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# IDENTIFICATION OF SEROTONERGIC ($5H_{1A}$-TYPE) RECEPTORS IN BROILER SMALL INTESTINE BY APPLICATION OF SEROTONIN AND ITS AGONISTS AND ANTAGONISTS*

## *UTVRĐIVANJE SEROTONERGIČNIH RECEPTORA $5HT_{1A}$ TIPA U TANKOM CRIJEVU BROJLERA PRIMJENOM SEROTONINA I NJEGOVIH AGONISTA I ANTAGONISTA*

**Indira Mujezinović, V. Ćupić, A. Smajlović, M. Muminović****

*Serotonin or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter synthesised from L-tryptophan in serotonergic neurons and enterochromaffin cells of the gastrointestinal tract. This neurotransmitter is widely distributed in the animal and plant kingdom and regulates some central and peripheral functions through several types of specific serotonergic (5-HT) receptors. Since it is known that the effect of serotonin, especially in pathological conditions, is very important, we believe that determining the types of receptors for this substance would make it possible to use their agonist or antagonists, which would undoubtedly enhance the pharmacotherapy of functional disruption of the small intestine in broilers.*

*Investigations were carried out on isolated smooth muscle strips of the circular and longitudinal layer of the broiler small intestine (strip dimension 3-4 mm x 2 cm). The muscle strips were placed in an isolated organ bath. The mechanical activity of the preparations was recorded via an isotonic force transducer coupled to a pen recorder. This was done following the addition of serotonin (nonselective 5-HT agonist), 8-OH-DPAT (selective 5-$HT_{1A}$ agonist) and spiroxatrin (selective 5-$HT_{1A}$ antagonist). The sensitivity of the tissues to acetylcholine was tested before starting the experiments. Using the obtained results, it can be concluded that $5HT_{1A}$ type receptors are present in smooth*

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*muscles of the broiler small intestine, duodenum and ileum, especially in the longitudinal smooth muscle layer which reacted with contractions even to low serotonin concentration ($10^{-6}$), but not in the jejunum.*

*Key words: broilers, serotonin, serotonergic receptors, isolated smooth muscle, small intestine*

## Introduction / *Uvod*

Serotonin or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter synthesized from L-tryptophan in serotonergic neurons and enterochromaffin cells of the gastrointestinal tract. This neurotransmitter is widely distributed in the animal and plant kingdom. It occurs in vertebrates, tunicates, molluscs, arthropods and colelenterates and in edible fruits and nuts. It is also present in numerous venoms, includings those of the common stinging nettle and wasps and scorpions. About 95% of the serotonin in the body is found in the gastrointestinal tract, where it has been estimated that the total serotonin content is about 10 mg, 90% is in enterochromaffin cells and 10% in enteric neurons. The rest of 5-HT (5%) is found in the brain. Virtually all of the serotonin in the blood is derived from the GI tract. Serotonin is present in the enteric nervous system. Serotonergic neurons constitute about 2% of all myenteric neurons. Serotonin is released from the bowel when enteric nerves are stimulated. Serotonin is also an important constituent of platelets and coagulation of blood (Brunton, 2006; Rang *et al.*, 2007; Riviere and Papich, 2009).

In the gut, serotonin is released from enterochromaffin cells. It has diverse motor and sensory function in the GI tract through submucosal and myenteric neurons that respond to 5-HT initiated responses as diverse as nausea, vomiting, intestinal secretion and peristalsis, and plays a role in bowel physiology as an enteric neurotransmitter.

Serotonin produces its action by the effect on specific serotonergic (5-HT) receptors which are present in enteric neurons, enterochromaffin cells, GI smooth muscle and possibly on enterocytes and immune tissues. Seven types or families and multiple subtypes of 5-HT receptors have now been identified by a combination of pharmacological techniques and molecular cloning. The seven recognized types or families of 5-HT receptors are termed as 5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, 5-$HT_5$, 5-$HT_6$ and 5-$HT_7$. The last three receptors (5-$HT_5$, 5-$HT_6$ and 5-$HT_7$) are distributed predominantly in the brain (Borne, 1994; Brunton, 2006; Meneses, 1998; Pauwels, 2003).

Many studies have indicated the involvement of various subtypes of 5-HT receptors in gastronitestinal motility and secretion regulation. Kojima *et al*. (1992), Meulemans *et al*. (1993) and Takemura *et al*. (1999) have shown that 5-$HT_1$ receptors mediate relaxation in guinea-pig proximal stomach *in vitro*. 5-$HT_7$ receptors are found to mediate relaxation in both distal (Prins *et al*., 2001) and proxi-

mal (Janssen *et al*., 2002) parts of the canine stomach. Data from *in vivo* studies have also indicated that 5-$HT_1$ receptors mediate relaxation, since sumatriptan (a 5-$HT_1$ receptor agonist) relaxed the proximal stomach of the cat (Coulie *et al*., 1999) and man (Tack *et al*., 2000). To date, it is not known what mechanism underlies this effect linked to 5-$HT_1$ receptors. Takemura *et al*. (1999) demonstrated that the 5-HT-induced contraction of guinea-pig fundus strips were antagonized by ketanserin, suggesting 5-$HT_{2A}$ receptor involvement. 5-$HT_{2A}$ receptors were also found to mediate contraction in the canine proximal stomach (Janssen *et al*., 2002). However, rat gastric fundus contraction is mediated by 5-$HT_{2B}$ receptors (Baxter *et al*., 1994). 5-$HT_3$ receptors mediate stomach contraction in the guinea-pig *in vitro* (Buchheit and Buhl, 1994). In conscious dogs, m-chlorophenylbiguanide (a selective 5-$HT_3$ receptor agonist) stimulates antral motility (Nagakura *et al*., 1997). 5-$HT_4$ receptor agonists potentiate electrically evoked contractions in the longitudinal muscle of dog gastric corpus (Prins *et al*., 2001) and the circular muscle of the guinea-pig gastric fundus and corpus (Hegde and Eglen, 1996). Kitazawa *et al*. (2006) have shown that 5-HT causes contraction of the proventriculus via 5-$HT_{2C}$-like receptors present on the smooth muscle and contraction of the ileum in the chicken.

Since it is known that the effect of serotonin, especially in pathological conditions, is very important, we believe that determining the types of receptors for this substance is just as important since it would allow the use of their agonist or antagonists, which would undoubtedly enhance the pharmacotherapy of functional disruption of the small intestine in broilers. If we add that disturbances of bowel function are common in broilers, and if we know that this mentioned disfunction causes great damages to the poultry industry due to the high mortality, the results of this research could form the basis for the introduction of new drugs in the pharmacotherapy of this species.

## Material and methods / *Materijal i metode rada*

During the experiment the following drugs were used: acetylcholine, 5-hydroxytriptamine creatinine sulphate (non selective 5-HT agonist), 8-OH-DPAT hydrobromide (selective 5-$HT_{1A}$ agonist) and spiroxatrin (selective 5-$HT_{1A}$ antagonist). All substances were obtained from the Sigma Chemical Co. (Germany) and Tocris Cookson Ltd., (Bristol, UK). The material used in the experiment was the small intestine of poultry (broilers) strain Cobb 500 taken at the time of slaughter at a private poultry slaughterhouse. The gut was dissected and immediately placed in cold, freshly prepared Kreb's bicarbonate solution (composition in mM: NaCl 118,4; KCl 4,7; $CaCl_2$ 2,5; $MgSO_4$ 1,2; $NaHCO_3$ 25; $KH_2PO_4$ 1,2 and glucose 11,5; pH 7,3-7,4) and transferred to the laboratory for isolated organs. The experiments were performed with the smooth muscle of the circular and longitudinal layer from the broiler small intestine – duodenum, jejunum and ileum (strip dimension 3-4 mm x 2 cm). Segments were placed in a 10 ml bath for isolated organs filled with

Krebs bicarbonate solution, gassed with 95% $O_2$ and 5% $CO_2$ and maintaned at cca 40 °C. Tissues were suspended under a resting tension of 2 g and were allowed to equilibrate for 45-60 minutes. The mechanical activity of the preparations was recorded via an isotonic force transducer (Ugo Basile, 7003, Italy) coupled to a pen recorder (Ugo Basile, 7050). All strips were studied on the day of preparation. The sensitivity of the tissues to acetylcholine ($10^{-5}$ M) was tested before starting the experiments. All experiments were repeated on five to six different preparations obtained from different animals. Segments of the small intestine were incubated with serotonin, and its $5HT_{1A}$ agonist - 8-OH-DPAT hydrobromide, for 1 minute and the time between two applications was 20 minutes. Antagonist spiroxatrin was added to the bath 3-4 minutes before the agonists.

## Rezultati / *Results*

Results are expressed as percentages of the maximum response (expressed as 100%) produced by acetilcholyne on the longitudinal layer of the smooth muscle of broiler duodenum with a concentration of $10^{-5}$ M.

Serotonin (at a concentration of $10^{-7}$ to $10^{-3}$ M) and its analogue 8-OH-DPAT, (at a concentracion of $10^{-7}$ to $10^{-4}$ M) induced dose-dependent contraction in the isolated longitudinal layer of the smooth muscle of the duodenum and ileum of broilers. Neither the circular nor the longitudinal layer of the smooth muscle of the jejunum had significant results. The circular layer of the smooth muscle of the small intestine (duodenum and ileum) showed lower sensitivity to histamine and its agonist betahistine in equal concentrations as in the longitudinal layer (Fig. 1 and 2).

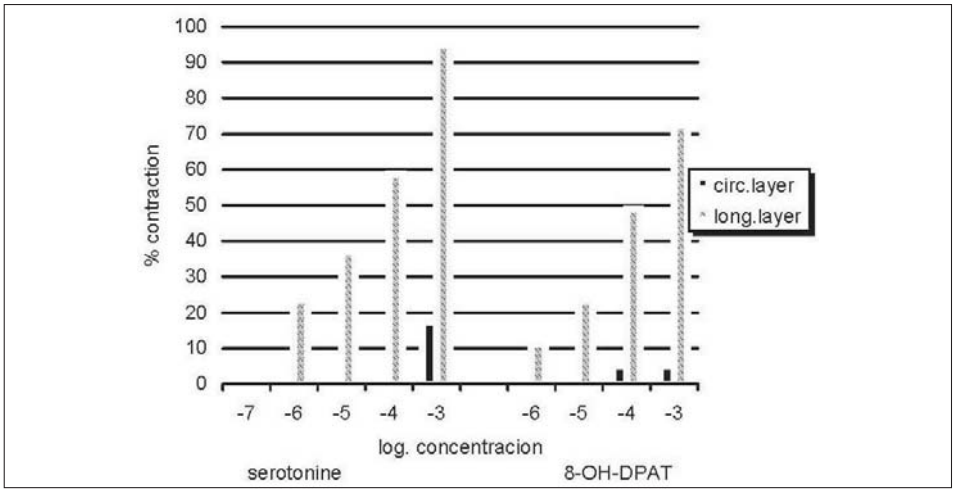


Figure 1. *Effect of various concentrations of 5-HT and 8-OH-DPAT, on circular and longitudinal layer of smooth muscles of broiler duodenum. Contractions were expressed as percentage of muscle strip preparation to acetylcholine ($10^{-5}$ M)*

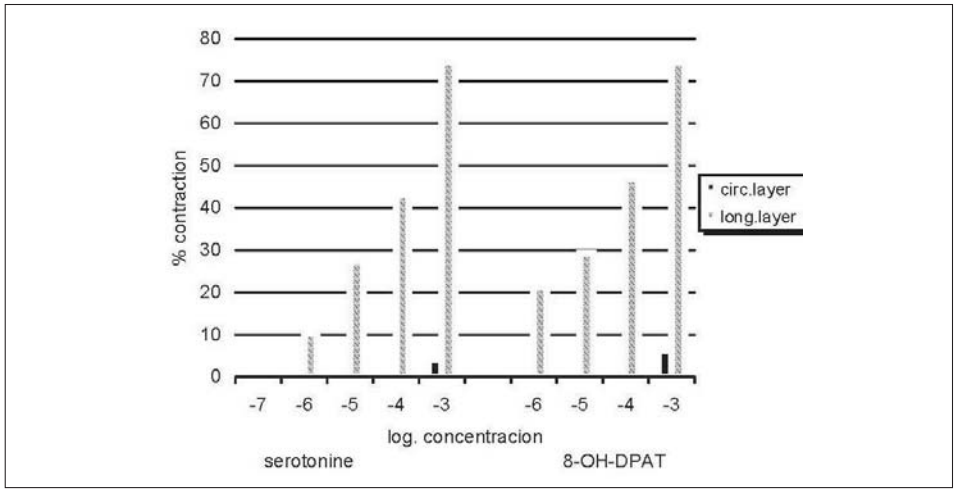


Figure 2. *Effect of various concentrations of 5-HT and 8-OH-DPAT on circular and longitudinal layer of smooth muscles of broiler ileum. Contractions were expressed as percentage of muscle strip preparation to acetylcholine ($10^{-5}$ M)*

In the experiment designed to investigate effects of antagonists of 5-HT receptors, spiroxatrin (selective 5-$HT_1$ antagonist) was added in a concentration of $10^{-7}$ M to $10^{-4}$ M) to antagonized 8-OH-DPAT-induced contraction at a concentration of $10^{-3}$ M (Fig. 3 and 4).

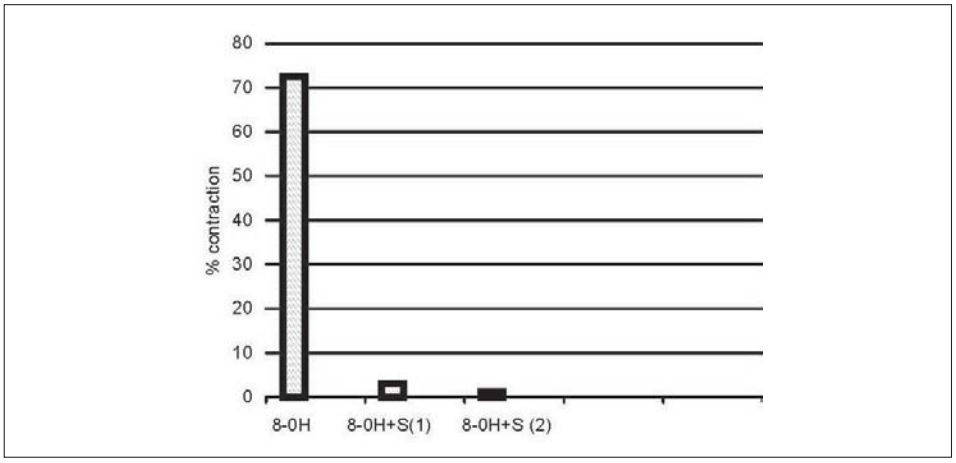


Figure 3. *Contractile effect of 8-OH-DPAT (8-OH) on smooth muscles of duodenum alone and in presence of spiroxatrin (8-OH+S) in concentration of $10^{-6}$ M (1) and $10^{-5}$ M (2)*

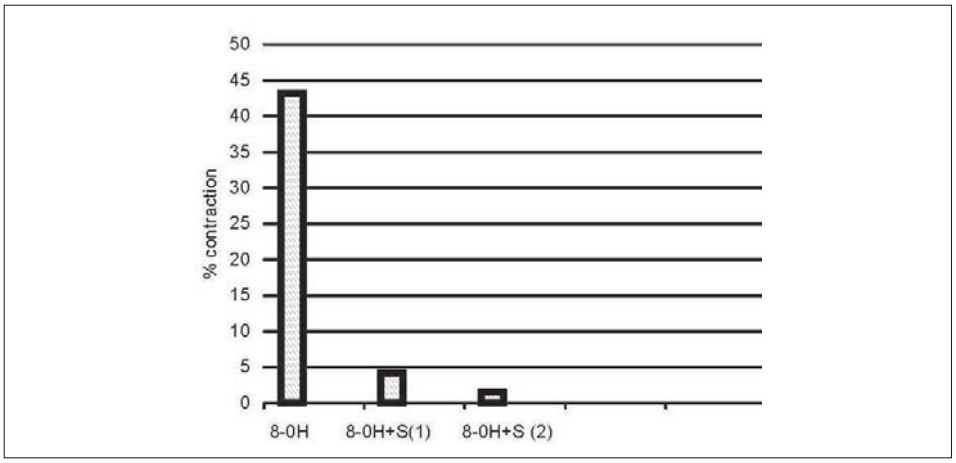


Figure 4. *Contractile effect of 8-OH-DPAT (8-OH) on smooth muscles of ileum alone and in presence of spiroxatrin (8-OH+S) in concentration of $10^{-6}$ M (1) and $10^{-5}$ M (2)*

## Discussion and conclusion / *Diskusija i zaključak*

Between 80 and 90% of the total content of serotonin in the body is located in the gastrointestinal tract, i.e enterochromaffin cells isolated from the stomach and intestines (Rang *et al*. 2007; Riviere and Papich, 2009). It is peripheral and the most evident effect of serotonin on smooth muscles of the gastrointestinal tract (Born, 1994, Rang *et al*., 2007). Serotonin, among other substances, causes contractions of smooth muscles of the bovine rumen (Muminović *et al*., 2000), regulates the motility of the digestive system, enhances secretion, etc. (Brunton, 2006; Hegde and Eglen, 1996; Rang *et al*., 2007). A number of authors tried to identify the 5-$HT_1$ receptors subtypes in isolated smooth musclse of organs from the gastrointestinal tract, such as the bovine rumen (Muminović *et al*., 2000), the porcine and human small intestine (Schworer and Ramadori, 1998), the rabbit ileum (Turabi *et al*.,2006), the guinea-pig ileum (Buccheit and Buhl, 1993) etc., but not in smooth muscles of the broiler small intestine.

In our experiments, the circular layer of the smooth muscle of the broiler small intestine (duodenum and ileum) showed considerably lower sensitivity to serotonin and 8-OH-DPAT used in equal concentrations as in the longitudinal layer. Neither the circular nor the longitudinal layer of the smooth muscle of the jejunum had significant results.

In the first part of our experiment we used serotonin (non-selective 5-HT agonist) in concentrations from $10^{-7}$ to $10^{-3}$ M. Considering the obtained results, it can be concluded that serotonergic receptors are highly present in smooth muscles of the circular and longitudinal layer of the broiler small intestine.

With the increase in concentration, we obtained responses in the form of stronger (higher) intensity contractions.

In the next part of our experiment, we tried to determine the presence of $5HT_1$ type receptor in intestinal smooth muscles of broilers. We used 8-OH-DPAT (selective $5HT_{1A}$ agonist) in concentrations from $10^{-7}$ to $10^{-3}$ M. In this *in vitro* study, 8-OH-DPAT evoked dose-dependent contractions on circular and longitudinal layers of the smooth muscle of the broiler small intestine (duodenum and ileum). Longitudinal muscles of the duodenum displayed about 15-25 % lower sensitivity to the same concentration than to serotonin, but longitudinal muscles of the ileum displayed the same sensitivity as to serotonin.

The 8-OH-DPAT effects (in a concentration of $10^{-3}$ M) on the longitudinal layer of the duodenum and ileum was blocked by spiroxatrin (selective $5HT_1$ antagonist) in concentrations of $10^{-6}$ and $10^{-5}$ M. Considering the obtained results, it can be concluded that a $5HT_{1A}$ type of receptor is present in the smooth muscle of both layers of the broiler duodenum and ileum, but not in the jejunum.

By summarizing all the effects in this assay, it can be concluded that $5HT_{1A}$ type receptors are present in smooth muscles of the broiler small intestine (duodenum and ileum), especially in longitudinal smooth muscles since this layer reacted with contractions even to low serotonin concentration ($10^{-6}$). In the light of these findings, we suggest that the investigated substances may have considerable physiological and therapeutic implications in disturbed functions of the small intestine of broilers.

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## SRPSKI

### UTVRĐIVANJE SEROTONERGIČNIH RECEPTORA $5H_{1A}$ TIPA U TANKOM CREVU BROJLERA PRIMENOM SEROTONINA I NJEGOVIH AGONISTA I ANTAGONISTA

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Serotonin ili 5-hidroksitriptamin (5-HT) je monoaminski neurotransmiter kojeg iz L-triptofana sintetišu serotonergični neuroni i enterohrmofine ćelije gastrointestinalnog trakta. Ovaj neurotransmiter se nalazi kod velikog broja životinja i biljaka i reguliše neke centralne i periferne funkcije posredsvom nekoliko tipova specifičnih serotonergičnih (5-HT) receptora. Pošto je poznato da je efekat serotonina,posebno u patološkim uslovima, veoma značajan, mi mislimo da određivanje tipa receptora za ovu supstancu može učiniti mogućom upotrebu njenih agonista ili antagonista, što bi nedvosmisleno povećalo farmakoterapiju poremećaja funkcije tankog creva kod brojlera.

Istraživanja su izvršena na izolovanim trakama glatkih mišića kružnog i uzdužnog sloja tankog creva brojlera (dimenzije trake 3-4 mm x 2 cm). Trake mišića su bile stavljane u kupatila za izolovane organe. Mehanička aktivnost preparata je registrovana pomoću izotoničnog transdjusera povezanog sa štampačem. Registrovanje je vršeno posle dodavanja serotonina (neselektivni 5-HT agonist), 8-OH-DPAT (selektivni agonist) i spiroksatrina (selektivni 5-$HT_{1A}$ antagonist). Osetljivost tkiva na acetilholin testirana je pre početka eksperimenta. Imajući u vidu dobijene rezultate može se zaključiti da su receptori $5HT_{1A}$ tipa prisutni u glatkim mišićima tankog creva brojlera, duodenumu i ileumu, posebno u uzdužnom sloju glatkih mišića koji je reagovao kontrakcijama čak i na niske koncentracije serotonina ($10^{-6}$), ali ne i u jejunumu.

Ključne reči: brojler, serotonin, serotonergički receptori, izolovni glatki mišić, tanko crevo

## РУССКИЙ

### УТВЕРЖДЕНИЕ СЕРОТОНЭРГИЧНЫХ РЕЦЕПТОРОВ $5H_{1A}$ ТИП В ТОНКОЙ КИШКЕ БРОЙЛЕРОВ ПРИМЕНЕНИЕМ СЕРОТОНИНА И ЕГО АГОНИСТОВ И АНТАГОНИСТОВ

**Индира Муезинович, В. Чупич, А. Смайлович, М. Муминович**

Серотонин или 5-гидрокситриптамин (5ГТ) монаминский нейротрансмитер, которого из Л-триптофана синтезируются серотонэргичные нейроны и энтерохроматонкие клетки гастроинтестинального тракта. Этот нейротрансмиттер находится у большого числа животных и растений и регулирует некоторые центральные и периферические функции посредством несколько типов специфических серотонэргичных (5-ГТ) рецепторов. Так как известно, что эффект серотонина, отдельно в патологических условиях, очень значительный, мы думаем, что определение типа рецептора для этой субстанции может сделать возможной употребление её агонистов или антагонистов, что бы недвусмысленно увеличивало фармакотерапию расстройств функции тонкой кишки у бройлеров.

Исследования совершены на изолированных лентах гладких мышц кружного и продольного слоя тонкой кишки бройлеров (размеры ленты 3-4 мм х 2 цм). Ленты мышц были кладываны в ванные для изолированных органов. Механическая активность препарата регистрирована с помощью изотонического трасдюсера, связанного с печатателем. Регистрация совершена после добавления серотонина (неселективный 5-ГТ агонист), 8-OH-DPAT (селективный агонист) и спироксатрин (селективный 5-$ГТ_{1A}$ антагонист). Чувствительность ткани на ацетилхолин тестирована до начала эксперимента. Имея в виду, полученные результаты можно сделать вывод, что рецепторы $5ГТ_{1A}$ типа присутствующие в гладких мыщах тонкой кишки бройлеров, двенадцатиперстной кишке подвздошной кишке, отдельно в продольном слое гладких мышц, реагированный контракциями даже и на низкие концентрации серотонина ($10^{-6}$), но не в тощей кишке.

Ключевые слова: бройлеры, серотонин, серотонэргические рецепторы, изолированная гладкая мышца, тонкая кишка