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Effects of cholinergic receptor antagonists on gastrointestinal growth factors in newly hatched chickens

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ABSTRACT. The early post-hatching period is the most critical phase in the life of chickens due to its important impact on the physical and functional development of the gastrointestinal tract. The activity of the brain-gastrointestinal axis is regulated by the vagus nerve (acetylcholine, ACh) and hormones modulating rapid growth and development of intestinal cells. Co-regulation of insulin like growth factor 1 (IGF-1) and somatostatin (STS) plays a unique role during extensive cell proliferation. However, the relationship between the cholinergic system and growth factors in the intestine of newly hatched chickens remains unclear. Therefore, an *in vitro* experiment (6-h tissue culture) was conducted to examine the impact of cholinergic receptor antagonists (atropine and hexamethonium) on IGF-1 and STS secretion in the duodenum of the newly hatched chickens 2 and 24 h after hatching (Day 0 and Day 1). Additionally, the activity of cholinergic muscarinic receptor 3 (CHRM3) in the duodenum was visualised using immunofluorescence. The results showed that the magnitude of the effect of cholinergic receptors inhibition on IGF-1 and STS release and CHRM3 expression depended on the antagonist and time after hatching. In conclusion, the findings clearly demonstrated a connection between the cholinergic system and growth factors in the digestive tract of newly hatched chickens.

Introduction

The early period after hatching is the most critical stage in the life of chickens due to its significant influence on the physical and functional development of the gastrointestinal tract (Kinstler et al., 2024). This period, particularly first 2 days, is crucial for intestinal development, affecting long-term growth, metabolism, and the interaction of the neuronal, immune, and endocrine systems, and ultimately chicken health. Recently, extensive research has focused on the influence of genetics on metabolic mechanisms and disorders associated with selection of fast-growing chickens (Soglia et al., 2021; Siegel, 2023). However, the influence of the interaction of the neuronal system, particularly cholinergic innervations, and hormonal growth factors on the intestine development and function in newly hatched chickens remains unexplored. The maturation that occurs during this period influences the activity of the brain-gastrointestinal axis, which shapes long term-growth, metabolism, and health. In recent years, several noteworthy studies have highlighted the involvement of acetylcholine (ACh) in modulating the brain-gastrointestinal axis, suggesting its potential use as a marker for digestive system diseases

(Nautiyal et al., 2023; Fujita et al., 2024). ACh acts through muscarinic and nicotinic receptors present in the gastrointestinal tract (Jaszcza et al., 2020; Igarashi-Hisayoshi et al., 2023). Many cholinergic receptor agonists and antagonists have already been synthesised (Wang et al., 2021), with atropine (targeting muscarinic receptors) and hexamethonium (targeting nicotinic receptors) being the most widely used in both *in vivo* and *in vitro* studies (Liu et al., 2020). Atropine has been found to inhibit motilin-induced contractions of the chicken small intestine and ghrelin-stimulated contractions of parts of the chicken glandular stomach *in vitro* (Kitazawa et al., 2007). The activity of the brain-gastrointestinal axis is regulated by ACh and various hormones modulating rapid growth and development of intestinal cells. These hormones include growth hormone (GH), insulin like growth factor 1 (IGF-1), somatostatin (STS), ghrelin, and leptin. Importantly, the co-regulation of IGF-1 and somatostatin plays a unique role during periods of extensive cell proliferation. IGF-1 is particularly important in tissues undergoing rapid proliferation and apoptosis, such as the gastrointestinal tract. IGF-1 has also been shown to affect energy metabolism, body weight and carcinogenesis (Pollak et al., 2004). Research conducted on rats, mice, pigs and sheep, both *in vivo* and *in vitro*, has indicated a stimulatory effect of IGF-1 on intestinal epithelial proliferation. This growth factor is also an important regulator of muscle development and metabolism in chickens (Yu et al., 2015; Nakashima et al., 2017). Comprehensive studies in hens have shown that restrictive feeding leads to reduced blood levels of IGF-1, accompanied by an increase in *IGF1* mRNA expression (Nakashima et al., 2017), underscoring its role in food intake regulation (Fujita et al., 2019). These outcomes suggest the involvement of IGF-1 in the modulation of metabolism in chickens and mature hens. Somatostatin is an oligopeptide occurring in two molecular forms composed either of 14 (somatostatin 14, STS-14) or 28 amino acids (somatostatin 28, STS-28). The highest concentration of STS has been documented in nerve cells, gastrointestinal D cells, pancreatic delta cells, brain, and enteroendocrine cells (Martinez, 2013). Somatostatin is an inhibitor of several physiological processes and acts as a neurotransmitter, hormone and paracrine regulator of gastrointestinal function. Initially, identified primarily as an inhibitor of growth hormone secretion, somatostatin is now recognised for its broader role in the digestive system. It inhibits key processes such as stomach emptying, secretion of gastric juice, intestinal motility and the release of gastrointestinal hormones (Maev et al., 2022). In birds, somatostatin significantly impacts metabolism by stimulating glucose uptake by small intestinal enterocytes and suppressing glucagon-stimulated lipolysis in chicken adipose tissue. Recently, growing interest in the influence of various nutrients, supplements, probiotics, prebiotics, and feed additives on the brain-gastrointestinal axis has demonstrated an important role of cholinergic receptors and growth factors. These findings may have potential useful application in the prevention and treatment of gastrointestinal disorders in rat and chicken (Dupak et al., 2020; 2022; Zaid et al., 2023; Kinstler et al., 2024). However, to the best of our knowledge, no studies have been published examining the interplay between the cholinergic system and growth factors in newly hatched chickens. The primary objectives are to determine if the interaction between cholinergic receptors and growth factors occurs within the first few hours after hatching and whether this interaction remains stable or changes over the subsequent 24 h.

To this end, the present study determined the effect of cholinergic receptor inhibition on IGF-1 and STS secretion in the duodenum of newly hatched chickens. Additionally, it examined the expression of the muscarinic receptor CHRM3 following treatment with cholinergic receptor antagonists in the duodenum of chickens.

Material and methods

Ethical approval

The experiments on chickens involved euthanising animals solely for the use of their organs or tissues. Consequently, the *in vitro* studies performed did not require the consent of the Local Ethics Committee, in accordance with Article 2.1. from the Act of Law of January 15, 2015 (Journal of Laws of 2015, item 266) on the protection of animals used for scientific or educational purposes.

Animals

Forty Ross 308 chickens (Aviagen) of both sex were divided into two experimental groups on the day of hatching: Day 0 (n = 20) and Day 1 (n = 20). Birds were placed in plastic cages (60 × 60 × 40 cm) and kept in a room maintained at a temperature of 30 °C with a 10D:14L lighting schedule. Neither group was fed, and additionally the Day 0 group did not have access to water. Birds from each group were further divided into 4 sub-groups: control/basal (C),

atropine (A), hexamethonium (H) and atropine plus hexamethonium (A + H). Two hours (Day 0 group) and 24 h (Day 1 group) after hatching, the chickens were weighed ($\bar{x}$ = 37 ± 4.2 g) and euthanised by cervical dislocation. Subsequently, tissues were collected and immediately used for analyses.

Methodology

In vitro secretion of IGF-1 and STS from the chicken duodenum

The secretion process was carried out using a modified method of Kowalski and Giraud (1993). Collected duodenal fragments were subjected to a 6-h *in vitro* tissue culture in Eagle's medium (Eagle's medium with L-glutamine and $NaHCO_3$ for laboratory use, BIOMED-Lublin, Poland) supplemented with an antibiotic (Sigma-Aldrich, Saint Louis, MO, USA). Incubation was designed to determine basal and cholinergic receptor antagonist-stimulated secretion (atropine, hexamethonium, atropine plus hexamethonium). Each well contained 2 ml of Eagle's medium, with 100 nM atropine and/or hexamethonium added for stimulation. After incubation, 2 ml of medium was collected from all wells and transferred to test tubes. The culture medium was stored at −80 °C until determination of IGF-1 and STS concentration. IGF-1 and STS levels were measured using radioimmunoassay commercial kits (DRG Instruments GmbH, Marburg, Germany). For IGF-1, the RIA-4702 kit was used, which had a standard curve range of 0.45 to 47 ng/ml and a sensitivity of 0.25 ng/ml. The intra- and inter- assay errors were 9.2 and 7.8%, respectively. For somatostatin, the RIA-3017 kit was utilised, featuring a standard curve range from 3.9 to 250 pmol/l and a sensitivity of 6 pmol/l. The intra- and inter-assay errors were 5.55 and 4.85%, respectively. The radioactivity of the samples was measured using a 5-channel, computer-coupled Wizard gamma radiation counter (LKB, Vienna, Austria).

Haematoxylin and eosin staining

For histological analysis, deparaffinised and rehydrated 6-µm-thick sections of the chicken intestine were stained according to the standard haematoxylin and eosin method. The process involved staining with a Harris haematoxylin solution for 20 min, differentiation in 70% ethanol containing 1% HCl, and subsequent staining with a 1% eosin aqua solution with acetic acid, as described by Grzegorzewska et al. (2020).

Periodic Acid-Schiff staining

The Periodic Acid-Schiff (PAS) staining procedure, adapted from Leśniak-Walentyn and Hrabia (2016), involved deparaffinising the preparations in xylene and rehydrating the small intestine sections (6-µm-thick) in a graded series of ethanol. The sections were then incubated with 0.5% periodic acid (Leica Biosystems, San Diego, CA, USA) for 5 min, transferred to distilled water, and incubated with Schiff's reagent in a humid chamber (15 min) until a light pink colour appeared. Following this, the slides were placed in lukewarm distilled water (5 min) and the colour changed from light pink to dark pink. Mayer's haematoxylin (Leica Biosystems, San Diego, CA, USA) was then applied for 1 min, and the sections were rinsed in distilled water and dehydrated in a graded series of ethanol and xylene, and subsequently mounted in DPX (Sigma-Aldrich, Saint Louis, MO, USA).

Immunohistochemical analysis of CHRM3 expression

The expression of the CHRM3 receptor in the duodenum of chickens on Days 0 and 1 was evaluated using a method modified by Grzegorzewska et al. (2022). Briefly, duodenal sections (6-µm-thick), sliced using an RM2245 semi-automatic rotary microtome (Leica Biosystems, San Diego, CA, USA), were deparaffinised in xylene and rehydrated in a graded series of ethanol (from 100% to 50%). Subsequently, the sections were heated in citrate buffer (pH = 6.0) at 75 °C for 5 min, followed by washing in TBS buffer. To block nonspecific binding, the slides were treated with 5% normal goat serum in TBST buffer (Sigma Aldrich, Saint Louis, MO, USA) and incubated at room temperature for 90 min in a humid chamber. After removing the serum, the slides were incubated for 12–14 h at 4 °C with a specific primary rabbit polyclonal antibody against CHRM3 (dilution 1:400, PA5-77485; Thermo Fisher Scientific, Waltham, MA, USA). A negative control was performed by replacing the antibody with TBST solution. After 12 h, the tissues were washed three times in TBS and incubated for 90 min with a secondary fluorescent antibody (dilution 1:150, goat anti-mouse conjugated with AlexaFluor 555, A-21424; Thermo Fisher Scientific, Waltham, MA, USA). The slides were then mounted in VECTASHIELD® HardsetTM Antifade Mounting Medium with DAPI (Vector Laboratories, Newark, CA, USA). The preparations were observed and analysed (n = 5 in each group) using an AxioScope fluorescence microscope with an Axiocam 503 camera and ZEN 2.3 pro software (Carl Zeiss, Oberkochen, Germany).

Statistical analysis

Analysis of IGF-1 and STS secretion was performed in duplicate. The data were statistically analysed using one-way ANOVA (treatment effect) followed by Duncan's multiple range test. Values were expressed as means ± SEM, and differences were considered significant at $P < 0.05$. The calculations were performed using SigmaPlot v.14. 2017 (Systat Software Inc., GmbH, Düsseldorf, Germany).

Results

Effects of cholinergic receptor antagonists on *in vitro* secretion of IGF-1 from the duodenum of chickens on Days 0 and 1

The basal secretion of IGF-1 *in vitro* from the duodenum collected from chickens on Days 0 and 1 (Figure 1) was 31.2 ± 1.1 ng/g tissue and 10.6 ± 0.5 ng/g tissue,respectively.

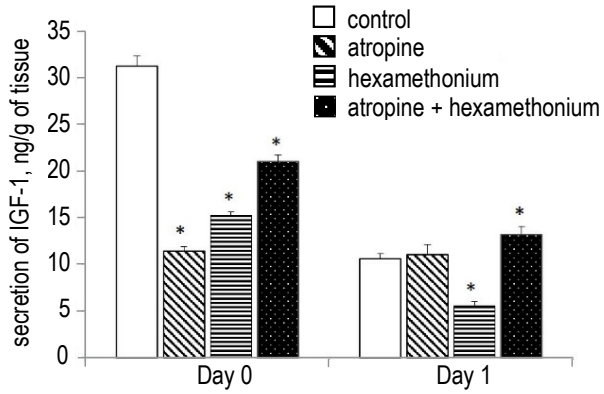

Figure 1. Effect of cholinergic receptor antagonists on *in vitro* insulin like growth factor 1 (IGF-1) secretion from the duodenum of chickens on Days 0 and 1 of tissue collection (X ± SEM, ng/g tissue, *$P < 0.05$ compared to control)

Treatment with atropine and hexamethonium significantly reduced ($P < 0.05$) IGF-1 secretion from the duodenum on Day 0 by 63.5% and 51.3%, respectively. The combined addition of muscarinic and nicotinic receptor antagonists resulted in a significant decrease in IGF-1 secretion from the duodenum on Day 0 by 32.7% compared to the basal level ($P < 0.05$).

On Day 1, atropine did not affect IGF-1 secretion from the duodenum collected from chickens, while hexamethonium significantly decreased its secretion by 48.1% ($P < 0.05$). In contrast, the combined supplementation of both cholinergic receptor antagonists significantly increased IGF-1 secretion by 24.5% compared to the basal level ($P < 0.05$).

Effects of cholinergic receptor antagonists on *in vitro* secretion of somatostatin from the duodenum of chickens on Days 0 and 1

The basal *in vitro* secretion of STS from the duodenum collected from chickens on days 0 and 1 (Figure 2) was 8.7 ± 0.2 ng/g tissue and 5.8 ± 0.1 ng/g tissue, respectively.

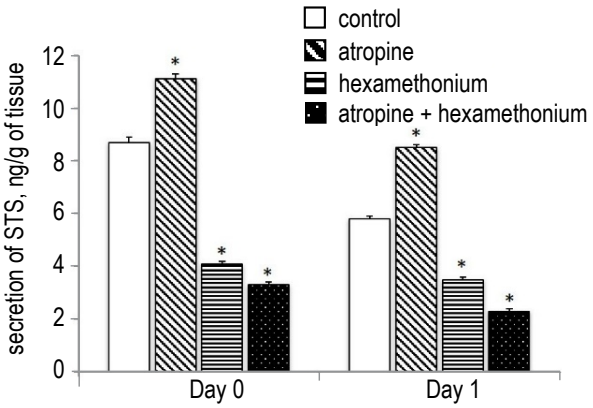

Figure 2. Effect of cholinergic receptor antagonists on *in vitro* somatostatin (STS) secretion from the duodenum of chickens on Days 0 and 1 of tissue collection (X ± SEM, ng/g tissue, *$P < 0.05$ compared to control)

On Day 0, atropine significantly increased ($P < 0.05$) STS secretion from the duodenum by 27.6%, while hexamethonium treatment decreased its secretion by 52.9% ($P < 0.05$). Finally, the combined supplementation of both cholinergic receptor antagonists reduced STS secretion from the duodenum of chickens on Day 0 by 62.1% ($P < 0.05$) compared to the control group.

On Day 1, the muscarinic receptor antagonist caused a significant ($P < 0.05$) increase in STS secretion from the duodenum of chickens by 46.5%. Similarly to Day 0, hexamethonium significantly decreased the secretion of STS by 39.6% ($P < 0.05$), while incubation of duodenum with both cholinergic antagonists caused a 60.3% ($P < 0.05$) reduction in STS secretion compared to the control/basal secretion.

Histological analysis of the duodenum of chickens on Days 0 and 1

Haematoxylin and eosin, and PAS staining

Haematoxylin and eosin staining of duodenal fragments collected from chickens on Days 0 and 1 was performed to illustrate morphological differences between tissue structures depending on the day of chickens life. The PAS reaction is a histochemical technique used to detect neutral polysaccharides that contain hexose rings. During PAS staining, glycol residues exposed to periodic acid are oxidised and the

resulting aldehyde groups react with Schiff's reagent. A positive PAS reaction, indicated by a purple-red colour, is observed in both cellular and tissue structures with higher concentrations of polysaccharides, such as basement membranes, glycocalyx of brush borders, intracellular glycogen deposits, mucus, and glycoprotein secretory granules. No morphological differences or positive PAS staining were detected in the duodenum of chickens on Day 0 and Day 1 (Figure 3A, B, C, D).

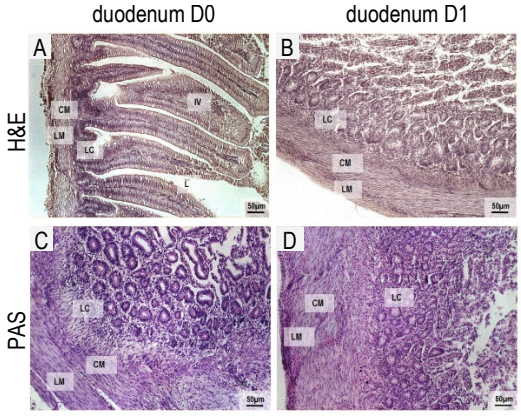

Figure 3. Duodenal samples collected from chickens on Days 0 and 1 (A, B – haematoxylin and eosin (H&E) staining; C, D – Periodic Acid-Schiff (PAS) staining). Description of structures according to Hodges (1974)

CM – circular muscle, IV – intestinal villi, L – lumen between the intestinal villi, LC – Lieberkühn crypts, LM – longitudinal muscle, D0 – Day 0 (2 h after hatching), D1 – Day 1 (24 h after hatching)

Effects of cholinergic receptor antagonists on CHRM3 expression in the duodenum of chickens on days 0 and 1

CHRM3 receptor was localised in the duodenum collected from chickens on Days 0 and 1. Immunopositive (cytoplasmic) reactions were obtained in the intestinal villi, Lieberkühn crypts, epithelium with a cuticular border and goblet cells in tssue samples collected from control chickens on Days 0 and 1 (Figure 4). No differences were found in the immunolocalisation and expression of CHRM3 between the control groups on Day 0 and Day 1. Atropine treatment induced a decrease in immunocytochemical CHRM3 expression only on Day 0; however, this effect was not observed in the duodenum collected from chickens on Day 1. Hexamethonium treatment did not alter CHRM3 expression in chicken tissues on either day. When both cholinergic receptors were blocked with atropine and hexamethonium on Day 0, a decrease in immunocytochemical CHRM3 expression was noted, and this effect was also visible in the duodenum collected from chickens on Day 1 (Figure 4, 5).

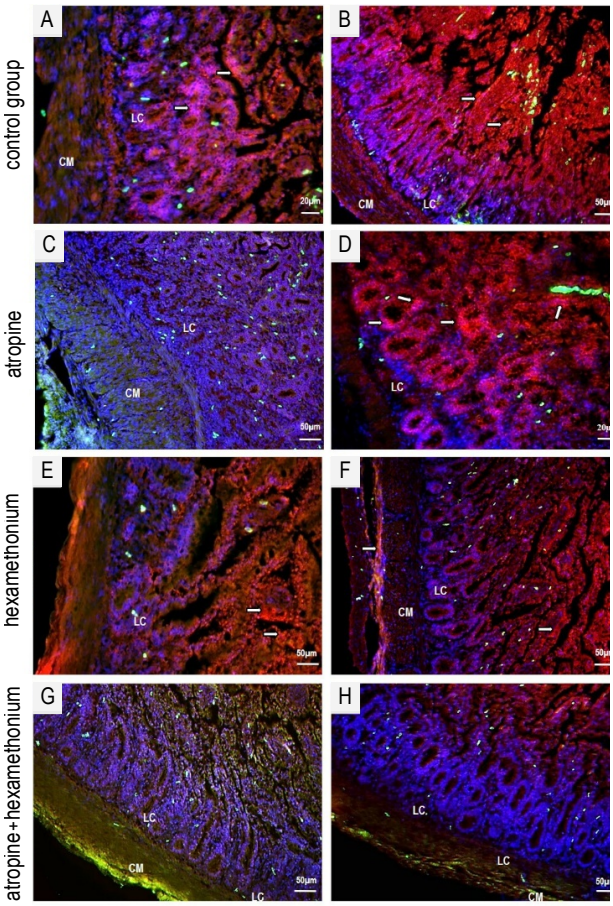

Figure 4. Immunohistochemical localisation of cholinergic receptor muscarinic 3 (CHRM3) in the duodenum of chickens on Days 0 and 1. Control (A, B) – tissues incubated in medium without the addition of antagonists. Atropine (C, D) – tissues incubated with atropine, a muscarinic receptor antagonist. Hexamethonium (E, F) – tissues incubated with hexamethonium, a nicotinic receptor antagonist. Atropine and hexamethonium (G, H) – tissues incubated with atropine and hexamethonium. White arrows indicate specific immunopositive reaction for CHRM3 (red fluorescence). Description of structures according to Hodges (1974)

CM – circular muscle, LC – Lieberkühn crypts. D0 – Day 0 (2 h after hatching), D1 – Day 1 (24 h after hatching)

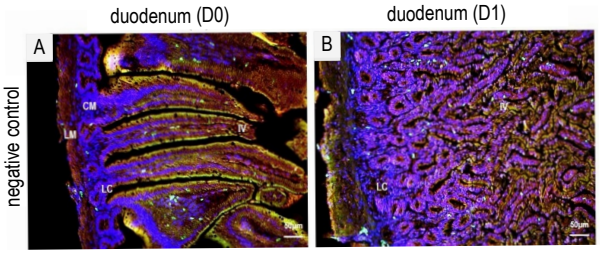

Figure 5. Immunohistochemical localisation of cholinergic receptor muscarinic 3 (CHRM3) in the duodenum of chickens on Days 0 (A) and 1 (B). Negative control – primary antibody omitted. Description of structures according to Hodges (1974)

CM – circular muscle, IV – intestinal villi, LC – Lieberkühn crypts, LM – longitudinal muscle, D0 – Day 0 (2 h after hatching), D1 – Day 1 (24 h after hatching)

Discussion

Small intestinal function is controlled by both the nervous and endocrine systems. Previous studies have demonstrated the presence of cholinergic receptors in the gastrointestinal tract of birds, mammals and other vertebrates (Jaszcza et al., 2020; Fujita et al., 2024). Enteroendocrine cells have been shown to be involved in the regulation of many gastrointestinal processes through paracrine, autocrine and endocrine mechanisms. These cells release hormones and regulatory peptides that affect nerve endings or enter the bloodstream, influencing the activity of the brain-gastrointestinal axis. Such interactions are essential for effective food processing and utilisation, as well as for regulating the growth and development, and protecting the organism against harmful chemicals or toxins, particularly during the first hours of life.

The *in vitro* experiment demonstrated that disrupting cholinergic signalling in duodenal cells using receptor antagonists, atropine and hexamethonium, led to alterations in the secretion of IGF-1 and somatostatin from intestinal explants collected from newly hatched chickens. On Day 0, the secretion of IGF-1 from intact explants was significantly higher than on Day 1, where a decrease of 66% was recorded. This observation suggests several possibilities: 1) tissue secretion of IGF-1 *in vivo* was increased; 2) IGF-1 was derived from outside the duodenum and was depleted before the experiment; 3) the protective mechanism of progressive neoplasmia induced by high IGF-1 levels was activated. In mammals, new bioactive preparations derived from milk and colostrum containing IGF-I have been developed as adjunctive treatments for certain bowel disorders. However, the results have demonstrated that in order to precisely understand the role of the IGF-1 axis in cancer, research should involve investigating the antagonism of IGF-1/receptor interactions as a significant strategy for cancer prevention and risk reduction (Howarth, 2003). Previous studies have demonstrated that ACh is involved in modulating major components of the somatotropic axis during mammalian development. However, there is a lack of research on the effects of ACh on the structures of the brain-gastrointestinal axis in growing chickens. Studies in a mouse model showed that endogenous ACh affected the concentrations of circulating GH and IGF-1 during embryonic and postnatal stages (Lecomte et al., 2018).

The present *in vitro* experiment showed that the addition of atropine (A) and hexamethonium (H) either individually or in combination (A + H) to chicken duodenal explants from Day 0 significantly decreased IGF-1 secretion by 63.5% and 33%, respectively. The observed reduction in IGF-1 secretion due to hexamethonium, a nicotinic cholinergic receptor antagonist, suggests that vagal innervation of the duodenum is active as early as 2 h after hatching. This finding highlights the unique sensory and motor innervation of the duodenum and suggests implications for the organisation of vagovagal reflexes controlling gastrointestinal function in chickens. This hypothesis is further supported by the results obtained during IGF-1 release from the duodenum of Day 1 chickens. Inhibition of muscarinic receptors by atropine did not affect IGF-1 secretion; however, antagonising nicotinic receptors reduced IGF-1 release by 48%. Unexpectedly, simultaneous inhibition of both types of cholinergic receptors caused an increase in IGF-1 release from duodenal explants, confirming the hypothesis that acetylcholine is one of the main regulators of hormonal activity in the gastrointestinal tract. Acetylcholine also modulated the levels of GHRH and STS in the hypothalamus, thereby affecting the peripheral levels of these hormones. The results demonstrate that ACh may be a potential regulator of the somatotropic axis during development (Lecomte et al., 2018). It is reasonable to infer that the presence of ACh and the activity of cholinergic receptors are necessary for the secretion of IGF-1 from the duodenum.

The pattern of *in vitro* secretion of somatostatin from control duodenal explants was similar to that of IGF-1. Tissue collected from Day 1 chickens spontaneously released 33% less somatostatin compared to those from Day 0 chickens. Inhibition of muscarinic receptors and the absence of acetylcholine stimulated STS release, which likely contributed to the observed 63% decrease in IGF-1 secretion on Day 0. STS release from Day 1 explants was significantly higher than that from Day 0 (48.5 vs. 27.6%), potentially diminishing IGF-1 release levels, which remained unchanged compared to Day 0. The addition of hexamethonium, either alone or in combination with atropine, reduced STS secretion from duodenal explants collected on both Day 0 and Day 1. It may be speculated that acetylcholine affects the release of somatostatin from the intestine through muscarinic and nicotinic receptors, with its effects varying depending on the time after hatching. Previous studies have

demonstrated that ACh stimulates STS secretion from pancreatic δ cells *via* the M1 muscarinic receptor in humans. Additionally, ACh and exogenous cholinergic receptor agonists have been found to increase insulin secretion from pancreatic β islet cells, probably through M3 muscarinic receptors (Molina et al., 2014). The inhibitory effect of atropine on ACh secretion by α cells of the islets of Langerhans has also been confirmed (Rodriguez-Diaz et al., 2011). The results of the present study indicate that cholinergic receptor antagonists act in line with the tonic release of acetylcholine from nerve endings or other cells. These findings demonstrate an interaction between gastrointestinal peptides and the cholinergic system.

The expression of muscarinic receptor CHRM3 in the duodenum of chickens was visualised by the immunolocalisation and was found to be similar on Day 0 and Day 1, suggesting that it is active at the time of hatching. It should be noted that due to lack of feed stimulation, the activity of receptors was spontaneous. Contrary to expectation, the supplementation of atropine significantly decreased CHRM3 expression only on Day 0 but not on Day 1. The addition of hexamethonium to duodenum *in vitro* culture did not affect receptor activity, proving that the number of nicotinic receptors present in the gastrointestinal tract was low. Unexpectedly, the incubation of duodenal explants with both cholinergic receptors antagonists decreased the expression of CHRM3 receptor on both Day 0 and Day 1. It may be speculated that on Day 1, hexamethonium potentiated the inhibitory effect of atropine during 6 h of incubation. The interaction between nicotinic and muscarinic cholinergic receptors has been postulated in earlier publications (Stenlake, 1979), as well as in a more recent study (Sampaio Moura et al., 2024).

The current *in vitro* experiments demonstrated the important role of cholinergic receptors in regulating the activity of the brain-gastrointestinal axis in growing chickens. Nicotinic receptors are ionotropic and their activity differs from the activation of muscarinic receptors, which are G-protein coupled receptors (GPCRs). The duodenal explants are composed of smooth muscle cells, endocrine cells and enteric nervous system axons. Consequently, the function of muscarinic receptors in tissues innervated by the postganglionic parasympathetic fibres, can be modulated by ganglionic nicotinic receptors through stimulation of acetylcholine (ACh) release. In the ganglia, postsynaptic nicotinic receptor activity can be modulated by presynaptic muscarinic and nicotinic autoreceptors, which regulate ACh release. Additionally, functioning of muscarinic receptors can be modulated by desensitisation or blockade of nicotinic receptors. These two types of receptor interact with each other, maintaining a dynamic homeostasis of the cholinergic nervous system.

Conclusions

The results of the present study demonstrate a significant interaction between gastrointestinal peptides and the cholinergic system. Further elucidation of the physiological processes dependent on the functioning of the brain-gastrointestinal axis in birds may be beneficial for breeding practices, leading to the formulation of optimal feed compositions that enhance nutrient utilisation efficiency. Moreover, this knowledge is valuable for the prevention and treatment of gastrointestinal disorders in poultry.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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