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Responsiveness of duodenal smooth muscle to histamine and carbachol following *Escherichia coli* infection in chickens

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ABSTRACT

The contractile responses of the duodenal segments from healthy and *Escherichia coli* infected broiler chickens on exposure to exogenous histamine and carbachol were studied. Molar concentrations of the agonists were introduced into suspensions of the segments in Tyrodes solution at 37 °C and the responses recorded using kymograph. Both healthy and infected segments responded dose-dependently to the agonists. The healthy segments showed greater sensitivity to the agonists and produced significantly ($P < 0.05$) higher mean maximum responses in all cases. The threshold responses to molar concentrations of the drugs were lower for segments from infected chickens. It is suggested that *E. coli* infection may have altered the contractile responses of chicken duodenum to the agonists.

Key words: chickens, *E. coli*, histamine, carbachol

Introduction

Agonists such as histamine and carbachol have continued to attract attention from investigators of the pharmacology of the autonomic nervous system. Carbachol (carbamylcholine) has both nicotinic and muscarinic activity (EGHIANRUWA, 2002) and is not hydrolyzed by cholinesterase enzymes (BRANDER et al., 1982; LAURENCE et al., 1997). The pharmacological action of carbachol has been observed to mimic that of acetylcholine at neuronal junctions. Carbachol produced an increase in tone and amplitude of contraction of both isolated and intact guinea pig ileum (UEKI et al., 1983). This action

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is mediated by the muscarinic receptors and could be blocked by atropine. JIBIKE et al. (1994) observed that trypanosome infected tissues have higher sensitivity to carbachol than non-infected tissues.

Histamine (P-imidazolyl-ethylamine) is a powerful and consistent stimulant of smooth muscles (GADDUM and PICARELLI, 1957; EGHIANRUWA, 2002). It is also a powerful gastric secretagogue (GANONG, 1999) and induces the contraction of the smooth muscle of the gastrointestinal tract (EYRE and CHAND, 1979). In the intestinal longitudinal muscle of chicks, histamine is known to produce relaxations that were relatively resistant to mepyramine (a H¹ receptor antagonist) and appear to result from the release of adrenaline or noradrenaline (EVERETT and MANN, 1967).

The receptors mediating the action of agonists, such as histamine and carbachol, are known to be widely distributed in the gastrointestinal system, including the duodenum, (LAURENCE et al., 1997; GANONG, 1999). However, changes in receptor population (SCHRRURS et al., 1980) and responsiveness of tissues to agonists are known to occur in some disease conditions (VEENEDAL et al., 1985; DINA and AROWOLO, 1989; JIBIKE et al., 1994). Although much work has been done on the pharmacological effects of agonists such as histamine and carbachol and their antagonists in various animal species (ASH and SCHILD, 1966; LEVINE, 1990), there is no information regarding their effects on isolated smooth muscle preparation in *Escherichia coli* (*E. coli*) infected chickens. The responsiveness of the smooth muscle of the duodenum of *E. coli* infected broiler chickens to histamine and carbachol was therefore studied.

Materials and methods

Experimental animals. Ten broiler chicks aged about 4 weeks, obtained from Ramadam Poultry Farm, Maiduguri, Nigeria were used for the study. The birds were housed in clean cages at the Department of Veterinary Physiology and Pharmacology Laboratory, University of Maiduguri, Maiduguri, Nigeria, and were fed chick mash and given water ad libitum. The chicks were allowed one week's acclimatization before use. The birds were vaccinated against Newcastle disease (intra-ocular) and at three weeks of age (LaSota). Infectious bursal disease vaccine was also administered to the birds when two weeks old.

Drugs. The pharmacological agonists used were histamine acid phosphate (British Drug House Biochemicals, Poole, UK) and carbachol (carbamylcholine chloride) (Sigma Chemical Company, U. S. A.) The agonists were dissolved in sterile distilled water and stock solutions of 10⁻²M were prepared. Further dilutions (10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷ and 10⁻⁸ M) were made from the stocks as needed.

The *Escherichia coli* used was the haemolytic strain, isolated in the Department of Veterinary Microbiology and Parasitology, University of Maiduguri. The birds were fasted for 12 hours and then infected by oral administration with the organisms in drinking water. The birds were sacrificed 24 hours after *E. coli* administration when apparent clinical

signs of infection, such as anorexia and increased body temperature, became obvious, as confirmed by the Eijkman test (HENRIKSEN, 1954).

Experimental groups. The chicks were separated into two equal groups of 5 birds each, one consisting of healthy birds while the other was infected with *E. coli*.

Tissue preparation. The birds were killed and exsanguinated by cutting the jugular vein and carotid artery in the neck. They were then dissected and the duodenum removed. The duodenum was cut into 2-3 cm segments and immersed in aerated Tyrode's solution maintained at room temperature (25 °C). To observe the effect of the agonists on the duodenal segments the method of RAND and DALE (1989) was used. Following routine preparation of the segments, a thread was carefully attached to each end of the tissue segment without occluding the lumen. One end of the tissue segment was mounted on the organ bath (Scientific and Research Instrument Ltd, England) containing Tyrode solution maintained at 37 °C. The other end was linked to a kymograph (Harvard Instruments) via a frontal writing lever, with a resting tension of 2 g. The isometric contractions of the segments were first recorded for 1 minute with kymograph speed set at 0.025 mm/sec. Various concentrations of carbachol and histamine were prepared and each of them was tested, starting with the lowest concentration, and the response of the segments recorded.

Contact time for each concentration was 30 seconds, after which the drug was washed off the segment by draining and filling the bath with fresh Tyrode's solution at least 3 times. The procedure was carried out for each concentration of the agonist in triplicate, using randomly selected segments from healthy and *E. coli* infected birds. The amplitude of contractions was measured by the standard method and the degree of agonist effect expressed as a percentage.

Statistics. Data obtained were statistically analyzed using unpaired Student's *t*-test. Significant differences between the responses of infected and healthy segments were detected at 95% confidence limits (STEEL and TORRIE, 1980).

Results

The mean responses of both normal and *E. coli* infected duodenal segments to graded concentrations of carbachol and histamine are shown in Figs 1 and 2. The isolated duodenal preparations of the healthy and infected segments were sensitive to both agonists. The dose-response curves for carbachol are shown in Fig. 1, while that of histamine is shown in Fig. 2. In all cases, the dose-response curves of the segments from infected birds shifted to the right, while the peak responses were significantly ($P < 0.05$) lower than those of the healthy segments. While the healthy segments produced curves, which fit the sigmoid shape, the infected segments seemed to respond to the agonists in an irregular manner.

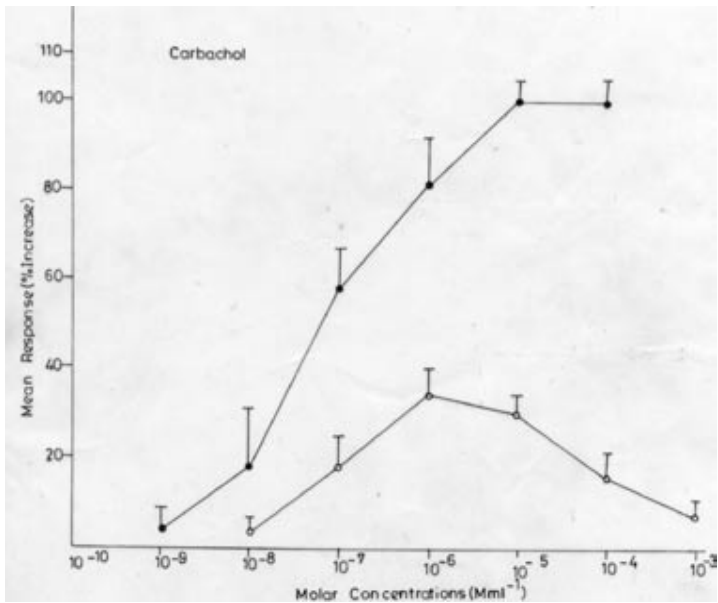


Fig. 1. Mean (\pm SD) contractile response of duodenal segment from healthy (\bullet) and *E. coli* infected (\circ) broiler chicks exposed to a graded concentration of carbachol.

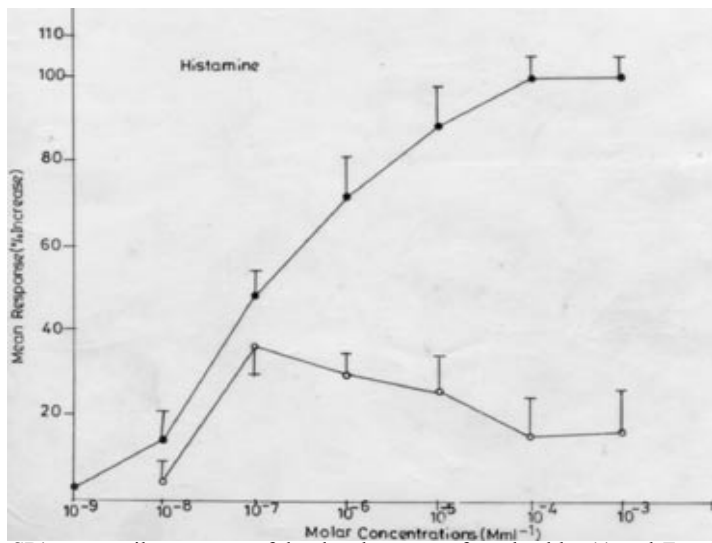


Fig. 2. Mean (\pm SD) contractile response of duodenal segment from healthy (\bullet) and *E. coli* infected (\circ) broiler chicks exposed to a graded concentration of histamine

Discussion

The result of this study showed that carbachol and histamine produced contractile responses in isolated duodenal segments of both *E. coli* infected and healthy chicks at a bath temperature of 37 °C, dose-dependently. Similar findings have been reported in *Gallus domesticus* caecal segments (AMEH et al., 1994). Concentration-dependent contractions of pulmonary artery or vein and bronchial artery of cattle to carbachol, histamine, and serotonin (5-hydroxytryptamine) have also been demonstrated by AROWOLO and EYRE (1979).

The contractions of the duodenal segments by carbachol are believed to be achieved through the muscarinic receptors (BOLTON and FIELD, 1977), while the histamine effect observed is obtained by stimulation of H¹ receptors present in duodenal smooth muscles (EYRE and CHAND, 1979).

E. coli infection appeared to alter significantly ($P<0.05$) the duodenal responses to the agonists used. There were qualitative and quantitative differences in the response of healthy and infected duodenal segments to the same concentration of the agonists. The infected segments demonstrated threshold responses to higher concentrations of the agonists with the resultant shifting of the dose-response curve to the right. Also, the peak response of the infected segments was significantly ($P<0.05$) lower than those of the healthy segments. It appears that *E. coli* infection renders the tissues less sensitive and weaker in response to carbachol and histamine. This observation agrees with the earlier finding that *E. coli* infection in pigs decreased gastrointestinal activity. Similarly, toxic metabolites and by-products of gut microflora were reported to decrease the activity of the gastrointestinal tract (KENWORTHY, 1976; DINA and AROWOLO, 1989). JIBIKE et al. (1994) have also observed decrease contractile activity of ileum and jejunum, respectively, in trypanosome infected guinea pigs. Infections with bacterial organisms such as *E. coli* are known to induce pathological changes and influence metabolic activity of tissues, including the duodenum. Such tissue damage may have affected the normal response of duodenal smooth muscle to carbachol and histamine, possibly due to changes in receptor population and characteristics due to the disease.

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SAŽETAK

Istraživane su kontraktilne reakcije pojedinih dijelova dvanaestnika zdravih tovnih pilića i pilića inficiranih bakterijom *Escherichia coli* na izloženost egzogenom histaminu i karbakolu. Molarne koncentracije agonista pripremljene su u Tyrodeovoj otopini na 37 °C, a odgovori su bilježeni pomoću kimografa. I zdravi i inficirani dijelovi reagirali su ovisno o dozi agonista. Zdravi dijelovi pokazivali su veću osjetljivost na agoniste i proizveli su u prosjeku značajno ($P < 0,05$) više reakcije u svim slučajevima. Granične reakcije na molarne koncentracije lijekova bile su niže u dijelovima inficiranih pilića. Smatra se da infekcija bakterijom *E. coli* može promijeniti kontraktilne reakcije pilećeg dvanaestnika na agoniste.

Ključne riječi: dvanaestnik, tovni pilići, histamin, karbakol, *E. coli*
