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THE EFFECTS OF CERTAIN SEROTONIN 5-HT$_3$ SUBTYPE RECEPTOR ANTAGONISTS IN A VISCERAL PAIN MODEL – EXPERIMENTAL DATA

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Abstract. Aim: Experimental researches on the effects of certain 5-HT3 serotonin receptor antagonists in visceral pain models in mice. **Material and method:** The experiments were carried out on white Swiss mice (20-25g), divided into 4 groups of 7 animals each, treated intraperitoneally with the same volume of solution, as follows: Group I (Control): distilled water 0,3ml; Group II (OND): ondansetron 1mg/kbw; Group III (TRO): tropisetron 0,5mg/kbw; Group IV (PAL): palonosetron 4μg/kbw. The experimental protocols were implemented according to the recommendations of the committee of research and ethics of the International Association for the Study of Pain. The models of visceral pain consist of writhing test with acetic acid 0,6% and colon inflammation test with capsaicin. Intraperitoneal injection of the irritant agent acetic acid causes a typical stretching response named writhing. Intracolonic administration of capsaicin triggered visceral pain-related nociceptive behavioral manifestations (licking the abdomen, stretching, contractions). In both tests, characteristic behavioral manifestations were observed, scored and the data was statistically analyzed. The data was presented as +/- SD and significance was tested by SPSS for Windows version 13.0 and by ANOVA method, followed by Neumann Keuls test as post hoc. **Results and conclusions:** In our experimental conditions, serotonin 5-HT$_3$ subtype receptor antagonist ondansetron, determined analgesic effects in both writhing test and colon inflammation model. 5-HT$_3$ receptor antagonists- tropisetron and palonosetron did not significantly influence nociceptive responses through chemical peritoneal irritation or colon inflammation.
Keywords: nociception, tropisetron, palonosetron, writhing test, colon inflammation

Introduction

The 5-HT$_3$ receptor antagonists competitively inhibit the binding of serotonin to 5-HT$_3$ receptors. Their antiemetic effects are postulated to stem from the blockade of 5-HT$_3$ receptors located on the nerve terminals of the vagus in the periphery and centrally in the chemoreceptor trigger zone of the area postrema. These drugs have little or no affinity for other serotonin receptors; for alpha or beta-adrenergic receptors; for dopaminergic or for histamine receptors. (Katzung B.G., 2007) (Gralla R.J. et al., 1999)

Literature data shows that various types of serotonin (5-HT) receptors participate in pain perceptions. A lot of experimental researches proved that 5-HT$_3$ and 5-HT$_4$ receptors can play a major role in the transmission of painful stimuli. These receptor subtypes have been localized in many parts of the body including the central nervous system, spinal cord, peripheral neurons on pain transmitting primary afferent fibers and it seems that they are likely to be primarily involved in the local mechanisms mediating the motor and secre-

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tor effects of 5-HT at peripheral level. Supporting evidence is that 5-HT$_3$ receptors are localized on intrinsic sensory neurons and extrinsic sensory nerve fibers that innervate the rat colon, whereas 5-HT$_4$ receptors exist in interneurons and motor and sensory neurons as well as in smooth muscle cells in the intestine of rodents, guinea pigs, and humans. Therefore, these 5-HT receptor subtypes are likely to be primarily involved in local mechanisms mediating the motor and secretor effects of 5-HT at peripheral level. (Pasricha et al., 2006) (Keith H. et al., 2006)

The 5-HT$_3$ antagonists are a class of medications that act as receptor antagonists on the 5-hydroxytryptamine-3 receptor (5-HT$_3$ receptor), a subtype of serotonin receptor found in terminals of the vagus nerve and in certain areas of the brain. (Katzung B.G., 2007)

Ondansetron is a 5-HT**3** antagonist which has been shown to be effective in treating and preventing chemotherapy-induced nausea and vomiting and also in controlling post-operative nausea and vomiting. Its effects are thought to be on both peripheral and central nerves. (Berger A.M. et al., 2005)

Tropisetron - chlorhydrate de 1 H-indole-3-carboxylate de (1R,3r,5S)-8-méthyl-8-azabicyclo[3.2.1] oct-3-yle is a serotonin 5-HT**3** receptor antagonist used mainly as antiemetic for treating nausea and vomiting following chemotherapy, although it has been used experimentally as an analgesic in cases of fibromyalgia. (Muller W. et al., 2004)

Palonosetron is the most effective of the 5-HT$_3$ antagonists in controlling delayed chemotherapy-induced nausea and vomiting (De Leon A., 2006)

So far, there are no data indicating that these two 5-HT$_3$ receptor antagonists entail effects on visceral nociception. This made us choose the research topic in order to study the effects of these serotoninergic agents in visceral pain.

Material and method

Substances: Ondansetron (Zofran – ampoules 4mg/2ml ondansetron dihydrochloride dehydrate, GlaxoSmithKline), tropisetron (Navoban - ampoules with 2mg/2ml tropisetrone chlorhydrate, Novartis), Palonosetron (Aloxi - ampoules 250µg/5ml, Helsinn Birex Pharmaceuticals Ltd., Irlanda). They were dissolved in distilled water, prepared immediately before use and injected in a volume of 0,3 ml. Capsaicin (Sigma Chemical Co), distilled water (Sicomed, Romania). Capsaicin 0,3% (Sigma Chemical Co) was dissolved in 10% ethanol and 90% saline. The control solution was distilled water (Sicomed, Romania).

Animals: Male white Swiss mice (20-25g) were used. Standard laboratory food and tap water were freely available, except during the time of the experiments. The experiment was performed according to the guidelines of the IASP Committee for Research and Ethical Issues. (Zimmermann, 1983)

Procedure: The mice were distributed into 4 groups of 7 animals, treated intraperitoneally with the same volume of solution as follows: Group I (Control): distilled water 0,3ml; Group II (OND): ondansetron 1mg/kbw, Group III (TRO): tropisetron 0,5mg/kbw; Group IV (PAL): palonosetron 4µg/kbw.

The models of visceral pain consist of the writhing test with acetic acid 0,6% and the colon inflammation test with capsaicin. Intraperitoneal administration of irritant chemical stimulus 0.6% acetic acid causes typical stretching responses (abdomen constrictions and full extension of hind limb- named writhes) as a pain reaction. Anti-nociception was recorded by counting the number of writhes after the injection of acetic acid for intervals of 5 minutes during a period of 30 minutes. The antinociceptive effects of the drugs were measured by calculating the mean reduction in the number of abdominal constrictions for each drug, as compared to the control group.

The visceral pain model is represented by chemical stimulation of the mice colon after intracolonic application of an irritant agent capsaicin, followed by pain-related behavioral manifestations of inflammatory visceral pain. (Laird J.M.A. et al., 2001) Prior to the experiment the mice were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room. Using a plastic cannula with a rounded tip (external diameter: 1,6 mm), attached to a 0,1 ml syringe, capsaicin 0,05ml/ mouse (0.3% solution dissolved in 10% ethanol) was administered into the colon 4 cm from the anus in the mouse after application of vaseline in the perianal area to avoid the stimulation of the somatic zones by contact with this irritant substance. Ethanol was used to break the mucosal barrier that normally protects the colon. The spontaneous behavior was observed for 20 min with a gradually decreasing intensity over time. Immediately after the administration of capsaicin the number of visceral pain-related nociceptive behaviors were counted every 5 min-

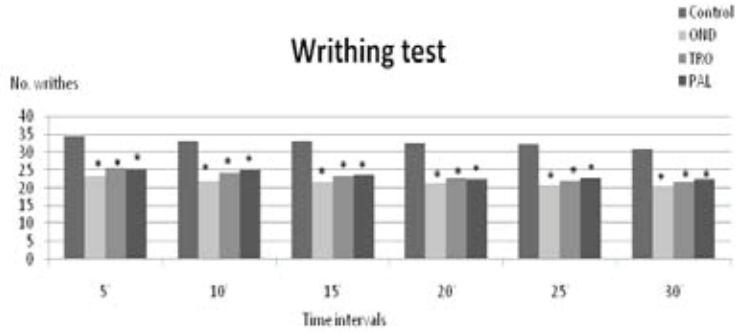

Figure 1. The effects of ondansetron, tropisetron and palonosetron in writhing test (* p<0.05)

utes for 20 minutes. Behavioral manifestations to chemical stimulation of the colon were defined as licking of the abdomen, stretching, squashing of the lower abdomen against the floor, abdominal retractions or contractions. The latency of the first such behavior was noted, as were the number and type of behaviors displayed. (Kamp E.H. et al., 2003) (Tarțău L., 2008)

In both tests, the values corresponding to each time period were statistically processed using SPSS for Windows version 13.0 and ANOVA method, followed by Neumann Keuls test as post hoc. P- values under 0,05 are considered statistically significant by comparison to those of the control.

Results

The results obtained in the writhing test are presented in **figure 1**.

Statistical analysis of the results obtained in the writhing test shows that:

- intraperitoneal administration of ondansetron (1mg/kbw) resulted in the gradual decrease of the number of writhes immediately after acetic acid injection, statistically significant (* p<0.05), compared with the control group in all time intervals of the experiment.
- in this experimental visceral pain model, both 5-HT_3 serotonin receptor antagonists decreases the number of behavioral manifestations as animals' response reaction to chemical peritoneal irritation, statistically significant (* p<0.05) compared with the control group, but less intense than those of ondansetron.

The results obtained for the colon inflammation test with capsaicin are presented in **figure 2**.

Statistical analysis of the results obtained in capsaicin induced colon inflammation shows that:

- in all observation intervals the 5-HT_3 antagonist ondansetron (1mg/kbw) decreased the number of painful behavioral manifestations due to colon chemical inflammation, statistically significant (* p<0.05) compared with the control group.
- intraperitoneal administration of tropisetron (0,5mg/kbw) or palonosetron (4μg/kbw) resulted in a tendency of reducing the behavioral modifications, but the influence on the hyperalgesic manifestations was not statistically significant compared with the control group.

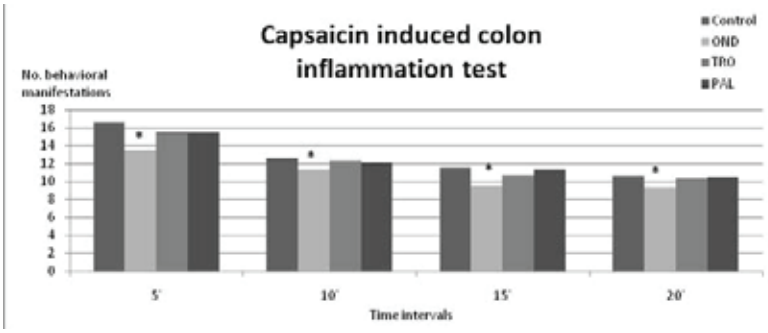

Figure 2. The effects of ondansetron, tropisetron and palonosetron in capsaicin induced colon inflammation test (* p<0.05)

Discussions

The finding of diversity and of sometimes controversial data regarding the influences of serotoninergic receptor antagonists on nociceptive processes was the reason that determined us to study the effects of 5-HT$_3$ receptor antagonists in visceral pain. Several studies have investigated the effects of different 5-HT$_3$ serotonin receptor antagonists on cutaneous and visceral nociception.

In one experiment the effects of the following 5-HT$_3$ antagonists were studied: granisetron, cilansetron and KC9946 on contractile responses of the colon and, as an index of nociception, the abdominal muscles to colorectal balloon distension before and during rectocolitis induced by rectal instillation of trinitrobenzene sulfonic acid. Thus, cilansetron and granisetron reduced the distension-elicited inhibition of the colonic contractile activity (or enhanced the activity so that the inhibition was less pronounced) but did not affect the nociceptive abdominal responses. (Morteau O. et al., 1993)

Further, it has been demonstrated that granisetron, ICS 205-930 and ondansetron administration resulted in antinociceptive action on the visceral pain reflex induced by duodenal distension in rats. (Moss H.E. et al., 1990)

Ondansetron also determined the potentiation of the analgesic effect of tramadol in a visceral pain model in mice. (Dürsteler C et al, 2006)

Another study in Wistar rats investigated the nociceptive responses of the abdominal muscles to the instillation of glycerol into the colon. The abdominal muscle responses were reduced by cilansetron ip in a dose- dependent manner. Cilansetron, granisetron and ondansetron were all effective ip and iv, but also intracolonically (ic), which suggests a local site of action. (Botella A. et al., 1998)

The effects of some 5-HT$_3$ receptor antagonists were also investigated in a visceral pain model consisting of intracolonic administration of glycerol. Contractions of the abdominal muscles, produced by irritant local action of chemical stimulus, represent indicatives of visceral nociception. Cilansetron, granisetron and ondansetron dose-dependently reduced the abdominal response, in both intravenous and intracolonic administration. (Botella A. et al., 1998)

A clinical trial showed that topical administration of ondansetron attenuated nociceptive and inflammatory effects of intradermal capsaicin in humans. (Giordano J. et al., 1998)

Another clinical study proved the efficacy of 5-HT$_3$ antagonist alosetron in the management of pain and gastrointestinal disturbances in patients with irritable bowel syndrome. (Cremonini F. et al., 2003)

There are also clinical studies that demonstrate the efficacy of tropisetron pain attenuation in patients with fibromyalgia. (Papadopoulos I.A. et al., 2000)

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