

**IDENTIFICATION OF HISTAMINIC (H₁-TYPE) RECEPTORS
IN SMALL INTESTINE OF BROILERS BY APPLICATION OF
HISTAMINE AND SOME OF ITS AGONISTS AND
ANTAGONISTS***

***UTVRĐIVANJE HISTAMINSKIH RECEPTORA H₁ TIPRA U TANKOM
CREVU BROJLERA PRIMENOM HISTAMINA I NEKIH NJEGOVIH
AGONISTA I ANTAGONISTA***

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Histamine is a biologically active amine (biogenic amine) that has a broad spectra of physiologic and pathologic reactions in the organism. Its effects are shown through 4 types of specific receptors (H₁, H₂, H₃ and H₄). Histamine is one of the main causes of intestine disorders and the occurrence of diarrhoea, both of which are very common in broilers. Whilst there is no information in scientific literature about the presence of histaminic receptors in smooth muscles of the small intestine wall of broilers (duodenum, jejunum and ileum), we tried to determine their presence, distribution and type in this kind of muscles. Investigations were carried out on isolated smooth muscles of the circular and longitudinal layer of the broiler small intestine (strip dimension 3-4 mm x 2 cm). The muscle strip was then placed in an isolated organ bath and the contractions obtained were registered with isometric transducers on a two-channel printer.

This was done following the addition of histamine, betahistine (H₁ agonist), and mepiramine (H₁ antagonist). Muscle vitality was checked by adding acetylcholine chloride. Using the obtained results, it can

* Rad primljen za štampu 20. 05. 2010. godine

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be concluded that H₁ types of histaminergic receptors are present in smooth muscles of the small intestine of broilers.

Key words: broilers, histamine, histaminic receptors, isolated smooth muscles, small intestine

Introduction / Uvod

Histamine is a basic amine, 2-(4-imidazolyl)-ethyl-amine and its formed from the decarboxylation of the amino acid histidine by a specific enzyme histidine decarboxylase. This enzyme is present in all cell types that contain histamine. Histamine is found in most mammalian tissues, but it is present in a high concentration in the lung, in the skin and in the gastrointestinal tract. At the cellular level, it is found largely in mast cells and basophils, associated with heparin (Adams, 2001; Hadžović and Muminović, 2001), and histaminocytes (non-mast-cell histamine) which occurs in the mucosa of the stomach and in histaminergic neurons in the brain. Histamine has a broad spectra of physiologic and pathologic reactions in the organism. In the presence of histamine different animals have a different reaction. This amine is released from mast cells by exocytosis during inflammatory or allergic reactions, and it is involved in inflammation, anaphylaxis, allergies, it regulates gastric secretion and has a role as a neurotransmitter (Hardmann and Limbird, 2001; Leurs and Timmerman, 2005; Rang *et al.*, 2007).

Histamine produces its action by the effect on specific histamine (H) receptors, which are of the four main types H₁, H₂, H₃ (Ćupić *et al.*, 2007; Hadžović, 2000; Hill *et al.*, 1997; Ramirez, 2003) and H₄ (Hardmann and Limbird, 2001; Rang *et al.*, 2007), and these receptors are present all over the body. H₁-receptors are distributed in a wide variety of tissues including: mammalian brain, smooth muscle from the airways, the gastrointestinal, the genitourinary and the cardiovascular system, adrenal medulla and endothelial cells and lymphocytes (Hadžović and Muminović, 2001; Hill *et al.*, 1997; Ramirez, 2003), and they have a role in allergy (Ramirez, 2003). H₂ receptors are present in the brain, gastric cells, cardiac tissue and in cells of the immune system. Histamine H₂-receptors have a potent effect on gastric acid secretion (Charbon, 1980, Ćupić *et al.*, 2007; Rang *et al.*, 2007). H₂-receptor-mediated smooth muscle relaxation has been documented in airways (Hemming *et al.*, 2000). H₃ receptors have been found in the lung, in several brain regions, (Ćupić *et al.*, 2007; Rang *et al.*, 2007), in the cardiovascular system, in mast cells of the stomach and enterochromaffin cells of the small intestine (Hill *et al.*, 1997). H₄ shows the highest expression in the brain and nervous tissues, in peripheral blood, in the bone marrow and in leukocytes, and moderate expression in spleen, thymus, lung, small intestine, colon and heart (Gantner *et al.*, 2002; Zhu *et al.*, 2001).

Histamine appears to be one of the major causes of disturbances of normal intestine function and diarrhoea. This is very often the case in broiler

chicken. The lack of knowledge of the existence of H₁ receptors types in the smooth muscle of the broiler small intestine induced us to try to establish their presence in this tissue using H₁ agonists and antagonists. Our opinion is that the obtained results could be taken as a basis for possible introduction of novel officiant drugs in the therapy of disturbed function of the broiler intestine.

Material and methods / Materijal i metode rada

Acetylcholine, histamine (nonselective agonist of H receptors), betahistine (selective H₁ agonist) and mepiramine (H₁ antagonist) were obtained from the Sigma Chemical Co. (Germany). Poultry (broilers) were obtained from private farms in Bosnia and Herzegovina and acclimatized for about 2-3 days, with food and water available *ad libitum*.

The experiments were performed with the smooth muscle of the circular and longitudinal layers from the broiler small intestine (strip dimension 3-4 mm x 2 cm). Segments were placed in a 10 ml bath for isolated organs filled with Krebs bicarbonate solution (composition in mM: NaCl 118,4; KCl 4.7; CaCl₂ 2.5; MgSO₄ 1.2; NaHCO₃ 25; KH₂PO₄ 1.2 and glucose 11.5; pH 7.3-7.4), gassed with 95% O₂ and 5% CO₂ and maintained at cca 40 °C. Tissues were suspended under a resting tension of 2 g and were allowed to equilibrate for 45-60 minutes. Contractions were recorded with an isotonic force transducer (Ugo Basile, 7003, Italy) coupled to a pen recorder (Ugo Basile, 7050). The sensitivity of the tissues to acetylcholine (10⁻⁶ M) was tested before starting the experiments. All experiments were repeated on five to six different preparations obtained from different animals. Segments of small intestine were incubated with histamine, and its agonists, betahistine and dimaprite-dihydrochloride for 1 minute and the time between two applications was 20 minutes. The antagonist mepiramine was added to the bath 3-4 minutes before the agonists.

Results / Rezultati

Results are expressed as percentages of the maximum response (expressed as 100%) produced by histamine on the longitudinal layer of the smooth muscle of broiler duodenum with a histamine concentration of 10⁻³ M.

Histamine (in concentrations of 10⁻⁷ to 10⁻³ M) and betahistine (in concentrations of 10⁻⁵ to 10⁻³ M) induced dose-dependent contraction in the isolated broiler small intestine (duodenum, jejunum and ileum). The circular layer of the smooth muscle of the small intestine showed lower sensitivity to histamine and its agonist betahistine in equal concentrations as in the longitudinal layer (Figures 1, 2 and 3).

In the experiment designed to investigate the effects of the antagonist of H₁ receptor type mepiramine (in concentrations of 10⁻⁶ M and 10⁻⁵ M) antagonized betahistine-induced contraction at a concentration of 10⁻³ M (Figure 4).

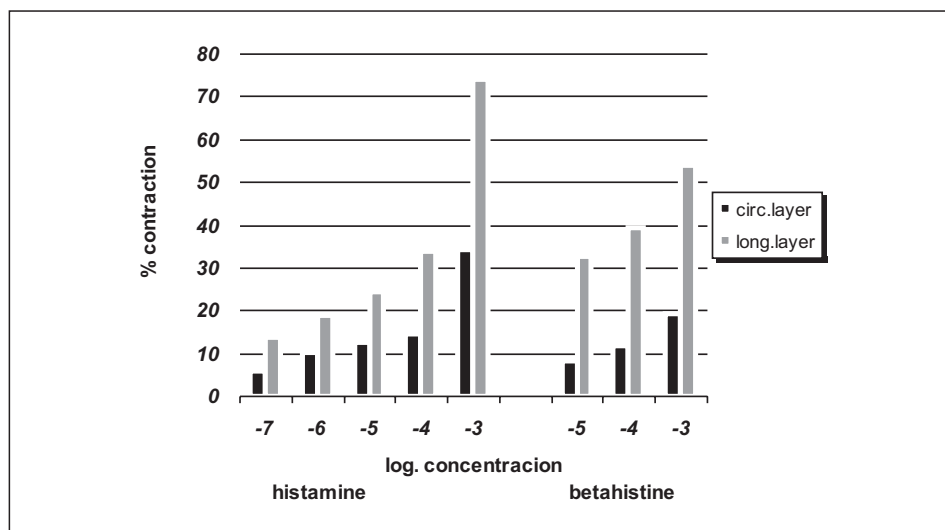


Figure 1. Effect of various concentrations of histamine (10^{-7} - 10^{-3} M) and betahistine (10^{-5} - 10^{-3} M) on circular and longitudinal layer of smooth muscles of broiler duodenum / Slika 1. Uticaj različitih koncentracija histamina (10^{-7} - 10^{-3} M) i betahistina (10^{-5} - 10^{-3} M) na cirkularni i longitudinalni sloj glatkih mišića u duodenumu brojlera

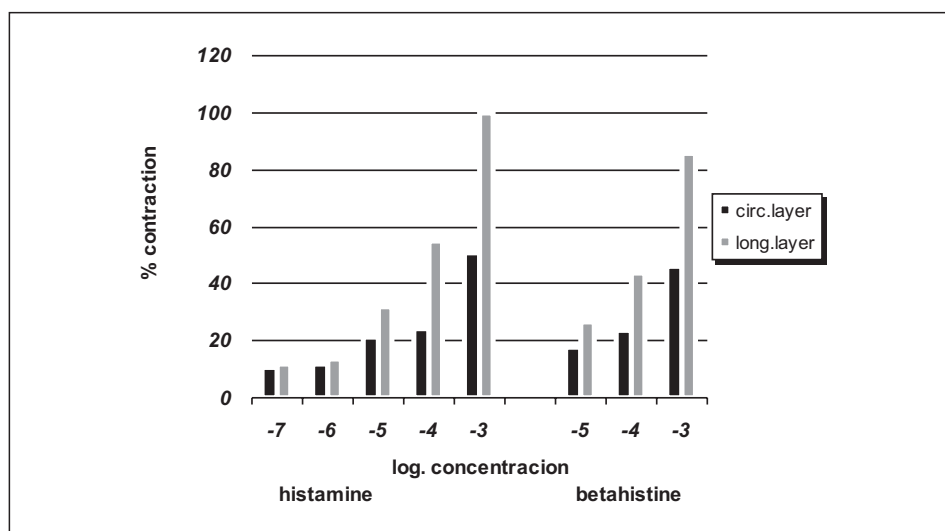


Figure 2. Effect of various concentrations of histamine (10^{-7} - 10^{-3} M) and betahistine (10^{-5} - 10^{-3} M) on circular and longitudinal layer of smooth muscles of broiler jejunum / Slika 2. Uticaj različitih koncentracija histamina (10^{-7} - 10^{-3} M) i betahistina (10^{-5} - 10^{-3} M) na cirkularni i longitudinalni sloj glatkih mišića u jejunumu brojlera

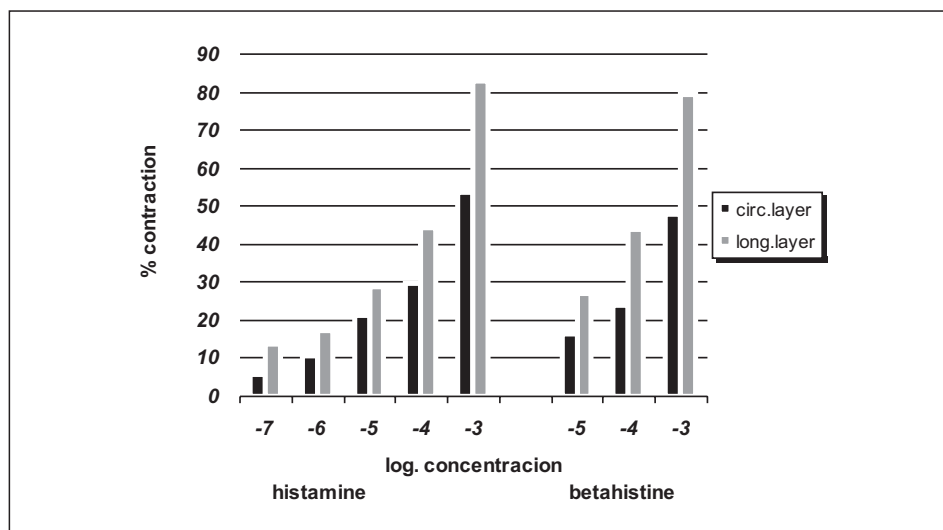


Figure 3. Effect of various concentrations of histamine (10^{-7} - 10^{-3} M) and betahistine (10^{-5} - 10^{-3} M) on circular and longitudinal layer of smooth muscles of broiler ileum /
Slika 3. Uticaj različitih koncentracija histamina (10^{-7} - 10^{-3} M) i betahistina (10^{-5} - 10^{-3} M) na cirkularni i longitudinalni sloj glatkih mišića ileuma brojlera

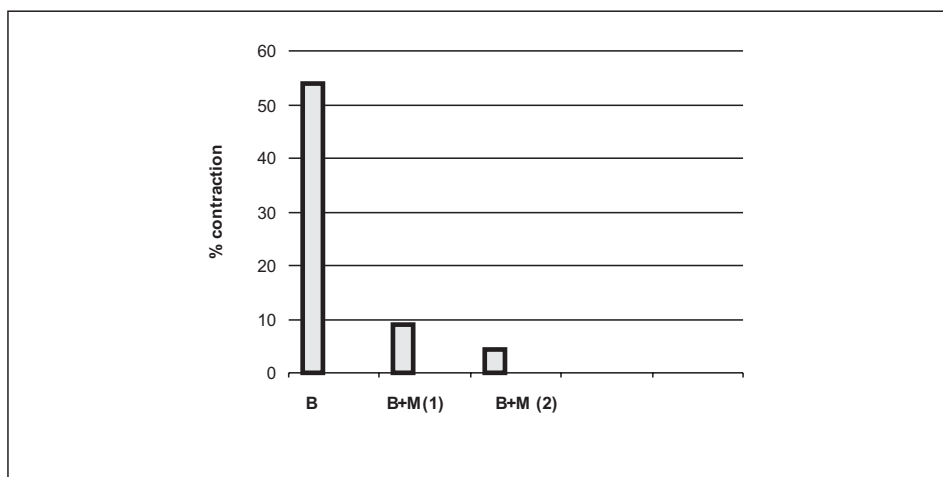


Figure 4. Contractile effect of betahistine (B) on smooth muscles of duodenum alone and in presence of mepiramine (B+M) in concentrations of 10^{-6} M (1) and 10^{-5} M (2) /
Slika 4. Kontraktilni efekat samog betahistina (B) na glatke mišiće duodeuma i u prisustvu mepiramina (B+M) u koncentracijama od 10^{-6} M (1) i 10^{-5} M (2)

Discussion and Conclusion / *Diskusija i zaključak*

In our experiments, the circular layer of the smooth muscle of the broiler small intestine (duodenum, jejunum and ileum) showed considerably lower sensitivity to histamine and betahistine used in equal concentrations as in the longitudinal layer.

In the first part of our experiment we used histamine (non-selective H agonist) in concentrations from 10⁻⁷ to 10⁻³ M. Using the obtained results, it can be concluded that histaminergic receptors are highly present in the smooth muscle in circular and longitudinal layers of the broiler small intestine. With the increase in concentration, we obtained responses in the form of stronger (higher) intensity contractions, which shows the presence of a dose-response relationship.

In the next part of our experiment, we attempted to find which kind of type H receptors are present in the smooth muscle of circular and longitudinal layers of the broiler duodenum. We used betahistine (H₁ agonist) in concentrations from 10⁻⁵ to 10⁻³ M. In this *in vitro* study, betahistine evoked dose-dependent contractions on the circular and longitudinal layers of the smooth muscle of the broiler small intestine (duodenum, jejunum and ileum). Longitudinal muscles displayed at least 30% lower sensitivity to the same betahistine concentration than to histamine.

The betahistine effects (at a concentration of 10⁻³ M) on both layers of the duodenum was blocked by mepiramine (H₁ antagonist) at concentrations of 10⁻⁶ and 10⁻⁵ M. Using the obtained results, it can be concluded that the H₁ type of receptor is present in the smooth muscle of both layers of the broiler duodenum. We obtained similar results on the other parts of the broiler small intestine (jejunum and ileum).

Summarizing all the effects in this assay, it can be concluded that H₁ type receptors are present in the smooth muscles of the broiler small intestine (duodenum, jejunum and ileum), especially in the longitudinal smooth muscle since this layer reacted with contractions even to a low histamine concentration (10⁻⁷). In light of these findings, we suggest that assay substances may have considerable physiological and therapeutic implications in disturbed functions of the small intestine of broilers.

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SRPSKI

**UTVRĐIVANJE HISTAMINSKIH RECEPTORA H₁ TIPA U TANKOM CREVU
BROJLERA PRIMENOM HISTAMINA I NEKIH NJEGOVIH AGONISTA I
ANTAGONISTA**

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Histamin je biološki aktivni amin (biogeni amin) koji ima širok spektar fizioloških i patoloških delovanja u organizmu. Svoje delovanje ispoljava preko četiri tipa specifičnih receptora (H₁, H₂, H₃ i H₄). Histamin je jedan od glavnih uzroka poremećaja funkcije creva i nastanka proliva, koji su kod brojlera česti. Pošto u stručnoj literaturi nema podataka o zastupljenosti histaminskih receptora u glatkoj muskulaturi zida tankog creva (duodenuma, jejunuma i ileuma) brojlera, pokušali smo utvrditi njihovu zastupljenost, distribuciju i vrstu u ovoj muskulaturi. Ispitivanja su vršena na izoliranoj glatkoj muskulaturi cirkularnog i longitudinalnog sloja sva tri dela tankog creva brojlera (strip dimenzija 3-4 mm x 2 cm). Mišićni strip je postavljen u kupatilo za izolirane organe, a izazvane kontrakcije su registrovane na dvokanalnom pisaču izometrijskim transdjuserima, nakon dodavanja histamina, betahistina (H₁ agonist) i mepiramina (H₁ antagonist). Vitalnost glatke muskulature tankog creva brojlera smo proveravali dodavanjem acetilholina. Na osnovu dobijenih rezultata se može zaključiti da su histaminergični receptori H₁ tipa zastupljeni u glatkoj muskulaturi sva tri segmenta tankog creva brojlera.

Ključne reči: brojleri, histamin, histaminski receptori, izolovana glatka muskulatura, tanko crevo

УТВЕРЖДЕНИЕ ГИСТАМИНЫХ РЕЦЕПТОРОВ Г1 ТИПА В ТОНКОЙ КИШКЕ БРОЙЛЕРОВ ПРИМЕНЕНИЕМ ГИСТАМИНА И НЕКОТОРЫХ ЕГО АГОНИСТОВ И АНТАГОНИСТОВ

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

Гистамин биологический активный амин (биогенный амин), имеющий широкий спектр физиологических и патологических действий в организме. Своё действие проявляет через четыре типа специфических рецепторов (Г1, Г2, Г3 и Г4). Гистамин одна из главных причин расстройств функции кишок и становления поносов, которые у бройлеров частые. Так как в специальной литературе нет данных о представленности гистаминовых рецепторов в гладкой мускулатуре стенки токой кишки (двенадцатиперстной, тощей и подвздошной) бройлеров, мы попытались утвердить их представительство, дистрибуцию и вид в этой мускулатуре. Испытания совершены на изолированной гладкой мускулатуре циркулярного и продольного слоев всех трёх частей токой кишки бройлеров (стрип размеров 3-4 мм x 2 см). Мышечный стрип поставлен в ванную для изолированных органов, а вызванные контракции регистрированы на двуканальном писчике изометрическими трансдюсерами, после добавления гистамина, бетагистина (Г1 агонист) и мепирамина (Г1 антагонист). Витальность гладкой мускулатуры тонкой кишки бройлеров мы проверяли добавлением ацетилхолина. На основе полученных результатов можно сделать вывод, что гистаминергические рецепторы Г1 типа представлены в гладкой мускулатуре всех трёх сегментов тонкой кишки бройлеров.

Ключевые слова: бройлеры, гистамин, гистаминовые рецепторы, изолированная гладкая мускулатура, тонкая кишка