EXPERIMENTAL RESEARCH ON THE EFFECTS OF SEROTONIN AND ACETYLCHOLINE ON MECHANICAL ISOMETRIC ACTIVITY OF ISOLATED RAT INTESTINE AFTER THE DEPLETION OF ENDOGENOUS SEROTONIN

M. COSTESCU, H. PĂUNESCU*#, B.Ş. VELESCU**, A. ZUGRAVU*, OANA ANDREIA COMAN*, I. FULGA**

* Department of Pharmacology and Pharmacotherapy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, România, #e-mail: phpaunescu@yahoo.com
** Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Abstract. The actual research was conducted to assess the influences of pretreatment with different doses of serotonin on gastrointestinal motility (rat jejunum) induced by acetylcholine after the depletion of endogenous serotonin by para-chlorophenylalanine (PCPA) in order to evaluate the effect of increased serotonin. Rats of which jejunum was removed were pre-treated with 300 mg/kg bw para-chlorophenylalanine, for 3 days. The assessments were done using an isolated organ bath with isometric transducers, using tyrode solution, aerated with air, 37 °C. Different acetylcholine and serotonin doses were added to the bath in order to obtain concentrations of 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M and 10^{-4} M, respectively. Subsequently after one dose of serotonin of 10^{-8} M, increasing doses of acetylcholine were added in order to obtain bath concentrations in the same concentration mentioned above. The cycle was repeated with serotonin 10^{-7} M, 10^{-6} M, 10^{-5} M and 10^{-4} M. The contractile force, the rate of contractile force development during contraction, the rate of contractile force development during relaxation were assessed. Results were interpreted by calculating the mean and standard deviation for each pair of concentrations. In the present experimental conditions, a complex interaction between serotonin and acetylcholine on intestinal motility was shown

Key words: acetylcholine, serotonin, para-chlorophenylalanine, organ bath, isometric contraction.

INTRODUCTION

The enteric nervous system (ENS) is involved in the modulation of motility [5], intestinal blood flow [31], exocrine and endocrine secretions [19], as well as in the immune processes [4]. The enteric nervous system can function on its own, but

Received: May 2016;
in final form May 2016.

ROMANIAN J. BIOPHYS., Vol. 26, No. 1, P. 21–34, BUCHAREST, 2016

the central nervous system (CNS) plays an important role in modulating its functions. The CNS is connected to the ENS through both afferent and efferent pathways of the sympathetic (via the prevertebral ganglia) and the parasympathetic nervous systems [2]. The efferent parasympathetic pathways of the gastrointestinal tract consist of the vagus and pelvic splanchnic nerves [9].

All the preganglionic neurons are cholinergic and they have excitatory effects on enteric neurons through nicotinic receptors [11]. There are indications that the nicotinic receptors are involved mainly in propulsive motility [15]. This is sustained by the effects of atropine, hexamethonium, bethanechol on the gastrointestinal tract, respectively.

Therefore, when the antimuscarinic parasympatholytic atropine is administered, the gastrointestinal motility is mildly affected and the patient may experience constipation [1]. However, when hexamethonium, a nicotinic receptor antagonist is administered, the peristaltic reflex is abolished and, sometimes, intestinal paralysis is installed [16, 18]. On the other hand, bethanechol, a parasympathomimetic that selectively stimulates only the muscarinic receptors, has diarrhea amongst its adverse effects [21]. Also, anticholinesterases used in the treatment of myasthenia gravis (pyridostigmine bromide) or in the treatment of paralytic ileus have been reported to produce intolerable diarrhea [17, 25]. Moreover, administration of irreversible acetylcholinesterase inhibitors, like insecticides and nerve agents, leads to uncontrollable diarrhea [3].

Serotonin or 5-hydroxytryptamine (5-HT), a neurotransmitter derived from the amino acid tryptophan, also influences the motility of gastrointestinal tract, but with a lower intensity than acetylcholine.

Serotonin initiates peristaltic and secretory reflexes and transmits information to the central nervous system [15, 29]. This is confirmed by the adverse effects of some antidepressant drugs that target the serotonergic system. Thus, selective serotonin reuptake inhibitors (SSRIs) inhibit the reuptake of serotonin, therefore increasing serotonin levels in gastrointestinal tract [26]. Consequently, when SSRIs are administered, sometimes adverse gastrointestinal motility disturbances appear like diarrhea and vomiting [8, 10].

Several experiments which investigated serotonin's pharmacological effect on intestinal tract were done on isolated ileum and colon preparations [14, 24].

Isolated ileum is a research technique invented by Otto Magnus in 1904. Up to this day, this method is still one of the most widely used in experimental pharmacology [34].

During the past years, the modulation of serotonergic system both in the brain and in the gastrointestinal tract has become an important pharmacological target. Some ligands of 5-HT_3 and 5-HT_4 serotonin receptors were proved to have therapeutic value.

ANTAGONISTS OF 5-HT$_3$ RECEPTORS

Antagonists of 5-HT$_3$ receptors like tropisetron, ondansetron, granisetron, dolasetron and palonosetron are widely used in Europe [33] as antiemetics. 5-HT$_3$ receptors antagonists are also employed in treating diarrhea-predominant irritable bowel syndrome. 5-HT$_3$ receptor antagonists reduce abdominal pain, decrease gastrointestinal transit and increase the compliance of the colon to distension [6, 12]. Because of the adverse reactions (severe constipation, ischaemic colitis and even death) of some 5-HT$_3$ antagonists (*e.g.* alosetron), researchers are now focusing on developing agents which act as partial agonists of 5-HT$_3$ receptors [23].

AGONISTS OF 5-HT$_4$ RECEPTORS

Agonists of 5-HT$_4$ receptors stimulate peristalsis and electrolyte secretion [13]. They can be used in treating irritable bowel syndrome with constipation predominance. For instance, tegaserod, a 5-HT$_4$ receptor agonist, has been proved to decrease abdominal pain, stool consistency and potentiate peristalsis [27, 32]. However, 5-HT$_4$ receptor agonists have been linked to cardiovascular adverse effects (tegaserod was withdrawn in 2007 because its administration was linked with an increased risk of cardiovascular events [35]). Researchers are now working on new-generation 5-HT$_4$ receptor agonists (*e.g.* prucalopride), that lack these adverse effects [7, 20].

Another interesting aspect regarding the serotonergic system is the pharmacological relationship between serotonin and acetylcholine. Studies show that serotonin can increase the excitatory response of acetylcholine in the intestinal ganglia of sea slug [30].

According to the literature and our previous researches, serotonin increases the contractility of isolated intestine from rats depleted of endogenous stores of serotonin. Serotonin depletion could be accomplished by systemic administration of para-chlorophenylalanine [28].

AIM

The actual research was conducted to assess the influences of pretreatment with different doses of serotonin on gastrointestinal (GI) motility (rat jejunum) induced by acetylcholine after the depletion of endogenous serotonin by PCPA.

MATERIALS AND METHODS

ANIMALS

Male Wistar rats, 3 months old, weighing 300–350 g, $n = 8$, were pre-treated with para-chlorophenylalanine, 300 mg/kg bw, dissolved in Tween 80 1%, for 3 consecutive days before tests were performed. The animals were starved for one day before euthanasia. The experiments were conducted in accordance with ethical principles (the experiments were carried out according the 86/609 Directive of European Council).

SUBSTANCES AND SOLUTIONS

All the substances used were bought from Sigma. The tissue bathing fluid was composed of Tyrode solution with the following composition (g/L): NaCl (8.0), KCl (0.2), $CaCl_2$ (0.2), $NaHCO_3$ (1.0), $MgCl_2$ (1.0), NaH_2PO_4 (0.5), glucose (1.0); which was maintained at 37 °C and aerated with air. The first five substances were mixed separately before adding the solution composed of glucose and NaH_2PO_4.

The substances and solutions used as active agents for motility were:

– Acetylcholine: 10^{-2} M, 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M (1 Mol of acetylcholine as acetylcholine iodide = 273.11 g).

– Serotonin: 10^{-2} M, 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M (1 Mol of serotonin as serotonin hydrochloride = 212.68 g)

APPARATUS AND SOFTWARE

An Isolated Organ Bath with two chambers (Ugo Basile 4050) was used. The recordings were made through two Ugo Basile isometric transducers (7003 model) covering a force range from 0 to 50 g. The force exerted on a hollow carbon fibre beam was converted into proportional electrical signal. The transducers were connected to an acquisition system (*e.g.* Ugo Basile Data Capsule), with an appropriate connector. The recordings were stored in a laptop and interpretations of results were made using 17400 Data Capsule software.

PROCEDURE

Rats were sacrificed by cervical dislocation and bleeding from carotid arteries. Following an incision of the abdominal wall on the median line, a segment of the jejunum was removed after discarding the portion near to the duodenal bulb junction (5 cm distance). The content was removed and then washed with Tyrode solution (as described above).

After cleaning of adhering fat and connective tissues, the jejune strip (approximately 1 cm in length) was mounted vertically under resting tension of 1 g in a 10 mL organ bath with Tyrode solution as fluid which was maintained at 37 °C and aerated with air. Responses of the isolated jejunum were recorded isometrically. The tissue was allowed to equilibrate for 30 minutes during which the bathing fluid was replaced every 10 min and tension was adjusted 15 and 30 minutes before recordings.

A standard recording time, one minute length, was performed. A solution of 0.1 ml of acetylcholine with a minimum concentration 10^{-6} M was added into the organ bath. Thus, the organ bath achieved a concentration of 10^{-8} M and the response was recorded for 60 seconds. Subsequently, another dose of acetylcholine was added and the response was again recorded for 60 seconds. The concentration of acetylcholine was increased in geometric progression with a ratio 10 for every 60 seconds. This procedure continues until a concentration of acetylcholine, that gives a maximal response in accordance with anterior results of 10^{-4} M concentration in organ bath is found. We will term this method "the method of cumulative doses".

After administering the acetylcholine 10^{-4} M concentration in organ bath, the organ preparation was washed three times with Tyrode solution at 60 seconds intervals. Then, for reaccommodation, the organ preparation was maintained in the Tyrode solution for 20 minutes (the Tyrode solution was replaced once after the first 10 minutes).

The same method was applied for serotonin – "the method of cumulative doses".

We also investigated to what extent the administration of serotonin prior to acetylcholine influences the effect of acetylcholine. For this, different serotonin doses were added to the bath in order to obtain serotonin concentrations of 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M and 10^{-4} M, respectively. For each dose of serotonin, increasing doses of acetylcholine applying "the method of cumulative doses" were used.

Three parameters were determined:

- **The contractile force** (measured in gF – grams force), which was obtained from the subtraction of the maximum force of muscle contraction (the subtraction of the peak of the activity of smooth muscle obtained after administration of different substances and the corresponding basal tone) and the basal smooth muscle tone (the subtraction of the peak of the activity of smooth muscle obtained spontaneously, without stimuli, and the corresponding basal tone).
- **The rate of contractile force development during contraction** (measured in gF/s) and obtained by the ratio of the contractile force (gF) and the length of contraction (s).

- **The rate of contractile force development during relaxation** (measured in gF/s) and obtained by the ratio of the contractile force (gF) and the length of relaxation (s).

For each concentration of serotonin and acetylcholine, we determined three parameters: the contractile force (measured in gF), rate of contractile force development during contraction (measured in gF/s) and the rate of contractile force development during relaxation (measured in gF/s).

Results were statistically interpreted by calculating the mean and standard deviation for each pair of concentrations. The statistical significance of the differences was assessed by Student's t-test. We considered statistically significant only the differences in which $p < 0.05$.

RESULTS

The results are presented in a tabelar fashion. Table 1 and Table 2 present the evolution of the contractile force (gF), of the rate of contractile force development during contraction (gF/s) and of the rate of contractile force development during relaxation (gF/s) after the administration of progressively increasing doses of acetylcholine and setotonin, respectively.

Tables 3, 4 and 5 present the contractile force (gF), the rate of contractile force development during contraction (gF/s) and the rate of contractile force development during relaxation (gF/s), respectively after the administration of progressively increasing doses of acetylcholine in organ bath after preincubation with serotonin 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M. The preparation was washed with tyrode solution and allowed to reaccommodate between each dose of serotonin administered.

Table 1

The evolution of the contractile force (gF), of the rate of contractile force development during contraction (gF/s) and of the rate of contractile force development during relaxation (gF/s) after the administration of progressively increasing doses of acetylcholine of 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M

Parameter	Basal activity	Ach 10^{-8} M	Ach 10^{-7} M	Ach 10^{-6} M	Ach 10^{-5} M	Ach 10^{-4} M
Contractile force (gF)	0.080 ± 0.011	0.064 ± 0.009	0.121 ± 0.017 #	0.128 ± 0.018 #	0.352 ± 0.049 #	0.764 ± 0.107 #
Rate of contraction (gF/s)	0.018 ± 0.003	0.020 ± 0.003	0.016 ± 0.002	0.016 ± 0.002	0.026 ± 0.004 #	0.063 ± 0.009 #
Rate of relaxation (gF/s)	0.022 ± 0.003	0.022 ± 0.003	0.015 ± 0.002	0.011 ± 0.002	0.009 ± 0.001	0.029 ± 0.004

Legend: Ach = acetylcholine. Rate of contraction (gF/s) = Rate of contractile force development during contraction (gF/s). Rate of relaxation (gF/s) = Rate of contractile force development during relaxation (gF/s). # $p < 0.05$, t-test, *vs*. basal activity.

Table 2

The evolution of the contractile force (gF), of the rate of contractile force development during contraction (gF/s) and of the rate of contractile force development during relaxation (gF/s) after the administration of progressively increasing doses of serotonin 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M

Parameter	Basal activity	5-HT 10^{-8} M	5-HT 10^{-7} M	5-HT 10^{-6} M	5-HT 10^{-5} M	5-HT 10^{-4} M
Contractile force (gF)	0.041 ± 0.006	0.082 ± 0.011	0.103 ± 0.014	0.089 ± 0.012	0.019 ± 0.003	0.190 ± 0.027 #
Rate of contraction (gF/s)	0.006 ± 0.001	0.008 ± 0.001	0.012 ± 0.002	0.010 ± 0.001	0.017 ± 0.002 #	0.015 ± 0.002 #
Rate of relaxation (gF/s)	0.007 ± 0.001	0.006 ± 0.001	0.010 ± 0.001	0,010 ± 0,001	0.011 ± 0.002	0.013 ± 0.002 #

Legend: 5-HT = serotonin. Rate of contraction (gF/s) = Rate of contractile force development during contraction (gF/s). Rate of relaxation (gF/s) = Rate of contractile force development during relaxation (gF/s). # $p < 0.05$, t-test, *vs*. basal activity.

Table 3

The evolution of the contractile force (gF), after the administration of progressively increasing doses of acetylcholine with prior administration of a dose of 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M serotonin

5-HT concentration	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
Basal activity	0.062 ± 0.009	0.099 ± 0.014	0.068 ± 0.010	0.062 ± 0.009	0.041 ± 0.006
5-HT	0.110 ± 0.015 #	0.066 ± 0.008	0.098 ± 0.014	0.103 ± 0.014	0.169 ± 0.024 #
Ach 10^{-8} M	0.054 ± 0.008	0.096 ± 0.013	0.114 ± 0.016 #	0.151 ± 0.021 #	0.078 ± 0.011 #
Ach 10^{-7} M	0.096 ± 0.013 #	0.051 ± 0.007	0.167 ± 0.023 #	0.103 ± 0.014	0.142 ± 0.020 #

Table 3

(continued)

Ach 10^{-6} M	0.119 ± 0.017 #	0.057 ± 0.008	0.058 ± 0.008	0.100 ± 0.014	0.078 ± 0.011 #
Ach 10^{-5} M	0.099 ± 0.014 #	0.137 ± 0.029	0.096 ± 0.013	0.103 ± 0.014	0.105 ± 0.015 #
Ach 10^{-4} M	0.130 ± 0.018 #	0.119 ± 0.017	0.117 ± 0.016 #	0.142 ± 0.020 #	0.167 ± 0.023 #

Legend: 5-HT = serotonin, Ach = acetylcholine, # $p < 0.05$, t-test, *vs.* basal activity.

Table 4

The evolution of the rate of contractile force development during contraction (gF/s) after the administration of progressively increasing doses of acetylcholine with prior administration of a dose of 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M serotonin

5-HT concentration	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
Basal activity	0.007 ± 0.001	0.009 ± 0.001	0.006 ± 0.001	0.008 ± 0.001	0.006 ± 0.001
5-HT	0.009 ± 0.001	0.009 ± 0.001	0.018 ± 0.003 #	0.015 ± 0.002	0.044 ± 0.006 #
Ach 10^{-8} M	0.009 ± 0.001	0.009 ± 0.001	0.010 ± 0.001	0.010 ± 0.001	0.008 ± 0.001
Ach 10^{-7} M	0.004 ± 0.001	0.013 ± 0.002	0.011 ± 0.002	0.015 ± 0.002	0.009 ± 0.001
Ach 10^{-6} M	0.013 ± 0.002 #	0.010 ± 0.001	0.007 ± 0.001	0.009 ± 0.001	0.008 ± 0.001
Ach 10^{-5} M	0.018 ± 0.003 #	0.016 ± 0.002	0.013 ± 0.002	0.007 ± 0.001	0.008 ± 0.001
Ach 10^{-4} M	0.012 ± 0.002 #	0.006 ± 0.001	0.007 ± 0.001	0.010 ± 0.001	0.009 ± 0.001

Legend: 5-HT = serotonin, Ach = acetylcholine, # $p < 0.05$, t-test, *vs.* basal activity.

Table 5

The evolution of the rate of contractile force development during relaxation (gF/s) after the administration of progressively increasing doses of acetylcholine with prior administration of a dose of 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M serotonin

5-HT concentration	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
Basal activity	0.007± 0.001	0.017± 0.002	0.014± 0.002	0.008± 0.001	0.005± 0.001
5-HT	0.011± 0.002 #	0.011± 0.002	0.009± 0.002	0.010± 0.001	0.019± 0.003 #
Ach 10^{-8} M	0.014± 0.002 #	0.010± 0.001	0.017± 0.002	0.012± 0.002	0.011± 0.002 #
Ach 10^{-7} M	0.015± 0.002 #	0.017± 0.002	0.012± 0.002	0.007± 0.001	0.015± 0.002 #
Ach 10^{-6} M	0.011± 0.002 #	0.011± 0.002	0.016± 0.002	0.006± 0.001	0.013± 0.002 #
Ach 10^{-5} M	0.006± 0.001	0.009± 0.001	0.006± 0.001	0.010± 0.001	0.013± 0.002 #
Ach 10^{-4} M	0.015± 0.002 #	0.016± 0.002	0.011± 0.002	0.014± 0.002	0.013± 0.002 #

Legend: 5-HT = serotonin, Ach = acetylcholine, # $p < 0.05$, t-test, *vs.* basal activity.

From the data presented above, it can be observed that acetylcholine increases the contractile force in a dose-dependent manner. The values were statistically significant for all the concentrations of acetylcholine that were equal or greater than 10^{-7} M.

When a maximum concentration of 10^{-4} M of acetylcholine was administered, the force of contraction increased by 9 and half times (from an initial value of 0.080 gF to 0.764 gF).

Acetylcholine has also increased, in a dose-dependent manner, the rate of contractile force development during contraction of the isolated intestine. However, the values were statistically significant only for the concentrations of 10^{-5} M and 10^{-4} M acetylcholine. For the maximum concentration of 10^{-4} M

acetylcholine, the rate of contractile force development during contraction increased by 4 times (from an initial value of 0.018 gF/s to a value of 0.063 gF/s).

The value of the rate of contractile force development during relaxation was not modified in a statisically relevant manner.

Serotonin has modified the contractility of the isolated intestine. Serotonin has increased in a dose-dependent manner the contractile force. For the maximum concentration of serotonin administered, the contractile force was 4 and a half times larger than the initial value (from 0.041 gF to 0.190 gF).

As acetylcholine, serotonin has increased in a dose-dependent manner the rate of contractile force development during contraction. Thus, for the maximum concentration of serotonin administered, the rate of contractile force development during contraction increased by 2 and half times (from an initial value of 0.006 gF/s to 0.015 gF/s).

However, unlike acetylcholine, serotonin increased the rate of contractile force development during relaxation, although these results were statistically significant only for the maximum dose of 10^{-4} M serotonin. Also, this increase was slightly lower than twice the initial value.

After prior administration of a dose of serotonin of 10^{-8} M, the contractile force produced by a concentration of 10^{-8} M acetylcholine was 0.119 gF, meanwhile the contractile force produced by a concentration of 10^{-4} M acetylcholine was 0.130 gF.

After prior administration of a concentration of 10^{-4} M serotonin, the contractile force produced by a concentration of 10^{-7} M of acetylcholine was 0.142 gF, meanwhile the contractile force produced by a concentration of 10^{-4} M of acetylcholine was 0.167 gF, an increase of only 4 times which is equal to the increase produced by a concentration of 10^{-7} M acetylcholine which led to a force of contraction of 0.142 gF.

Generally, these small differences between the effect of very different concentrations of acetylcholine can be observed for all the administered concentrations.

Serotonin concentration of 10^{-6} M increased the rate of contractile force development during contraction from 0.006 gF/s to 0.018 gF/s/. The concentration of 10^{-5} M serotonin increased the rate of contractile force development during contraction from 0.008 gF/s to 0.015 gF/s. The dose of 10^{-4} M serotonin increased the rate of contractile force development during contraction of 0.006 gF/s to 0.044 gF/s.

Regarding the effect of acetylcholine to increase the rate of contractile force development during contraction, found and presented above, we can see that this effect is maintained at serotonin concentrations of 10^{-8} M. Concentrations of serotonin higher than 10^{-8} M make this effect disappear.

After administering a dose of 10^{-4} M of serotonin, the rate of contractile force development during relaxation grew from a base value of 0.005 gF/s to 0.019 gF/s and was subsequently maintained without significant statistical variations, recording values of 0.011 gF/s at a concentration of 10^{-8} M acetylcholine,

0.015 gF/s at 10^{-7} M acetylcholine, 0.011 gF/s at 10^{-6} M acetylcholine, 0.013 gF/s at a concentration of 10^{-5} M acetylcholine and 0.013 gF/s at concentration of 10^{-4} acetylcholine.

DISCUSSION

We can conclude, from the presented data, that both acetylcholine and serotonin administered alone, are increasing the intestinal contraction force dose dependently.

However, there are differences between the two substances. Both serotonin and acetylcholine increase the contraction force and the rate of contractile force development during contraction but, in higher doses, for the same molar concentration, acetylcholine effects are much more intense than the serotonin effects. In the higher administered dose of 10^{-4} M acetylcholine raised the contraction force twice as serotonin. In contrast to acetylcholine, serotonin raised also the rate of contractile force development during relaxation.

In the presence of serotonin, acetylcholine raised also the rate of contractile force development during contraction, but the increase manifested only for the low doses; in the high doses these parameters increase was much lower than their increase in the condition of administering acetylcholine alone.

This suggests that acetylcholine mechanism of action is different when it is administered in low doses than in the case of high doses. It is possible that in low doses, acetylcholine acts by muscarinic receptor stimulation at the terminal parasympathetic synaptic level and in high doses acetylcholine may stimulate also the nicotinic receptors from the myenteric plexus. The stimulation of these nicotinic receptors determines the release of acetylcholine in the parasympathetic nicotinic synapse. This phenomenon represents a spectacular amplification of the intensity of effect, knowing that at the enteric nervous system level, a nicotinic synapse corresponds to 8000 neuroeffector muscarinic synapses [22].

In these circumstances serotonin could antagonize the effects of high doses of acetylcholine by inhibiting its release from the muscarinic synapses, probably through presynaptic serotoninergic receptors, most likely 5-HT1 receptors.

The high dose nicotinic receptors stimulation correlates with the literature data, according to which the muscarinic receptors are much more sensitive to acetylcholine than the nicotinic receptors.

According to the results presented above, it can be assumed that serotonin could modulate the intestinal cholinergic tone.

In an organism with a high cholinergic intestinal tone, serotonin by reducing the cholinergic tone as a result of lowering the acetylcholine release in the neuroeffector synapses could reduce the intestinal transit. This assumption correlates with the usage of serotonin selective reuptake inhibitors in the treatment of irritable bowel syndrome. These medications could be effective in treating this condition not only by the classical central nervous system of improving the thymus status and by the anxiolytic effect, but also through a direct mechanism at the intestinal level.

In an organism with a low cholinergic intestinal tone, on the contrary serotonin increases the intestinal tone by its own effects of intestinal motility stimulation.

This correlates with the digestive disorders (nausea, vomiting, eventually diarrhea) caused by the antidepressant medication (serotonin selective reuptake inhibitors) and with the prokinetic action of the serotoninergic receptor stimulants.

Probably the interference is more complex. Serotonin raises the rate of contractile force development during contraction when it is administered alone. The administration of acetylcholine after serotonin reduced the rate of contractile force development during contraction at the levels before serotonin administration, thus we can conclude that acetylcholine has reversed the serotonin effect of raising the rate of contractile force development during contraction. It is difficult to evaluate the mechanism of this effect.

In our experimental conditions serotonin administered alone raises the rate of contractile force development during relaxation. Acetylcholine administered after serotonin did not influence the serotonin effect of modifying the rate of contractile force development during relaxation, regardless the dose of acetylcholine used in our experimental model.

CONCLUSIONS

Our research showed that serotonin and acetylcholine, two endogenous substances that stimulate the bowel motility, actually show complex interactions at this level. The bowel motility is most likely controlled by the cholinergic system and serotonin modulates the cholinergic tone at the bowel level.

When the cholinergic tone at the bowel level is increased, serotonin lowers this tone by decreasing the release of acetylcholine in the parasympathetic neuroeffector synapses. When the cholinergic tone at the bowel level is low and thus the bowel motility is also decreased, serotonin raises the bowel motility by direct effects. This explains the effectiveness of serotonin reuptake inhibitors antidepressant drugs in different pathological conditions. They are effective in the irritable bowel syndrome, characterized by increased motility, but also they can produce hypermotility, as side effect, when used as antidepressant drugs.

REFERENCES

1. BRUNTON L.L., B.A. CHABNER, B.C. KNOLLMANN, *Goodman & Gilman's the Pharmacological Basis of Therapeutics.* 12. ed., New York, NY, McGraw-Hill Medical, 2011, p. 231.
2. CARABOTTI, MARILIA, ANNUNZIATA SCIROCCO, MARIA ANTONIETTA MASELLI, CAROLA SEVERI, The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems, *Ann. Gastroenterol. Q. Publ. Hell. Soc.Gastroenterol*. 2015, **28**(2), 203–209.
3. COLOVIC, MIRJANA B., DANIJELA Z. KRSTIC, TAMARA D. LAZAREVIC-PASTI, ALEKSANDRA M. BONDZIC, VESNA M. VASIC, Acetylcholinesterase Inhibitors: Pharmacology and Toxicology, *Curr Neuropharmacol*., 2013, **11**(3), 315–335.
4. COOKE, H.J., Neuroimmune signaling in regulation of intestinal ion transport, *Am. J. Physiol*., 1994, **266**(2 Pt 1), G167–G178.
5. COSTA, M., S.J. BROOKES, The enteric nervous system, *Am. J. Gastroenterol*., 1994, **89**(8 Suppl), S129–S137.
6. DELVAUX, M., D. LOUVEL, J.P. MAMET, R. CAMPOS-ORIOLA, J. FREXINOS, Effect of alosetron on responses to colonic distension in patients with irritable bowel syndrome, *Aliment. Pharmacol. Ther*., 1998, **12**(9), 849–855.
7. FAYYAZ, M., J.M. LACKNER, Serotonin receptor modulators in the treatment of irritable bowel syndrome, *Ther. Clin. Risk Manag*., 2008, **4**(1), 41–48.
8. FERGUSON, J.M., SSRI antidepressant medications: Adverse effects and tolerability, *Prim. Care. Companion J. Clin. Psychiatry*, 2001, **3**(1), 22–27.
9. FLOCH, M.H., F.H. NETTER, *Netter's Gastroenterology*, Philadelphia, PA, Saunders/Elsevier, 2010, p. 258.
10. GOLDSTEIN, B.J., P.J. GOODNICK, Selective serotonin reuptake inhibitors in the treatment of affective disorders III. Tolerability, safety and pharmacoeconomics, *J. Psychopharmacol*., 1998, **12**(3 Suppl B), S55–S87.
11. GOYAL, R.K., I. HIRANO, The enteric nervous system, *N. Engl. J. Med*., 1996, **334**(17), 1106–1115.
12. HARRIS, LUCINDA A., LIN CHANG, Alosetron: an effective treatment for diarrhea-predominant irritable bowel syndrome, *Womens Health* (Lond. Engl.), 2007, **3**(1), 15–27.
13. HEGDE, S.S., R.M. EGLEN, Peripheral 5-HT4 receptors, *FASEB J. Off Publ. Fed. Am. Soc. Exp. Biol*., 1996, **10**(12), 1398–1407.
14. HOCK F.J. *Drug Discovery and Evaluation: Pharmacological Assays*, Springer, 2015, pp. 2422–2426.
15. HOFFMAN, J.M., ELICE M. BROOKS, G.M. MAWE, Gastrointestinal motility monitor (GIMM), *J. Vis. Exp*. 2010, **(46)**.
16. KADOWAKI, M., P.R. WADE, M.D. GERSHON, Participation of 5-HT_3, 5-HT_4, and nicotinic receptors in the peristaltic reflex of guinea pig distal colon, *Am. J. Physiol*., 1996, **271**(5 Pt 1), G849–G857.
17. KUMAR, V., H.J. KAMINSKI, Treatment of myasthenia gravis, *Curr. Neurol. Neurosci. Rep*. 2011, **11**(1), 89–96.
18. LOCKET, S, Paralytic ileus after hexamethonium, *Br. Med. J*., 1951, **1**(4718), 1331–1332.
19. LUNDGREN, O., J. SVANVIK, L. JIVEGÅRD, Enteric nervous system. I. Physiology and pathophysiology of the intestinal tract, *Dig. Dis. Sci*., 1989, **34**(2), 264–283.
20. MANABE, NORIAKI, BANNY S. WONG, M. CAMILLERI, New-generation 5-HT_4 receptor agonists: potential for treatment of gastrointestinal motility disorders, *Expert Opin. Investig. Drugs*, 2010, **19**(6), 765–775.

21. MEGENS, A.A., F.H. AWOUTERS, C.J. NIEMEGEERS, General pharmacology of the four gastrointestinal motility stimulants bethanechol, metoclopramide, trimebutine, and cisapride, *Arzneimittelforschung*, 1991, **41**(6), 631–634.
22. MILLER, R.D., L.I. ERIKSSON, L. FLEISHER, JEANINE P. WIENER-KRONISH, N.H. COHEN, W.L. YOUNG, *Miller's Anesthesia*, 7.–8. ed., Philadelphia, Churchill Livingstone, Elsevier, 2010.
23. MOORE, N.A., B.J. SARGENT, D.D. MANNING, P.R. GUZZO, Partial agonism of 5-HT3 receptors: a novel approach to the symptomatic treatment of IBS-D, *ACS Chem. Neurosci*, 2013, **4**(1), 43–47.
24. MYLECHARANE, E.J., J.A. ANGUS, I.S. DE LA LANDE, P.P.A. HUMPHREY, *Serotonin*, London, Palgrave Macmillan UK, 1989, p. 217.
25. NAIR, V. PRIYA, JENNIFER M. HUNTER, Anticholinesterases and anticholinergic drugs, *Contin. Educ. Anaesthesia, Crit. Care Pain*, 2004, **4**(5), 164–168.
26. NEMEROFF, C.B., M.J. OWENS, Treatment of mood disorders, *Nat. Neurosci.*, 2002, **5**(Supp), 1068–1070.
27. PEYTON, LAUREN, JOY GREENE, Irritable bowel syndrome: current and emerging treatment options, *P T A Peer-Reviewed J. Formul. Manag*., 2014, **39**(8), 567–578.
28. SEKIZUKA, H., H. NISHIZAKI, M. SUNAGAWA, K. SUGIHARA, S. NAGAI, G. KUDO, H. ISHIYAMA, T. MURAKAMI, Selective depletion of serotonin in the alimentary tract of rats with p-chlorophenylalanine treatment, and the effect of serotonin depletion on its movements and on the response to biogenic amines, *Gastroenterol. Jpn*., 1974, **9**(3), 235–244.
29. SIKANDER, A., S.V. RANA, K.K. PRASAD, Role of serotonin in gastrointestinal motility and irritable bowel syndrome, *Clin. Chim. Acta*, 2009, **403**(1–2), 47–55.
30. SIMMONS, L.K., J. KOESTER, Serotonin enhances the excitatory acetylcholine response in the RB cell cluster of *Aplysia californica*, *J. Neurosci. Off J. Soc. Neurosci*., 1986, **6**(3), 774–781.
31. SUPRENANT, A., Control of the gastrointestinal tract by enteric neurons, *Annu. Rev. Physiol*., 1994, **56**(1), 117–140.
32. TERRY, A.V. JR., J.J. BUCCAFUSCO, W.J. JACKSON, M.A. PRENDERGAST, D.J. FONTANA, E.H.F. WONG, D.W. BONHAUS, P. WELLER, R.M. EGLEN, Enhanced delayed matching performance in younger and older macaques administered the 5-HT_4 receptor agonist, RS 17017, *Psychopharmacology* (Berl)., 1998, **135**(4), 407–415.
33. THOMPSON, A.J., SARAH C.R. LUMMIS, The 5-HT_3 receptor as a therapeutic target, *Expert Opin. Ther. Targets*., 2007, **11**(4), 527–540.
34. VOGEL, H.G., *Drug Discovery and Evaluation: Pharmacological Assays*, Berlin, Springer, 2008, p. 1247.
35. Zelnorm. http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm103223.htm. Accessed at 12.05.2016.