

IN VITRO STUDY OF ACETYLCHOLINE AND HISTAMINE INDUCED CONTRACTIONS IN COLON AND RECTUM OF ADULT AND NEONATE RATS

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Abstract : Contractile mechanisms of different parts of the gut in adult and neonate may not be identical due to developmental processes. The present study was undertaken to investigate acetylcholine (ACh) and histamine induced contractile responses of colon and rectum in adult and neonatal albino rats. Contractile responses were recorded from isolated *in vitro* preparations. The dose-response curve for ACh (0.001-100 μ M) revealed dose dependent increase in contractile responses. A significantly ($P < 0.05$) greater contractile responses (g/g wet tissue) was observed in rectum as compared to colon. Atropine pretreatment significantly blocked ACh responses in both rectum and colon. The blockade was higher in adult preparations. The dose-response study for histamine (0.001-100 μ M) did not show any significant difference between rectum and colon. Histamine (100 μ M) induced contractions were significantly ($P < 0.05$) increased after pretreatment with pheniramine (100 μ M) in adult rectum. This potentiating response of pheniramine was absent in neonate rectum. Such effect was also not seen in colon of both adult and neonate. The present investigation indicates that the contractile responses induced by ACh are similar in both adult and neonate, excepting that the blocking effect of atropine in colon was more pronounced in adult as compared to neonate. Further, the results also indicated different mechanism of histamine action in adults and neonates as evidenced by the significant enhancement of contractions by pheniramine only in adult rectum. Therefore, the present results indicate the existence of a different cholinergic and histaminergic activity in adult and neonate as well as in rectal and colonic tissue.

Key words : acetylcholine
neonate rectum

histamine
colon rat

INTRODUCTION

Although the gut motility disorder in neonate is very common and important

cause of neonatal morbidity and mortality in human but there are very few studies available on gut contractility in neonate, especially those affecting colorectal area. It

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has been reported that contractile pattern of colon in rat changes during first few weeks of post natal life before attaining the adult pattern (1). Similarly, in neonatal mouse colon, spontaneous motor activity changes during first 10 days of postnatal period have been observed (2). Thus, a difference in contractility is possible in adult and neonatal gut.

Studies showed that acetylcholine causes contractions in different part of the gut obtained from adult of different species including guinea pig, human and rat etc. that could be blocked by atropine (3, 4, 5). Acetylcholine elicited contractions were also studied in adult human and rabbit distal colon, rat ileum, guinea pig ileum and colon, dog ileum and colon and human ileum and these contractions were mediated by muscarinic M_2 and M_3 receptors (3, 4, 5, 6, 7, 8, 9).

Histamine was also found to induce contractions in guinea pig small and large intestine (10). Another study reported that histamine induces contractions of guinea pig colon but not of rat colon (11) and these histamine induced contractions appeared to be mediated by H_1 receptor (10, 11). Histamine also induced smooth muscle contraction in adult human and rabbit distal colon (3). In one report which included neonatal rabbit ileum showed more sensitivity to histamine than the adult ileum (12).

Thus, most of the studies were carried out in adult animals and very little is known about the cholinergic and histaminergic activity of neonatal colon and rectum. Therefore, the present study was undertaken

to investigate the effect of acetylcholine and histamine and their antagonists atropine and pheniramine maleate (H_1 receptor antagonist) on rectum and colon contractile responses *in vitro* in neonate and adult rat.

METHODS

Animals

Adult and neonatal Albino rats of Charles Foster strain weighing 150–200 g (5-7 months) and 10-25 g (1-2 weeks) were used respectively. The animals were individually housed in a temperature, humidity and light controlled room (12 h light and 12 h dark) with an *ad libitum* supply of food and water. The animal experiments were performed after obtaining animal ethical clearance from the Ethical Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Dissection of animal

Adult Rats were sacrificed by cervical dislocation and exsanguinations while neonatal rats were sacrificed by decapitation. The area of gut was opened immediately and part of the gut like, colon and rectum were dissected out and placed in a petri dish containing chilled Krebs Ringer solution bubbled with 100% O_2 .

Mounting and recording of contractile response

After cleaning, longitudinal segments (1.5 cm) of either colon or rectum was dissected out and placed in an organ bath (10 ml) containing Krebs-Ringer solution maintained at $37\pm 1^\circ\text{C}$ and continuously bubbled with 100% O_2 . The tissue segments were mounted

vertically. One end of the tissue was fastened to a glass tube support, and the other end was fixed to a force transducer (MLT 0210, AD Instruments, Sydney, Australia) with an initial tension of 0.5 g.

The preparations were allowed to equilibrate for 30 minutes before taking the control recordings. Isometric contractions were amplified by bridge amplifier and digitized via an analog/ digital interface (Power Lab 4/ST system) to acquire onto a personal computer. The contraction recording was displayed and analyzed with the help of software Chart-5 for windows (AD Instruments, Sydney, Australia). Before, as well as after recording the contractile responses, calibration for the tension (0-10 g) was performed. After stabilization, the initial recordings of spontaneous contractions were made for 30 minutes without any external chemical interventions. Subsequently, the tissue segment was exposed to different concentrations of drugs and recording was continued for another 30 minutes.

Drugs and solutions

Krebs Ringer solution was prepared with following compositions (in mM/L): NaCl, 119; KCl, 4.7; CaCl₂.2H₂O, 2.5; KH₂PO₄, 1.2; MgSO₄.7H₂O, 1.2; NaHCO₃, 5; and glucose, 11 and pH of the solution was 7.4.

Acetylcholine chloride, histamine dihydrochloride and atropine sulphate were procured from Sigma Chemicals Inc (St Louis MO, USA) and histamine antagonist pheniramine maleate was obtained from Aventis Pharma Limited, Bangalore, India. The stock solution (10⁻² M) of all the drugs was prepared in distilled water. The final

dilution was made in the Krebs Ringer solution.

Experimental protocol

Adult animals were divided into two groups (each group of 5 rats). In the first group, after taking initial recording of contractile response from colon and rectum, the tissue was subjected to bath application of different concentration of histamine (0.001-100 µM) to record the dose-response relation. The tissue was washed with Krebs Ringer solution after obtaining dose-response with histamine and then preparations were treated with pheniramine maleate (100 µM) followed by histamine (100 µM) application to assess the effect of H₁ antagonist on histamine induced contractions.

In the second group, dose-response in rectum and colon segments was determined by application of different doses of acetylcholine (0.001-100 µM). Thereafter, ACh (100 µM) induced contractions were recorded after atropine (100 µM) pretreatment.

Similarly, neonatal rats were also divided into two groups (n=6 in each group) to assess histamine and Ach induced contractions by following same experimental protocol as used in adult rats.

Statistical analysis

The mean±SEM value of each group was calculated. The dose-response relationships between two groups were compared using two-way ANOVA followed by the Student-Newman-Keuls test for multiple comparisons. A Student's t-test for paired and unpaired

observations was also performed wherever applicable. A value of $P < 0.05$ was considered significant.

RESULTS

Acetylcholine (ACh) induced contractions

In neonate as well as adult rat rectum and colon, ACh (0.001-100 μM) produced concentration dependent contractions. ACh produced greater contractile responses in rectum as compared to colon ($P < 0.05$, two way ANOVA) in both adult and neonatal rat (Fig. 1 and 2). The contractile responses in adults were not significantly different from

that of neonates. Rectum and colon of adult and neonate produced maximum contractile responses at 100 μM concentration.

Effect of atropine on ach induced contractions

In neonatal rat rectum, atropine (100 μM) pretreatment blocked 63.29% of ACh (100 μM) induced contractions while in colon, atropine pretreatment blocked 49.77% of ACh induced contractions. In adult rat atropine pretreatment blocked 76.40% of ACh induced contractions in rectum, and 86.51% in colon (Fig. 3).

Atropine (100 μM) significantly ($P < 0.05$,

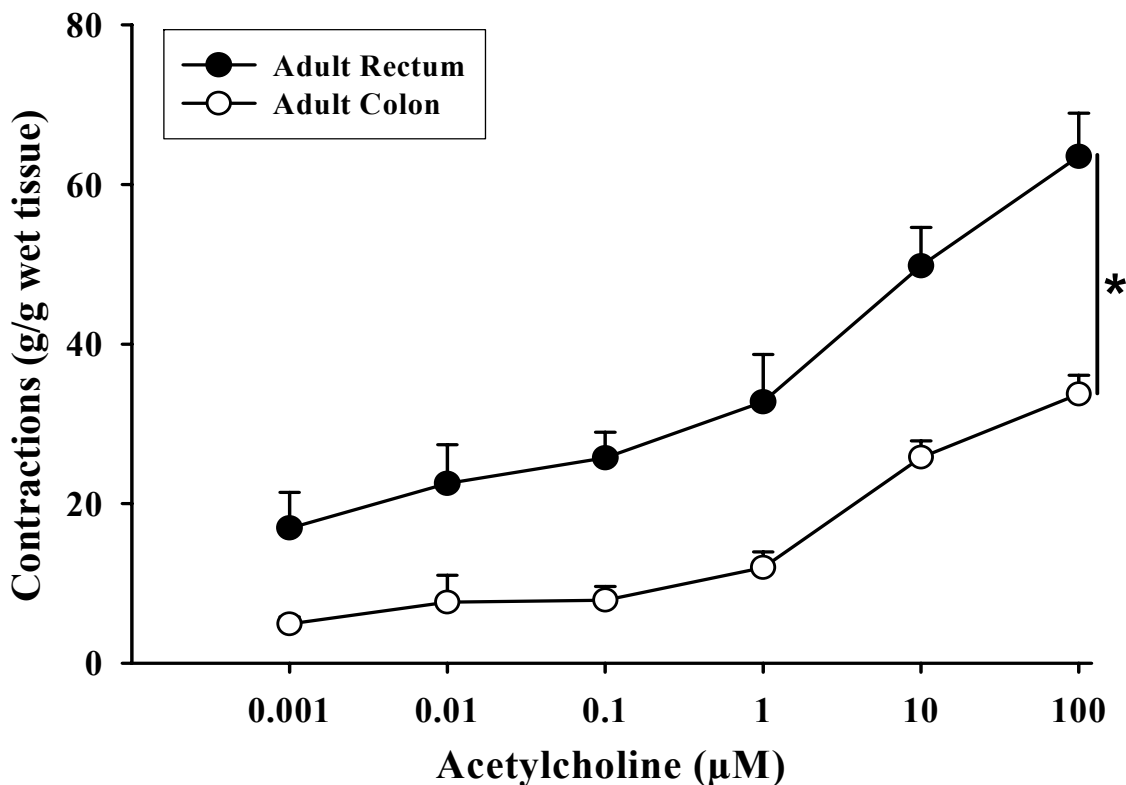


Fig. 1: The contractile responses (g/g wet tissue) of adult rat rectum and colon to the treatment with different concentration (0.001-100 μM) of acetylcholine (ACh). Data points indicate (mean \pm SEM, n=5) values. ACh induced contractions in neonatal rat rectum were significantly ($P < 0.05$) higher to that of colon, (Two way ANOVA as indicated by asterisk).

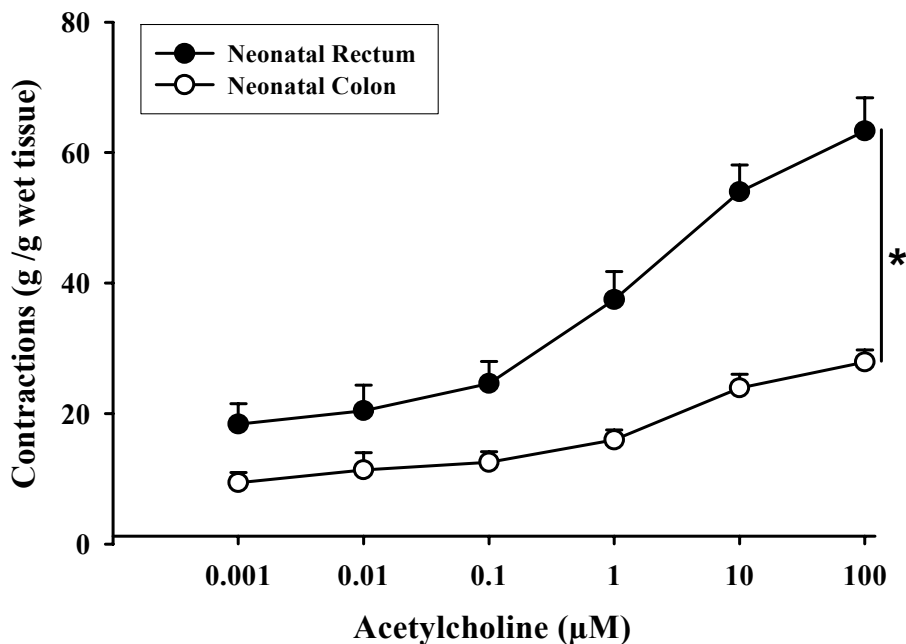


Fig. 2: The contractile responses (g/g wet tissue) of neonatal rat rectum and colon to the treatment with different concentration (0.001-100 μ M) of acetylcholine (ACh). Data points indicate (mean \pm SEM, n=6) values. ACh induced contractions in neonatal rat rectum were significantly ($P < 0.05$) higher to that of colon, (Two way ANOVA as indicated by asterisk).

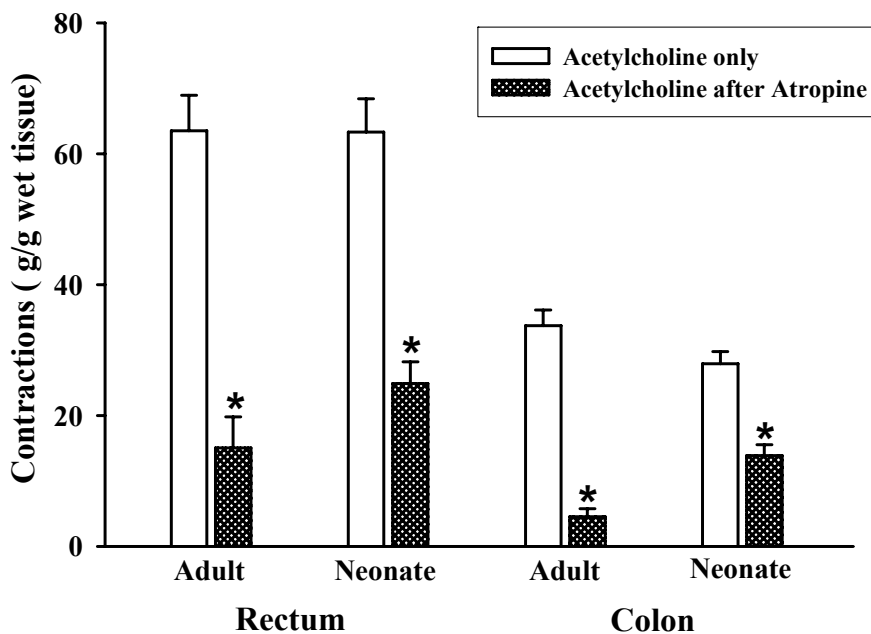


Fig. 3: Histograms showing the effect of Acetylcholine (ACh, 100 μ M) before and after atropine (100 μ M) application in adult and neonatal rectum and colon. Atropine pretreatment significantly blocked ACh induced contractions in both adult and neonatal rectum and colon. Asterisk (*) indicates significantly ($P < 0.05$, Student's *t*-test, paired) different values.

Student's *t*-test, paired) blocked ACh induced contractile responses in both neonate and adult rat rectum and colon (Table II and Fig. 3). ACh (100 μ M) induced contractile responses in rectum of adult was not significantly different ($P>0.05$, Student's *t* test, unpaired) from that of neonate (Table I). Further, these contractile responses in colon of adult and neonate were not significantly different ($P>0.05$, Student's *t* test, unpaired) while it produced significant higher blockade ($P<0.05$, Student's *t* test, unpaired, table I) in adult after pretreatment with atropine.

Histamine induced contractions

In adult as well as neonate, histamine (0.001-100 μ M) produced concentration-dependent contractions in rectum and colon (Fig. 4 and 5) with no significant difference ($P>0.05$, two way ANOVA). As compared to

TABLE I: Showing statistical significance after comparison of contractile responses (induced by chemical agents) between adult and neonatal rat (n=5-6) colon and rectum.

Chemical agents	Compared between	P value
Acetylcholine (100 μ M)	Adult vs. Neonate Rectum	>0.05 (0.979)
	Adult vs. Neonate Colon	>0.05 (0.080)
Atropine (100 μ M)+ Ach (100 μ M)	Adult vs. Neonate Rectum	>0.05 (0.115)
	Adult vs. Neonate Colon	<0.05 (0.001)*
Histamine (100 μ M)	Adult vs. Neonate Rectum	>0.05 (0.449)
	Adult vs. Neonate Colon	>0.05 (0.306)
Pheneramine (100 μ M)+ Histamine (100 μ M)	Adult vs. Neonate Rectum	>0.05 (0.660)
	Adult vs. Neonate Colon	>0.05 (0.912)

Student's *t*-test for unpaired observations was used. A P value <0.05 was considered as significant as indicated by asterisk. Values in parentheses indicate actual P value.

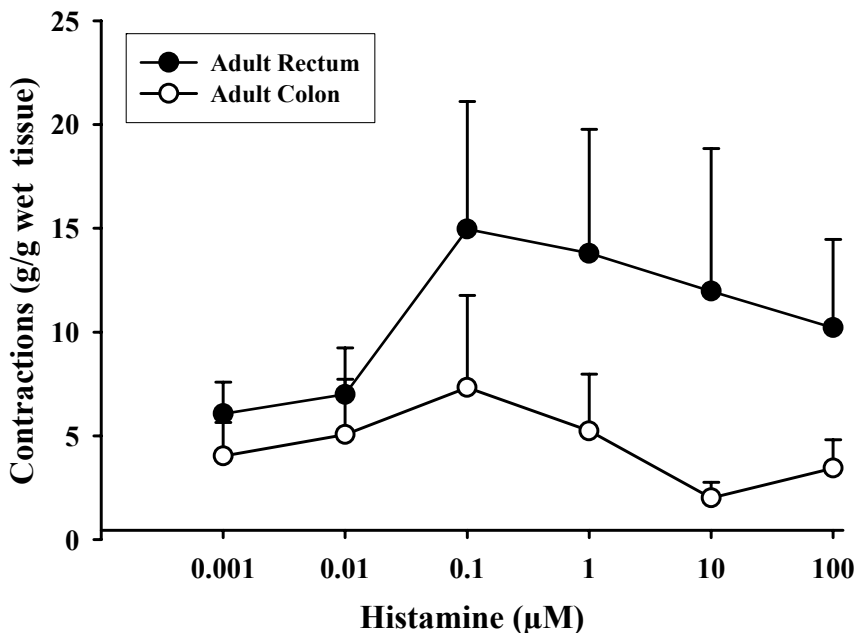


Fig. 4: The contractile responses (g/g wet tissue) of adult rat rectum and colon to the treatment with different concentration (0.001-100 μ M) of histamine. Data points indicate (mean \pm SEM, n=5) values of histamine induced contractions. Histamine induced contractions in adult rat rectum and colon were not significantly different ($P>0.05$, two way ANOVA).

TABLE II: Showing comparison of ACh and histamine induced contractile responses between colon and rectum in adult and neonate (n=5-6) before and after application of antagonist (atropine and pheniramine).

Treatment	Segment of gut	Contractile responses (g/g wet tissue) (mean±SEM)		P value
		Before	After	
ACh (100 µM) application before and after Atropine (100 µM)	Adult Rectum	63.51±5.41	15.03±4.74	<0.05*
	Adult Colon	33.73±2.37	4.55±1.23	<0.05*
	Neonate Rectum	63.31±.06	24.88±3.33	<0.05*
	Neonate Colon	27.90±1.86	13.86±1.68	<0.05*
Histamine (100 µM) application before and after Pheneramine (100 µM)	Adult Rectum	10.21±4.26	23.24±7.97	<0.05*
	Adult Colon	3.45±1.37	8.00±5.26	>0.05
	Neonate Rectum	14.56±3.46	27.13±3.08	>0.05
	Neonate Colon	6.10±2.01	8.73±3.70	>0.05

Data are expressed as mean±SEM. P<0.05 considered as significant (Student's *t*-test for paired observations)

colon both neonatal and adult rat rectum produced more contractile responses but without any statistical significance (P>0.05, two way ANOVA).

Effect of pheniramine on histamine induced contractions

In present study pheniramine pretreatment did not block histamine induced contraction but it potentiated the contractions. In case of neonate, pheniramine (100 µM) pretreatment enhanced histamine (100 µM) induced contractions by 86.33% in rectum and 43.11% in colon. In case of adult pretreatment with pheniramine, histamine induced contractions were increased by 127.5% in rectum while the increase was 31.88% in colon (Fig. 6).

However, the histamine (100 µM) induced contractile responses in rectum and colon (Table I) of adult was not significantly different (P>0.05, Student's *t*-test, unpaired) from those of neonate either before or after pretreatment with pheniramine (100µM).

Histamine (100 µM) induced contractions were significantly (P<0.05, Student's *t*-test, paired) increased after pretreatment with pheniramine (100 µM) in adult rectum only. This potentiating response of pheniramine was not seen in neonate rectum. Such effect was also not seen in colon of both adult and neonate (Table II and Fig. 6).

DISCUSSION

In present study it was observed that ACh and histamine (0.001-100 µM) produced dose-dependent contractions in rectum and colon of both adult and neonate. ACh produced greater response in rectum as compared to colon of both adult and neonates (Fig. 1 and 2). It may be noted that both adult and neonate produced similar type of contractile responses to ACh. Similar results were also reported in rabbit ileum where the slope of the dose-response curve and height of the maximum contraction on treatment with ACh were approximately same in adult and neonate (12). Thus it appeared that gut as a whole (small intestine

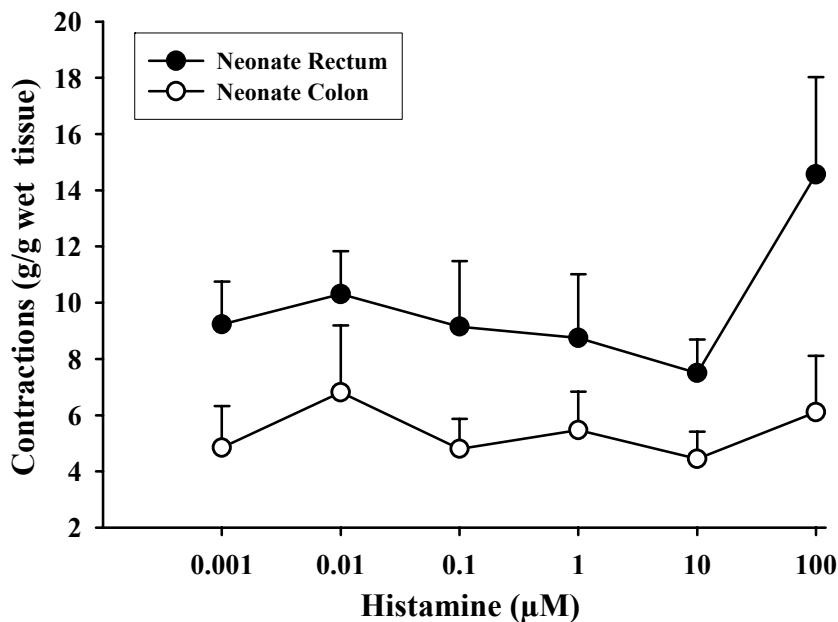


Fig. 5: The contractile responses (g/g wet tissue) of neonatal rat rectum and colon to the treatment with different concentration (0.001-100 μM) of histamine. Data points indicate (mean \pm SEM, n=6) values of histamine induced contractions. Histamine induced contractions in neonatal rectum and colon were not significantly different ($P>0.05$, two way ANOVA).

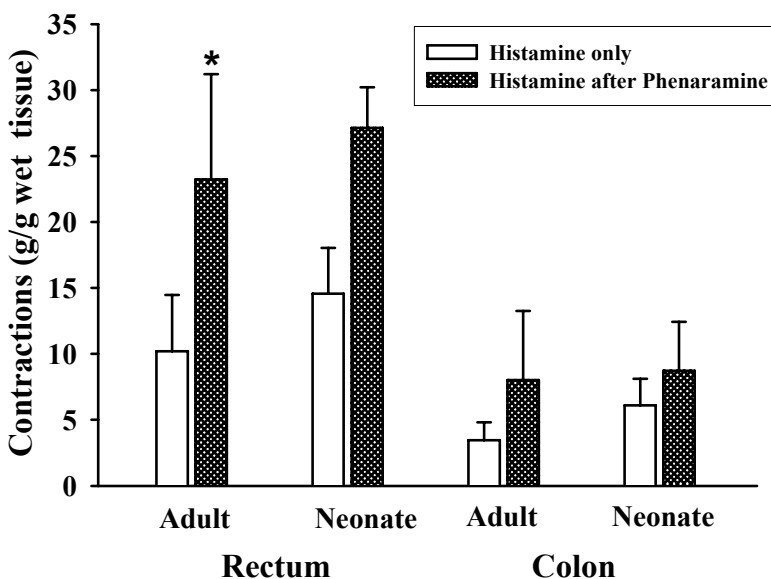


Fig. 6: Histograms showing the effect of histamine (100 μM) before and after pheneramine (100 μM) application in adult and neonatal rectum and colon. Pheneramine pretreatment did not significantly ($P>0.05$) block histamine induced contractions. However, it significantly enhanced the histamine induced contractions in adult rectum. Asterisk (*) indicates significantly ($P<0.05$, Student's *t*-test, paired) different values.

or large intestine) may show similar response to ACh in both rabbit and rats. Atropine (100 μM) pretreatment blocked ACh (100 μM) induced responses in both adult and neonatal rectum and colon indicating that cholinergic contractions were mediated by muscarinic receptors. The blocking effect of atropine was also reported in human newborn (13). Similar results of cholinergic agonists have been reported in healthy human and rabbit distal colonic smooth muscle (3). Earlier studies showed that ACh produced initially rapid phasic contractions, followed by a tonic contraction in human ileum (9), in adult rat ileum (4) and rectum (14). This, ACh elicited contractions are believed to be mediated through M_3 receptors in most of the species and different region of the gut including rat ileum, guinea pig ileum and colon, dog ileum and colon and human ileum (4, 5, 6, 7, 8, 9). In the present investigation greater blockade observed in the adult as compared to neonate may have resulted from the increase in number of ACh receptors or their sensitivity due to developmental process.

The histamine (0.001-100 μM) also produced dose dependent contractile responses without any significant difference between rectum and colon of both adult and neonate (Fig. 6 and 5). The response of histamine was substantially less than the effect of ACh and such reduced responsiveness to histamine was also observed in human newborn colon (13). Thus, there may not be any difference of mechanism of histamine actions in human and rat neonate. Histamine has been shown to produce its contractile effects mainly through H_1 receptors (15). Histamine was also reported to produce a dose-dependent

contraction of smooth muscles of large and small intestine in adult guinea pig (10) and in adult rabbit ileum (16). Other studies also reported that histamine and its analogue 2-pyridyl-ethylamine induced concentration-dependent contraction in adult guinea pig colon through direct activation of H_1 receptor (11).

In the present study pheniramine pretreatment (100 μM) significantly augmented histamine (100 μM) induced contractions in adult rectum. However, potentiating response of H_1 blocker has been also reported in adult rabbit ileum (16). While in case of human newborn colon it was reported that histamine induced contractions were blocked by pheniramine pretreatment and indicated involvement of H_1 receptors in histaminergic contractions (13). This signifies that the histaminergic mechanism of colon in human neonate and rat neonate may not be similar. The potentiating response of pheniramine was not seen in neonate rectum. Thus, observations in the present study indicated that the histaminergic system in adult and neonate rectum may not be identical.

It may be concluded that during developmental process the cholinergic sensitivity is altered and also colon and rectum have differential sensitivity and the histaminergic influence is different in adult and neonate rectum. Thus, these knowledge may be useful while selecting antihistaminic or anticholinergic drugs in adults and neonates for the treatment of their rectal or colonic ailments.

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