverine reduces the spontaneous phasic contractions of SM preparations from all regions of the GI tract (Table 3). No statistically significant differences (P > 0.05) were found in the reaction power between corresponding SM tissues from the control and IAH rats treated with equimolar concentrations of drotaverine.

5.2. On the frequency of phasic contractions

The drug substance does not influence the frequency of spontaneous contractions of SM preparations from stomach, duodenum and sigmoid colon in the control rats and from stomach and duodenum in the IAH rats. In single preparations from sigmoid colon of IAH rats a tendency to increasing frequency of spontaneous contractile activity after treatment with 1.10^{-4} and 1.10^{-3} mg/ml concentrations of drotaverine is observed.

**DISCUSSION**

Our research suggests that increase in the intra-abdominal pressure within the bounds defined in the experiment (25 mmHg, 60 min) does not cause statistically significant change in the character and power of the spontaneous phasic contractions of SM preparations from stomach, duodenum and sigmoid colon.

**Table 2.** Comparison of the strength of reactions (mN) triggered by equimolar concentrations of drotaverine on same type SM preparations from GI tract from control and IAH rats

<table>
<thead>
<tr>
<th>Drotaverine mg/ml</th>
<th>Smooth muscle preparations</th>
<th>Control rats</th>
<th>Intra-abdominal hypertension rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric corpus n = 7</td>
<td>Duodenum n = 7</td>
<td>Sigmoid colon n = 7</td>
</tr>
<tr>
<td>1.10^{-6}</td>
<td>-0.30 ± 0.06</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.10^{-5}</td>
<td>-0.34 ± 0.31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.10^{-4}</td>
<td>-0.62 ± 0.44</td>
<td>-0.30 ± 0.38</td>
<td>-0.16 ± 0.21</td>
</tr>
<tr>
<td>1.10^{-3}</td>
<td>-1.48 ± 0.33</td>
<td>-0.72 ± 0.35</td>
<td>-0.64 ± 0.55</td>
</tr>
</tbody>
</table>

"-" indicates relaxation; * P < 0.05.

**Table 3.** Comparison of the effects of equimolar concentrations of drotaverine on the strength of spontaneous phasic contractions (mN) of the different types of SM preparations from control and IAH rats

<table>
<thead>
<tr>
<th>Drotaverine mg/ml</th>
<th>Smooth muscle preparations</th>
<th>Control rats</th>
<th>Intra-abdominal hypertension rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric corpus n = 7</td>
<td>Duodenum n = 7</td>
<td>Sigmoid colon n = 7</td>
</tr>
<tr>
<td>Autocontrols</td>
<td>0.83 ± 0.24</td>
<td>1.85 ± 0.87</td>
<td>3.19 ± 0.81</td>
</tr>
<tr>
<td>1.10^{-6}</td>
<td>0.76 ± 0.27</td>
<td>1.76 ± 0.87</td>
<td>3.09 ± 0.87</td>
</tr>
<tr>
<td>1.10^{-5}</td>
<td>0.71 ± 0.20</td>
<td>1.78 ± 1.02</td>
<td>3.02 ± 0.61</td>
</tr>
<tr>
<td>1.10^{-4}</td>
<td>0.67 ± 0.30</td>
<td>0.89 ± 0.62</td>
<td>2.83 ± 0.81</td>
</tr>
<tr>
<td>1.10^{-3}</td>
<td>0.14 ± 0.05</td>
<td>0</td>
<td>2.34 ± 1.28</td>
</tr>
</tbody>
</table>

* P < 0.05.
Receptors, causes elevation of the cytosol level of Ca\(^{2+}\) in the SM cells and in turn enhances the contractile activity.\(^{13}\)

SM tissues from stomach, duodenum and sigmoid colon react with tonic contractions in both animal groups (control and IAH rats). The contractions are concentration-dependant and are most expressed in the stomach preparations. Although similar in character they differ in strength. Equimolar concentrations of galantamine cause contractions that are statistically significantly more expressed in SM preparations from IAH rats than in SM preparations from control animals.

A number of studies show that galantamine-induced tonic effects on the GI tract are associated with influence of the accumulated acetylcholine mainly on the muscarine cholinergic receptors (mAChR) of the SM cells that are effectively blocked by atropine.\(^{14}\) Given this, the difference in the strength of reactions can be accounted for by an IAH-induced change in the behaviour of these receptors. The unidirectional character of changes in all examined SM tissue types indicates most probably an IAH-provoked process causing, in broad terms, sensibilisation of mAChR.

The differences in the strength of the effects of galantamine on the amplitude of the phasic contractions of different SM tissue types are result from the effect of the endogenous acetylcholine mainly on the nicotinic cholinergic receptors (nAChR) located on the pre- and postganglionic synapses of the neurons in the intramural neuronal structures.\(^{15}\) Their activation stimulates release of neurotransmitters like glutamine, serotonin, dopamine, GAPA and/or VIP etc. with SM effects diverse in character.\(^{16}\) Some of these are proven activators while others are inhibitors of the contractile activity. The absence of statistically significant differences in the galantamine-induced effects on the strength and frequency of the spontaneous contractions of similar SM preparations between the two groups of rats indicates, in broad terms, no effect of IAH on nAChR.

Drotaverine inhibits the contractile activity of gastric and intestinal SM preparations by increasing the cytosol concentrations of cAMP and/or cGMP.\(^{17}\) The absence of statistically significant differences in the reactions of same type SM preparations between control and IAH rats shows that within the parameters of the present study the increased intrabdominal pressure does not influence the activity of phosphodiesterase system, which is the target of drotaverine action.\(^{18}\)

**CONCLUSIONS**

Intra-abdominal hypertension (25 mmHg, 60 min) does not cause statistically significant changes in the strength and character of spontaneous contractility of SM tissue from stomach, duodenum and sigmoid colon of rats.

Intra-abdominal hypertension causes statistically significant increase in the strength of galantamine-induced tonic contractions of the three SM tissue types (gastric corpus, duodenum and sigmoid colon) compared with the same type SM tissues of control animals. The latter is a reason to suggest an increase in the contractile effect of acetylcholine, which is a basic excitatory neurotransmitter in the GI tract.

**REFERENCES**

10. Blattner R, Classen HG, Dehnert H, Doring HG. Experiments on isolated smooth muscle prepara-