Identification of serotonergic 5HT-3B type receptors in broiler's small intestine

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ABSTRACT

The study was performed in order to identify the serotonergic 5HT3B type receptors in broiler's small intestine. Investigation were carried out on isolated smooth muscle of the circular and longitudinal layer of the broilers 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 were recorded via an isotonic force transducer coupled to a pen recorder. This was done following the addition of serotonin (nonselective 5-HT agonist), 2-Me-5HT (5-HT1B agonist) and Y-25130-hydrochloride (selective 5-HT3B antagonist). The research established a presence of serotonergic 5HT3B type receptors within the smooth musculature of the small intestines of broilers (COBB 500). The 5HT3B type receptors were present in smooth muscles of duodenum, jejnum and ileum, especially in longitudinal smooth muscles since this layer reacted even to low serotonin concentration (10^-6).

Statistical tests of obtained results showed significant differences (p< 0.001) in responses related to muscle layers, applied concentrations and intestinal parts which were observed. In the light of these findings, we suggest that investigated substances may have considerable physiological and therapeutic implications in disturbed function of small intestine of broiler’s.

Keywords: Broilers, serotonin, serotonergic 5HT-3B type receptors, isolated smooth muscle, small intestine

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Introduction

Serotonin (enteramine; 5-hydroxytryptamine; 5-HT), a well-characterized neurotransmitter in the central and peripheral nervous system, plays a crucial role in regulating some central and peripheral function such as mood, sleep/wake cycle, thermoregulation, food intake, nociception, locomotion, sexual behavior, sleep appetite, gastrointestinal motility and secretion, blood coagulation, cardiovascular homeostasis etc. (Cirilo et al., 2011; Kato, 2013; Muminović et al., 2000).

Serotonin regulates various functions in the body through its specific serotonergic (5-HT) receptors (Darmon et al., 2015; Cirilo et al., 2011; Kato, 2013; Kaufman and Milstein, 2013; Pytlak et al., 2011). In the recent 20 years, seven distinct families of 5-HT receptors have been identified and various subtypes have been described for several of these (Darmon et al., 2015; Kaufman and Milstein, 2013; Nichols and Nichols 2008; Rang et al., 2015). At least 20 subtypes of 5-HT
receptors have been cloned yet (Hannon and Hoyer 2002; Berger et al., 2009). These receptors are localized in the brain and in peripheral organs but their distribution is not homogeneous. The majority of 5-HT receptors are postsynaptic, with some exceptions, most notably 5-HT1A and 5-HT1B that are mainly presynaptic and modulate serotonin release. The signaling pathways to which these receptors are coupled are known, but it has not been possible to link direct clinical effects systematically to their stimulation. Serotonin receptors are coupled to G proteins except 5-HT3 receptors which are receptor-channels, also called ionotropic receptors, which, in the activated state, are open and permeable to sodium and potassium cations (Darmon et al., 2015; Pytliak et al., 2011; Rang et al., 2015).

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, as importantly, allow the use of their agonist or antagonists, which would undoubtedly enrich the pharmacotherapy of functional disruption of the small intestine in broilers. If we add that the disturbances of bowel function are common in broilers and if we know that mentioned dysfunction cause great loss to the poultry industry, due to the high mortality, the results of research could form the basis for the introduction of new drugs in the pharmacotherapy of this species. Thus, the aim of the present study was to try to determine the possible distribution of 5-HT3B receptors in small intestine of broilers (duodenum, jejunum and ileum).

**Material and Methods**

**Experimental animals**
The research was carried out on 20 broiler chickens (Cobb 500). Broilers had body weight between 2.1 and 2.3 kg, and of the age of up to 42 days. Broilers were obtained and transferred to the local private poultry slaughterhouse, where they were slaughtered in accordance with the regulations.

**In vitro organ bath experiments**
The following substances were used in the study: Kreb’s bicarbonate solution (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); Serotonin (3-(2-Aminoethyl)-1H-indol-5-ol) [Sigma-Aldrich, Germany]; 2-Me-5HT [3-(2-aminoethyl)-2-methyl-1H-indol-5-ol hydrochloride] (5-HT3 agonist); Y-25130-hydrochloride (N-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydropyridine-2H-1,4-benzoazine-8-carboxamide hydrochloride) (selective 5-HT3 antagonist) (Tocris Cookson Ltd., Bristol, UK); 2-(Acetyloxy)-N,N,N-trimethylmethylethanaminium chloride (a solution that the viability of the strips was tested with) (F. Hoffmann-la Roche & Co. Ltd. Basle Switzerland). All the substances used in the experiment were dissolved in distilled water. The sensitivity of the tissues to acetylcholine was tested before starting the experiments.

After the animals were sacrificed, parts of small intestine were taken; i.e. the strips of the first parts of duodenum, jejunum, ileum in length of 5 cm. They were then immersed in a cold, freshly prepared Krebs bicarbonate solution and transferred to a laboratory. The tissue strips were taken to the procedure 20 minutes after the animal was sacrificed. Small intestines were cleansed of fatty and connective tissue, followed by a preparation of circular and longitudinal strips (2 cm in length and a width of about 3-4 mm) and placed in an isolated organ bath with a volume of 10 ml.

In order for conditions to be as close as possible, 4 strips were used simultaneously. The strips were placed in isolated organ baths having 10 ml volume, (Ugo Basile, Model 4050 Two-chamber, Italy). Such suspended strips were aerated in Kreb’s bicarbonate solution with a mixture of oxygen and carbon dioxide (95 % O2 and 5 % CO2) at a constant temperature of 41 °C. Tissues were suspended under a resting tension of 2 g and were allowed to equilibrate for 45-60 minutes and were rinsed every fifteen minutes. Movement registration was performed on a single-channel printers of isometric transducers (Ugo Basile, Italy). Viability of the strips was verified by adding acetylcholine at a concentration of 10^{-5} M at the beginning and/or the end of the experiment.

After the acetylcholine solution was washed, tissue strips were leave to rest for 20 minutes, and then serotonin the (non-selective agonist) was added to the bath using an insulin syringe to achieve sufficient concentrations (10^{-7} -10^{-3} M). In next part of experiment was used 2-Me-5HT receptor agonist at concentration from 10^{-6} to 10^{-3}. Each concentration of serotonin and 2-Me-5HT was maintained in the bath for one minute and the other concentration was applied after washing. The solution of serotonin was then washed after one minute. The period between the individual applications
lasted for about 20 minutes. For statistical data processing, application of a single substance was repeated at least six times on different preparations obtained from different animals. The 5-HT3 receptor antagonist, Y-25130 at concentration of \(10^{-6}\) and \(10^{-5}\) was added to the bath 3-4 minutes before the agonist.

**Statistical Analysis**

Basic statistical data diagnostics was conducted by using Microsoft Excel® (Microsoft Office package, Microsoft, USA). Values of standard deviations of obtained results are presented in the tables. Testing the significance of the differences among obtain results was performed by ANOVA tests, f-tests and t-tests.

**Results**

Results are expressed as percentages of the maximum response (expressed as 100%) produced by serotonin (10-3) on longitudinal layer of smooth muscle of broiler’s duodenum, due to a fact that we obtained the best respond to the action of serotonin in this part of small intestine, so we used it as a positive control for all other intestinal parts in experiment (Table 1.; Fig. 1., 2. and 3).

Results are expressed as percentages of the maximum response (expressed as 100%) produced by selective agonist 5-HT3B type serotonergic receptor (2-Me-5HT at concentration of \(10^{-7}\) to \(10^{-3}\) M) on longitudinal layer of smooth muscle of broiler’s duodenum and ileum, due to a fact that we obtained the best respond to the action of 2-Me-5HT in this part of small intestine, so we used it as a positive control for all other intestinal parts in experiment (Table 2.; Fig. 1, 2 and 3).

**Discussion**

Approximately 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 (Kato, 2013; Rang et al. 2015; Riviere and Papich, 2009). It is peripheral and the most evident effect of serotonin on smooth muscles of gastrointestinal tract (Penkova and Nikolova, 2017; Rang et al., 2015). Serotonin, among other substances, causes contractions of smooth muscles of bovine rumen (Muminović et al., 2000), regulates the motility of the digestive system, enhances secretion, etc. (Rang et al., 2015). 5-HT3 receptors in the enteric nervous system regulate gut motility, secretion, and peristaltic movements and are involved in information transfer in the gastrointestinal tract (Galligan, 2002). Some authors
tried to identify the 5-HT3 receptors in isolated smooth muscles from gastrointestinal tract of rats (Glatzle et al., 2002), jejunum of rats (Veeresh et al., 2009), bovine rumen (Muminovic et al., 2000), human small intestine (Coleman et al., 2003), human stomach (Penkova and Nikolova, 2017), smooth intestine musculature during the turkey fattening phase (Katica, 2015) and effect of 5-HT3 receptors on gastric motility in fasted and fed dogs (Nagakura et al., 1997), but not in smooth muscles of broilers small intestines. There is a general agreement that 5-HT3 receptors in the intestinal tract are located on enteric sensory neurons and activate a cholinergic mechanism to stimulate secretion (Hendriks et al., 1989).

Receptor 5-HT3, more precisely its subtype 5-HT3B which was investigated in tunica muscularis of small intestine, is bound to membrane ionic channels, for difference to the other serotonergic receptors (Katica, 2015, Pauwels, 2003, Siegel et. al., 1999). Peripheral effects which appears after 5HT3B activation, are reflecting in cardio-vascular system, control of intestinal tonus and secretion as well as in contraction of bladder’s smooth muscle in cat. Central effects are manifested as psychological disorders and vomiting (Hardman and Limbrid, 2001; Katica, 2015).

Table 1. Effects of different serotonin concentrations on both layers of smooth intestinal muscles of broiler.

| Conc. | Duodenum | Jejunum | Ileum |
|-------|----------|---------|-------|
|       | Circ. Layer | SD | Long. Layer | SD | Circ. Layer | SD | Long. Layer | SD | Values |
| 10^-7 | 0 | 0 | 0 | | 0 | 0 | 0 | | |
| 10^-6 | 0 | 0 | 18.9 | 1.394 | 0 | 0 | 6.6 | 0.261 | 0 | 0 | 13.3 | 0.200 | 0.001 |
| 10^-5 | 0 | 0 | 32.2 | 1.271 | 0 | 0 | 13.9 | 0.385 | 0 | 0 | 24.4 | 1.265 | 0.001 |
| 10^-4 | 0 | 0 | 55.6 | 0.635 | 0 | 0 | 35.5 | 2.15 | 0 | 0 | 42.2 | 1.289 | 0.001 |
| 10^-3 | 20 | 1.133 | 100 | 0 | 3.3 | 1.088 | 64.4 | 1.54 | 3.3 | 0.089 | 83.3 | 0.089 | 0.001 |

SD = Standard deviation, Conc. = Concentrations, Circ. = Circular, Long. = Longitudinal

Results obtained for different serotonin concentrations (nonselective agonist of serotonergic receptors) indicated on evident presence of serotonergic receptors in smooth intestinal muscles of broiler. Application of different serotonin concentrations showed significant differences in distribution of serotonergic receptors among the intestinal parts (duodenum, jejunum and ileum) as well as among the layers of tunica muscularis in broiler’s small intestine (p < 0.001). The highest response was obtained in longitudinal
duodenal layer under the concentration $10^{-3}$. The lowest values were obtained in the circular layer. Statistically significant differences among the different intestinal parts were recorded under the same serotonin concentrations (Table 1).

Results obtained by application of Me-2-5HT (selective agonist of 5HT$_{3B}$ type receptor) indicated the significant contractions in longitudinal layer of tunica muscularis in duodenum and ileum, under the concentration of $10^{-3}$ (Table 2). These responses were the highest and expressed by maximal percent. Effects Me-2-5HT on circular layer were lower than those obtained for longitudinal layer which were in accordance with the results obtained by serotonin application (Table 1 and Table 2). Statistically significant differences were recorded for the results obtained in different part as well as in different layers of broiler’s small intestine ($p < 0.001$).

According to the study of Mujezinović et.al (2011), it could be concluded that 5H71A type receptors were present mainly in smooth muscles of the broiler broiler’s small intestine, duodenum and ileum, especially in the longitudinal smooth muscle layer which reacted with contractions even to low serotonin concentration ($10^{-6}$). Similar reaction was not recorded in the jejunum.

Results for duodenum and jejunum obtained in this study partly corresponded with similar study of serotonergic receptors distribution, as well as with the study of 5 HT$_{3B}$ in tunica muscularis of small intestine during the turkey fattening phase (Katica, 2015), while the results for both muscular layers of ileum showed high accordance with the results from the same study.

The research established general a presence of serotonergic, receptors within the smooth musculature of the small intestines of broilers (COBB 500). The 5HT-3B type receptors are present in smooth muscles of duodenum, jejunum and ileum, especially in longitudinal smooth muscles since this layer reacted even to low serotonin concentration ($10^{-6}$). In the light of these findings, we suggest that investigated substances may have considerable physiological and pathophysiologival implications in functions of small intestine of broiler’s.

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