



Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

FIRST REPORT OF FUNCTIONAL IDENTIFICATION OF MUSCARINIC RECEPTORS IN THE SMALL INTESTINE OF JAPANESE QUAIL (*Coturnix coturnix japonica*)

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Received – December 25, 2019; Revision – February 03, 2020; Accepted – February 24, 2020

Available Online – February 25, 2020

DOI: [http://dx.doi.org/10.18006/2020.8\(1\).71.75](http://dx.doi.org/10.18006/2020.8(1).71.75)

KEYWORDS

Quail

Muscarinic receptors

EC50

pD2

Ileum

ABSTRACT

Motility of small intestine which determines the efficacy of digestion and assimilation of nutrients and thus the rate of growth in the animals and birds is modulated mainly by muscarinic cholinergic receptor system. The efficacy of agonists and antagonists in modulating the gastrointestinal motility is governed by subtypes of muscarinic receptors present in the organs. So far, there are no reports of muscarinic receptors of quail intestine. Hence the current study was conducted for identifying the muscarinic receptor subtypes for better pharmacological management of dysfunctions of the small intestine of Japanese quail. Eight healthy quails of either sex were raised under uniform management conditions. Birds were euthanized and two to three centimetres length ileum was separated from a region five centimetres away from the ileo-caecal junction and transferred to Tyrode solution at 37.2 °C. The ileum tissue was mounted under 1 g tension in an organ bath chamber with constant aeration. The contractile responses to the agonist alone, agonist in presence of antagonists and relaxant effect of muscarinic receptor antagonists with submaximal contraction of ACh were recorded with isometric transducer connected to a recorder. The median effective concentration 50 (EC50) and pD2 values were determined. From the results it is evident that muscarinic acetylcholine receptors are present in the ileum of Japanese quail which, as per our knowledge is the first report of this receptor in Japanese quail intestine. The EC50 values of acetylcholine alone in ileum of Japanese quail varied from 1.235×10^{-7} M

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

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value of 1.701×10^{-7} M and pD₂ value of 6.769. It was also confirmed that Tyrode solution is better suited for assessment of intestinal motility of quail. This model can be useful as a cheap and effective replacement for rat intestinal experiments.

1 Introduction

The history of cholinergic receptors start with the experiments performed independently by Henry Dale and Otto Loewi, whose spectacular works identified the neurotransmitter acetylcholine that is responsible for vasodepressor effects on frog heart preparation (Dale, 1914; Loewi, 1921). The muscarinic acetylcholine receptors are G protein coupled receptors with seven transmembrane-spanning domain acting through second messengers (Bonner et al., 1987; Alexander et al., 2017). There are five different subtypes of muscarinic receptors namely M₁, M₂, M₃, M₄ and M₅ which are pharmacologically different based on the competition between antagonists and agonists (Gholamnezhad et al., 2018). The odd-numbered muscarinic acetylcholine receptors i.e. M₁, M₃ and M₅ receptors caused the activation of phospholipase C, generating two secondary messengers inositol 1, 4, 5-trisphosphate (IP₃) and diacylglycerol (DAG) eventually leading to an intracellular increase of calcium, while the even-numbered muscarinic acetylcholine receptors vi., M₂ and M₄ inhibit adenylatecyclase, thereby decreasing the production of the second messenger cyclic adenosine monophosphate (cAMP). Muscarinic receptors play an important role in the motility of gastrointestinal tract; their presence was reported in small intestine of most of the livestock and chicken (Delvalle et al., 2018). But, so far, there are no reports of cholinergic receptor response and their subtypes present in the small intestine of Japanese quail. Hence the current study was conducted to assess functional activity of cholinergic system by finding the median effective concentration (EC₅₀) of acetylcholine (ACh) in the ileum of Japanese quail.

2 Material and Methods

2.1 Preparation of quail ileum for isolated organ bath experiment

Eight healthy three to six months old Japanese quail (*Coturnix coturnix japonica*) weighing 110-120 g were procured from Kozhikode district, Kerala, India and maintained three weeks for acclimatization under standard uniform managemental conditions. All the experiments involving quails and quail tissues were reviewed and approved by the Institutional Animal Ethics Committee (IAEC/COVAS/PKD/3/2019) and conformed to the CPCSEA guidelines. The birds were euthanized by anaesthetic overdose and two to three centimetres length ileum was separated from a region five centimetres away from the ileo-caecal junction and was transferred to Tyrode solution kept at 37.2 °C. The lumen

and surface of ileum was washed and flushed with Tyrode solution. The fascia adhering to the tissue were carefully removed and one end of the ileum was fixed to tissue holder which was then mounted in to the tissue chamber of organ bath containing 20 ml Tyrode solution and the other end of ileum was tied to an isometric force transducer. The bath temperature was maintained at 37.2 °C and constantly aerated with atmospheric air till the end of the experiment. The tension was recorded using a polygraph digital data acquisition system linked to isometric force transducer connected to a recorder (M/s INCO, India). The ileum was mounted under 1 g resting tension and allowed to equilibrate for 60 minutes before the commencement of the experiment. During the equilibration period of 60 minutes, Tyrode solution was changed every 15 minutes. The contractile response to the agonist acetylcholine was recorded with isometric transducer connected to a recorder (Apu et al., 2016). The composition of Tyrode solution was as follows: 137 mM sodium chloride (NaCl); 2.7 mM potassium chloride (KCl); 1.8 mM calcium chloride (CaCl₂); 0.5 mM magnesium chloride (MgCl₂); 11.9 mM sodium bicarbonate (NaHCO₃); 0.4 mM sodium dihydrogen phosphate (NaH₂PO₄); and 5.55 mM glucose. The ileum tissue was mounted under 1 g constant resting tension and allowed to equilibrate for 60 minutes before commencing the experiment, changing the solution every 15 minutes (Ghosh, 2014).

2.2. Dose- response curve of acetylcholine

A dose response curve was constructed by adding successive cumulative concentrations of ACh ranging from 10^{-7} M to 10^{-2} M, the procedure was repeated after giving 30 minutes rest for the tissue and same is followed for remaining quails to get the uniform responses (Ghosh, 2014).

2.3. Assessment of functional response of quail ileum to acetylcholine

Contractile responses to ACh in quail ileum were converted to percentage and the graph was plotted against the logarithmic concentration of ACh. The effective concentration (EC₅₀) and pD₂ value were calculated by non-linear regression analysis using GraphPad Prism (Ver.5.0.)

3 Results

The present study was conducted to find the median effective concentration (EC₅₀) of ACh in ileum of Japanese quail.

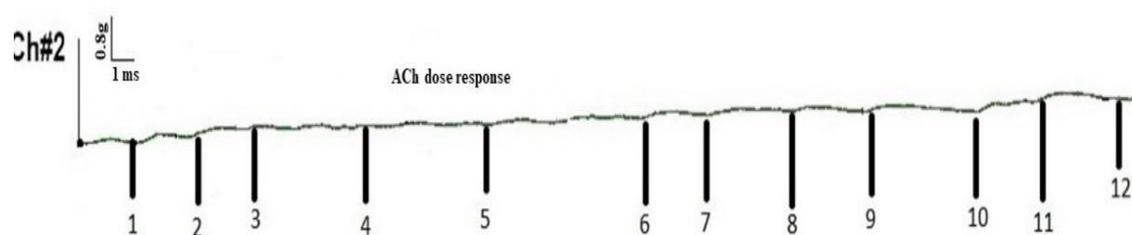


Figure 1: Representative physiographic recording of contractile effect of ACh on quail ileum. The numbers on the graph indicate the cumulative doses as given below Dose 1 = 0.5×10^{-9} M; Dose 2 = 1.5×10^{-9} M; Dose 3 = 0.5×10^{-8} M; Dose 4 = 1.5×10^{-8} M; Dose 5 = 0.5×10^{-7} M; Dose 6 = 1.5×10^{-7} M; Dose 7 = 0.5×10^{-6} M; Dose 8 = 1.5×10^{-6} M; Dose 9 = 0.5×10^{-5} M; Dose 10 = 1.5×10^{-5} M; Dose 11 = 0.5×10^{-4} M; Dose 12 = 1.5×10^{-4} M

Representative physiographic recording of cumulative dose response curve of acetylcholine is represented in Figure 1. Mean cumulative graded dose response is given in the table 1. The EC₅₀ value of ACh varied from 1.235×10^{-7} to 2.344×10^{-7} with mean value of 1.701×10^{-7} at 95 % confidence interval (Table 2). The mean pD₂ value of quail ileum for acetylcholine was 6.78 ± 0.08 (Table 3). The cumulative log dose response curve is depicted in Figure 2.

4 Discussion and conclusion

From the results it is evident that muscarinic acetylcholine receptors are present in the ileum of Japanese quail which, as per our knowledge is the first report of muscarinic receptor in Japanese Quail intestine. The EC₅₀ values of acetylcholine alone in ileum of Japanese quail varied from 1.235×10^{-7} M to 2.344×10^{-7} M with mean value of 1.701×10^{-7} M and pD₂ value of 6.769. Since there are no reports of muscarinic receptors in quail, comparison was made with the chicken ileum, where the mean EC₅₀ and pD₂ values of carbachol, another muscarinic receptor agonist was reported to be 3.54×10^{-6} M and 5.45 with (Darroch et al., 2000). The mean EC₅₀ and pD₂ values of acetylcholine were 1.94×10^{-7} M and 6.71 and for carbachol, the mean EC₅₀ and pD₂ values were 5.12×10^{-8} M and 7.29 in chicken proventriculus (Kitazawa et al., 2016). This results conforms to the present findings in quail. In other species of animals the mean EC₅₀ and pD₂ values were 1.23×10^{-6} M and 5.91 in ileum, 9.77×10^{-7} M and 6.01 in trachea and 7.24×10^{-7} M and 6.14 in oesophageal muscularis mucosa for pilocarpine (guinea pig). The mean EC₅₀ and pD₂ values were 1.86×10^{-7} M and 6.73 in ileum, 2.54×10^{-7} M and 6.61 in trachea and 1.54×10^{-7} M and 6.81 in

Table 1 Mean percent contraction of quail ileum with respect to log dose of Ach (n=6)

Acetylcholine Conc. [Log M]	% Contractile response
-9.30103	0
-8.823909	12.49 ± 0.91
-8.30103	22.43 ± 0.89
-7.823909	33.66 ± 1.51
-7.30103	43.93 ± 1.92
-6.823909	53.75 ± 1.88
-6.30103	64.57 ± 1.31
-5.823909	76.38 ± 1.49
-5.30103	89.96 ± 0.56
-4.823909	100
-4.30103	100
-3.823909	100

Table 2 EC₅₀ of ACh on ileum of Japanese quail

Parameters	Mean
LogEC ₅₀	-6.769 ± 0.06974
EC ₅₀	1.701×10^{-7}
95% Confidence Intervals	
LogEC ₅₀	-6.908 to -6.630
EC ₅₀	1.235×10^{-7} to 2.344×10^{-7}

Table 3 pD₂ values of ACh with mean \pm SE

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	Mean \pm SE
ACh	7.02	6.59	6.52	6.96	6.75	6.87	6.78 ± 0.08

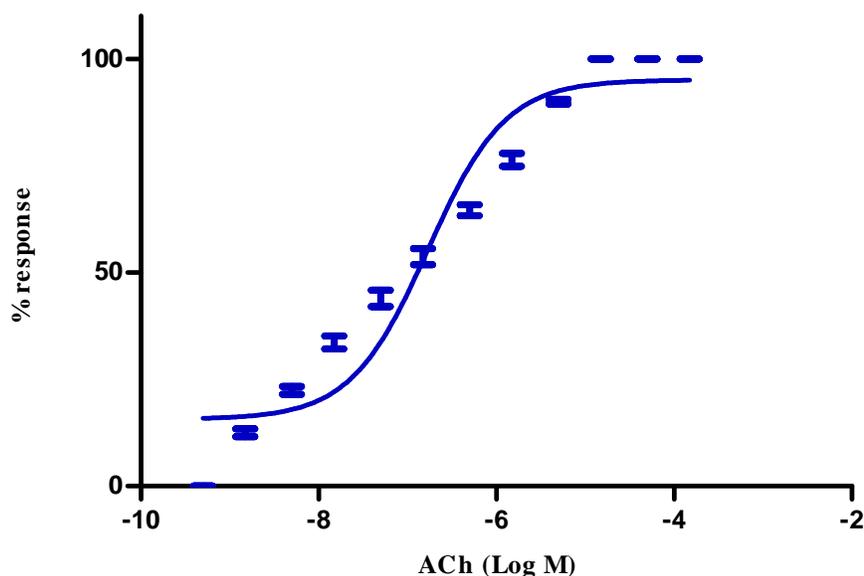


Figure 2 Graded dose response curve of ACh on ileum of Japanese quail

oesophageal muscularis mucosa for carbachol (Eglen & Whiting, 1988). Kamikawa et al. (1985) reported the mean EC₅₀ and pD₂ values were 6.30×10^{-14} M and 13.2 for carbachol and 1.25×10^{-8} M and 7.9 for ACh in longitudinal smooth muscle of guinea pig ileum. All these results indicate that muscarinic receptors are distributed with the same propensity in quails similar to that of other avian and mammalian species.

Muscarinic receptors are widely expressed in many tissues, from neuromuscular junctions, cardiovascular, respiratory and reproductive system to cortical regions of the brain involved in learning and memory (VanPatten & Al-Abed, 2017). Muscarinic receptors play a significant role in maintaining gastrointestinal motility as well as its secretions (Alexander et al., 2017). Besides, antibiotics like aminoglycosides and macrolides should not be given at higher doses for quails as they have inhibitory effect on gastrointestinal motility (Paradelis, 1981). Better understanding of the receptor sub systems in the gastro intestinal tract of quail will help in the judicious selection of therapeutic agents for various ailments in commercial farming, since the gastro intestinal motility is the major determinant of the digestion and absorption of feed and thus modulate the feed conversion ratio of the quails. Hence any drug which can modulate the gastro-intestinal motility should be carefully administered in quails.

Acknowledgement

The author acknowledges the assistance from Indian Council of Agricultural Research (ICAR), New Delhi through post graduate

scholarship (NTS). Financial supports from ICAR through research project (F No.7 (2)/-2011-EPD) and National Bank for Agriculture and Rural Development G.O.(Rt)No.100/12/ AD RIDF XVI KERALA) are thankfully acknowledged.

Conflicts of interest

The authors declare that there are not any conflicts of interests

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