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In vitro Study of Adrenaline-induced Contractile Tension and Frequency of Colon and Rectum in Neonate and Adult Rats.

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ABSTRACT

Adrenaline is known to induce relaxation in different part of the gastrointestinal tract in various species. However, it is not clear whether its effects on large gut contractility in neonates are similar to adult. Therefore, present study was undertaken to examine the effect of adrenaline on colon and rectum of neonate and adult rats. *In vitro* isometric contractions were recorded using organ bath preparations in colon and rectum from isolated large gut segments (colon and rectum) from neonate and adult albino rats in presence or absence of adrenaline and its antagonist. Adrenaline caused dose-dependent relaxation in colon and rectum of both adult and neonate rats. However, colon was more sensitive to adrenaline as EC_{50} was 0.44 µM as compared to rectum where EC_{50} was 3.2 µM. In case of neonate, rectum has greater susceptibility to the relaxing effect of adrenaline and EC_{50} was more than 100 µM in both colon and rectum of neonate rats. Further, adrenaline treatment decreased frequency of contraction in colon and rectum of both adult and neonate and more decrease was observed in adult rectum than colon. It is concluded that adrenergic mechanism exists right from the birth but the differences in the sensitivity and responses to adrenaline in adult and neonate indicated the changes in receptors or other signaling molecules during the process of development from neonate to adulthood.

Keywords: Neonate rat, adrenaline, propranolol, labetalol, colon, rectum

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INTRODUCTION

Adrenaline is a hormone and neurotransmitter produced by adrenal glands as well as by certain neurons. It is one of the most important catecholamines known to inhibit gut motility in different species. Bayliss and Starling were first to report inhibitory sympathetic influence on gut motility in the small intestine of dogs [1]. Adrenaline exerts its action on diverse body functions through α and β-receptors with their subtypes (α_1-α_2 and β_1- β_3). The structure, distribution and functions of these receptors in various species are described elsewhere [2-11]. Stimulation of these α and β adrenergic receptors produced mainly inhibitory response [12]. The importance of the two receptor populations varies in different parts of the alimentary tract; β-adrenoceptors on the smooth muscle are almost always inhibitory and α-adrenoceptors may mediate excitation or inhibition [12-15]. Further, studies in human colon, rat jejunum and ileum reported that α_1 and β_1-β_3 receptors were involved in inhibition of contractile activity [5, 8, 16]. Studies in human isolated taenia coli demonstrated that stimulation of α- and β-receptor by adrenaline and other agonist caused relaxation [17]. Agonists for α-β-receptors including adrenaline or stimulation of sympathetic nerves are known to induce relaxation in different parts of the gut in different species e.g. rat colon, human and rabbit taenia coli, rabbit ileum, sheep small intestine, mice colon [8, 13, 15, 18-21].

Relaxation induced by adrenaline or other agonists in rat colon, human and rabbit taenia coli could be blocked by β-blocker propranolol and oxprenolol [18, 19]. In sheep small intestine inhibitory effect of isoprenaline was abolished by propranolol [21], whereas in mice colon propranolol along with prazosin (α-blocker) inhibited the relaxation to adrenaline [13]. Study in human sigmoid colon also reported involvement of β-adrenoceptor in relaxation of gut [22]. Further, blockade of β-adrenoceptor reported to increase the propulsive force associated with small intestinal contractions in humans. [23] Inhibition of longitudinal muscle contractility of rat colon by sympathetic nerve stimulation was also blocked by propranolol [15].

All of the above studies were reported in adult animals but the action adrenaline in neonate gut is not known. Since, various transmitters, receptors and signaling mechanisms have been shown to develop or change during postnatal period, the action of adrenaline on neonate gut may not be same as that of adult. Therefore, the present study was carried out to evaluate the effect of adrenaline and its antagonist propranolol and labetalol on large gut for better understanding of its physiological role in large gut motility, especially in neonate.

MATERIAL AND METHODS

The animal experiments were performed as per guideline of the Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Animals

Adult Albino rats of *Charles Foster* strain of 4-6 months and neonate of 10-16 days of same strain were used. The animals were 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.

Dissection of animal

Adult rats were sacrificed by cervical dislocation and exsanguinations while neonate rats by decapitation. The abdomen was opened by midline incision and part of the gut (colon and rectum) was dissected out. Thereafter they were cleaned by flushing out the gut contents and placed in a petri dish containing chilled Krebs-Ringer solution bubbled with 100% O_2.

Mounting and recording of contractile response

The detailed procedure for recording of contractions has been described earlier elsewhere [24, 25]. In brief, 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 recordings were analyzed with the help of software 'Chart-5 for Windows' (AD Instruments, Sydney, Australia). After stabilization, spontaneous contractions were

recorded for 30 minutes at 37ºC. Subsequently, the gut segments were exposed to different concentration of adrenaline (0.01-10 µM) in a cumulative manner. Propranolol (10 µM), an adrenaline β-blocker and labetalol (10 µM), an α-β-blocker was used to assess the blocking effect on adrenaline action on contractile response.

After recording of contractions, the segment of tissue was removed from the organ bath and placed on blotting paper for soaking the extra water from the tissue. The wet tissue was then weighed in a fine balance to express the contractile response per unit weight of tissue (g/g wet tissue).

Drugs and solutions

Adrenaline bitartrate was procured from S.D. Fine-Chemi. Ltd. Mumbai, India. Propranolol was obtained from Abbott Healthcare Pvt. Ltd.,HP, India. Labetalol was bought from Samarth life sciences Pvt. Ltd., HP, India. A stock solution (10 mM) of Adrenaline bitartrate, Propranolol and Labetalol were prepared in distilled water. Further dilution was made in Krebs-Ringer solution prior to experiments. Krebs-Ringer solution was prepared with following compositions (in mM/L): NaCl, 119; KCl, 4.7; $CaCl_2.2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 1.2; $NaHCO_3$, 5; and glucose, 11 and pH of the solution was 7.4.

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EXPERIMENTAL PROTOCOL

Two sets of experiments were carried out involving both adult and neonate rats. In the first set of experiments, after obtaining initial recording of spontaneous contractions from colon and rectum of adult (n = 4-6) and neonate (n = 4-6) rats, the tissue was subjected to different concentration of adrenaline (0.01-10µM) for 10 minutes in a cumulative manner to obtain the dose-response curve.

In the 2nd set of experiments, adult (n = 4-6) and neonate rats(n = 4-6) tissue was pretreated with propranolol (10 µM, n=4-6) or labetalol (10 µM, n=4-6) for 10 minutes. Subsequently, gut segment was subjected to different concentration of adrenaline as mentioned above in the presence of propranolol and labetolol.

STATISTICAL ANALYSIS

The contractile tensions were expressed in the terms of % of initial tension (g/g wet tissue) of control experiment and contractile frequency was expressed in term of contractions/min. The mean ± SEM values of % of initial contractile tension (g/g wet tissue) and frequency (contractions/min) in both colon and rectum of adult and neonate rats were calculated. Dose-response relationships between two groups were compared by using two-way ANOVA (Graph Pad Prism 4 software). A Student's t-test for paired and unpaired observations was also performed wherever applicable. A value of $P \leq 0.05$ was considered as significant.

RESULTS

1: Effect of adrenaline on contractile response in colon and rectum of adult and neonate rats:

1a. In adult, adrenaline decreased contractile tension in a dose-dependent manner

Adrenaline (0.01 µM- 10 µM) caused decrease in contractile tension in both colon and rectum of adult rats in a concentration-dependent manner. Contractile tensions were decreased by 69% and 64%, on application of 10 µM of adrenaline in colon and rectum, respectively. However, no difference in contractile response between colon and rectum to adrenaline was observed ($p>0.05$, two-way ANOVA, Fig. 1 A & C). EC_{50} was more in rectum i.e. 3.2 µM as compared to colon where EC_{50} was 0.44 µM.

1b. In neonate, adrenaline also caused decrease in contractile tension in a dose-dependent manner

Exposure of tissue to adrenaline produced decrease in contractile tension in both colon and rectum of neonate rats (0.01 µM - 10 µM, Fig. 1 B & D). Contractile tension decreased by 24% and 42% at 10 µM of adrenaline in colon and rectum respectively. When compared, there was significant decline in contractile response in rectum than colon ($p<0.05$, two-way ANOVA, Fig. 1 D). EC_{50} was more than 100 µM in both colon and rectum of neonate.

2: Effect of propranolol (10 µM) pretreatment on adrenaline-induced relaxation in colon and rectum of adult and neonate rats:

2a. *In adult, propranolol (10 µM) pretreatment blocked adrenaline (0.001-10 µM) –induced relaxation in rectum but not in colon.*

In this group (n=4), experiments were performed by pretreating colonic and rectal segments with propranolol (a beta blocker). Propranolol per se failed to block adrenaline activity in colon ($p>0.05$, two-way ANOVA, Fig. 2 A & D) but inhibited its activity in rectum leading to increase in contractile tension ($p<0.05$, two-way ANOVA, Fig. 2 B & E).

2b. In neonate, propranolol (10 µM) pre-application inhibited adrenaline-induced relaxation in both colon and rectum.

Propranolol pretreatment blocked adrenaline-induced decrease in contractile tension in both colon and rectum. Rather it caused increase in contractile tension in colon and rectum of neonate rats ($p<0.05$, two-way ANOVA, Fig. 2 C, F).

3. Effect of labetalol (10 µM) pretreatment on adrenaline-induced contractile response in colon and rectum of adult and neonate rats:

3a. Labetalol (10 µM) pretreatment blocked adrenaline-induced relaxation in adult rectum only

In this group (n=4-5), experiments were performed by pretreating colonic and rectal segments with labetalol (an alpha-beta blocker). Labetalol per se failed to block adrenaline activity in colon ($p>0.05$, two-way ANOVA, figure 6 & 8) but inhibited its activity in rectum leading to increase in contractile tension ($p<0.05$, two-way ANOVA, Fig. 3 A, B, D, E).

3b. In neonate, labetalol (10 µM) pre-application inhibited adrenaline-induced decrease in contractile activity in both colon and rectum

In this group (n=6), pretreatment of labetalol inhibited adrenaline-induced relaxation in contractile tension in both colon and rectum of neonate ($p<0.05$, two-way ANOVA, Fig. 3 C, F).

4. Effect of adrenaline on contractile frequency in colon and rectum of adult and neonate rats before and after propranolol and labetalol treatment:

Contractile frequency decreased as concentration of adrenaline increased and more decrease was observed in adult rectum than colon ($p<0.05$, two-way ANOVA, n=5-7, Fig.4 A) but there was no difference between neonate colon and rectum ($p>0.05$, two-way ANOVA, n=5-7, Fig. 4 D).

Further, propranolol and labetalol (10 µM) pre-application failed to inhibit adrenaline-induced decrease in contractile frequency in colon and rectum of adult rats ($p>0.05$, two-way ANOVA, n=4-7, Fig. 4 B & C and 5 A & B).

However, pre-incubation with propranolol (10 µM) inhibited adrenaline-induced decrease in contractile frequency in both colon and rectum of neonate rats ($p<0.05$, two-way ANOVA, n=4, Fig. 4 E, F). Whereas, pre-treatment with labetalol (10 µM) produced the same effect only in rectum but not in colon ($p<0.05$, two-way ANOVA, n=5-6, Fig. 5, C, D).

DISCUSSION

This is the first study to report the role of adrenaline and its mechanism in the large gut of the neonate. Present investigation showed that adrenaline caused dose-dependent relaxation of large gut segments (colon and rectum) of both adult and neonate rats (Fig. 1 A, B, C & D). Adrenaline (10 µM) produced relaxation in colon and rectum of adult rats by 69% and 64% respectively and there was no difference in

contractile tension between colon and rectum (Fig.1 C). However, colon was more sensitive to adrenaline as EC_{50} was 0.44 µM as compared to rectum where EC_{50} was 3.2 µM. In case of neonate, 10 µM of adrenaline produced decrease in contractile tension by 24% and 42% in colon and rectum respectively (Fig. 1 D). This indicated the greater susceptibility of rectum to the relaxing effect of adrenaline. However, EC_{50} was more than 100 µM in both colon and rectum of neonate rats. Thus, adults were more sensitive to adrenaline as compared to neonates. In earlier studies, similar dose-dependent decrease in contractile response to adrenaline was also seen in adult rat colon, human and rabbit taenia coli, rabbit jejunum, mice colon and rat gastric fundus muscle [13, 19, 20, 26]. In absence of any reports on effect of adrenaline in neonate gut activity, at present, this is the first report to demonstrate that adrenaline-induced relaxation is a little different in neonate gut i.e. adult gut is more sensitive to effect of adrenaline and the difference in sensitivity between colon and rectum are reversed in adult. This change in sensitivity appears to be dependent on the developmental and maturation process.

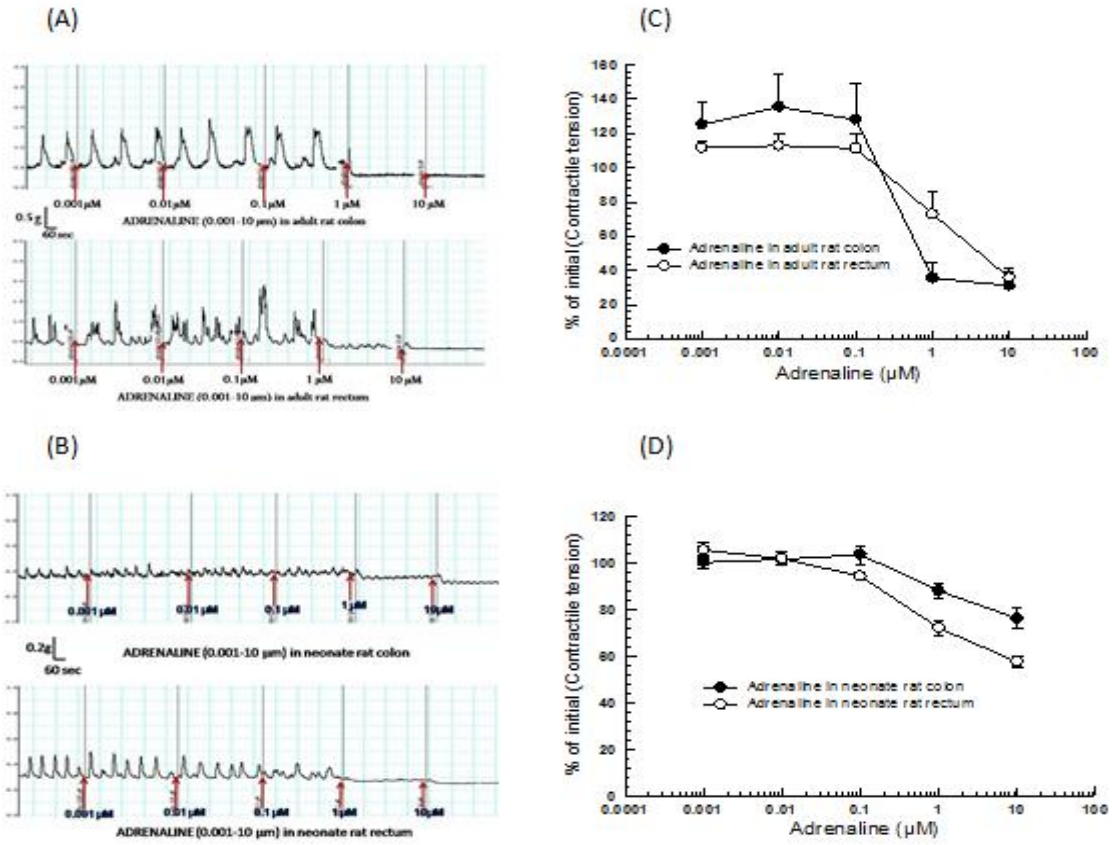

Fig. 1: Left panel showing original representative recordings of contractile response at different concentration of adrenaline (0.001-10 µM) in colon and rectum of adult (A) and neonate (B) rats. Arrows indicate point of administration of adrenaline.

Right panel shows dose-response curves for adrenaline (0.01 µM- 10 µM) on contractile tension (% of initial) in colon and rectum of adult (C) and neonate (D) rats. Adrenaline caused dose-dependent decrease in contractile tension in colon and rectum of adult and neonate rats. There was no difference in responses between colon and rectum to adrenaline in adult rats (C) ($p>0.05$, two way ANOVA, n = 5-6). There was significant decrease in contractile tension in rectum of neonates (D), ($p<0.05$, two way ANOVA) Data points indicate mean ± SEM.

To find out adrenaline-induced relaxation mechanism, in a set of experiments, colon and rectum of adult and neonate were exposed to propranolol, a non-selective β-blocker and labetalol a non-selective α-β-blocker as they are known to inhibit relaxation of gut smooth muscle. It was observed that propranolol and labetalol pre-application blocked adrenaline-induced decrease in contractile tension in adult rat rectum but not in colon (Fig. 2 A, B, D, E & Fig. 3 A, B, D, E). In contrast to earlier reports which showed that β-blocker could prevent adrenaline-induced relaxation in adult rat colon, human and rabbit taenia coli, rabbit jejunum, mice colon [12, 19, 20]. The present investigation failed to demonstrate same in adult colon. However, in case

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of adult rectum, propranolol inhibited adrenaline induced relaxation. This suggested the role of β-adrenoceptor in adult rat rectum but not in colon. The inconsistency in the blocking effect by β-blocker in colon partly may be attributed to high dose of adrenaline used in the study. Because, in an experiment on rat colon, it was reported that high concentration of adrenaline may not get reversed by β-blocker [19]. Inhibition of longitudinal muscle contractility of rat colon by high sympathetic nerve stimulation also could not be blocked completely by propranolol [15]. However, relaxation of human sigmoid colon was reported the involvement of β-adrenoceptor as propranolol was seen to enhance colonic motility [22] and blockade of β-adrenoceptor revealed an increased propulsive force in human small intestine [23].

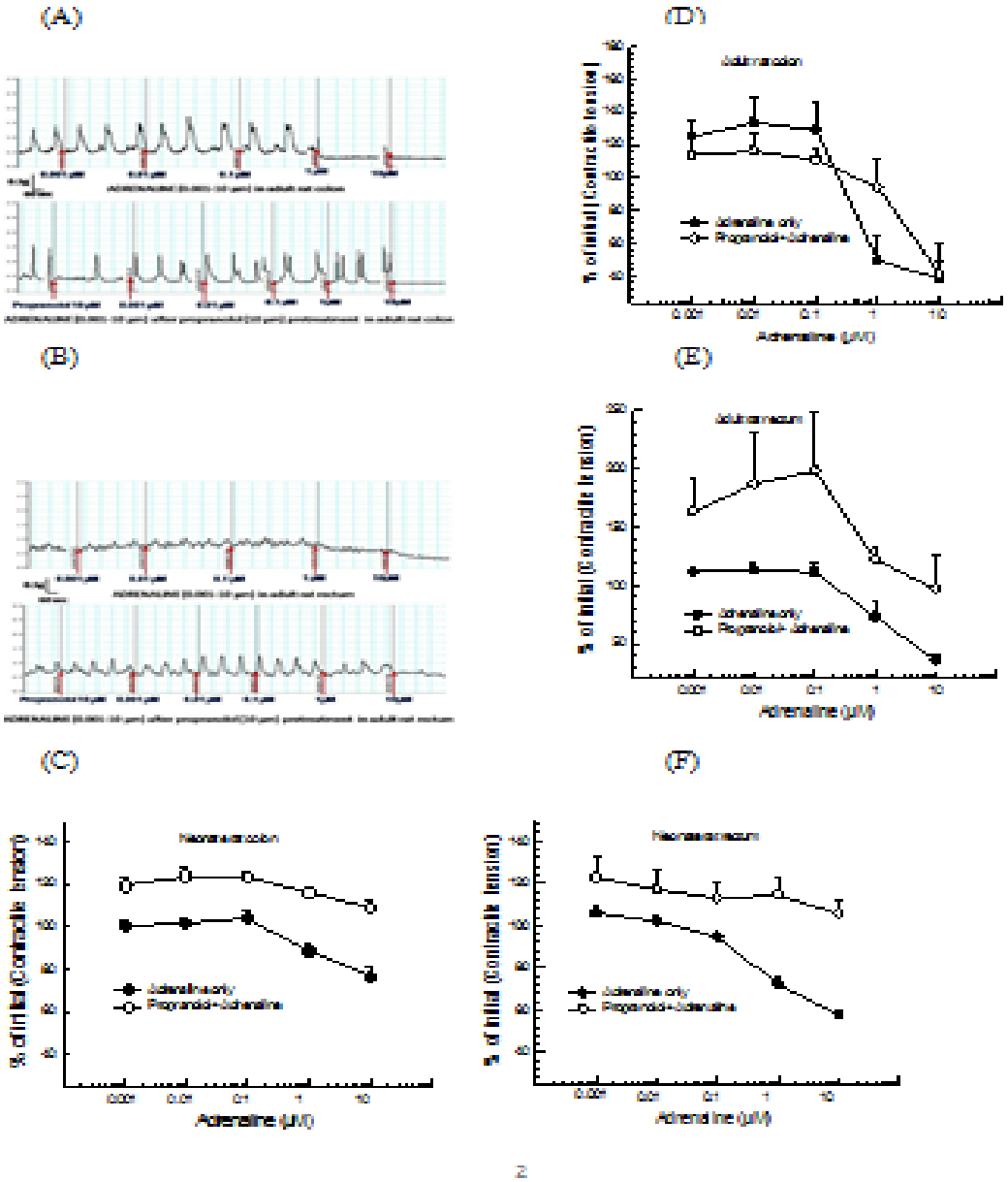

Fig. 2: Showing original representative recording of contractile response at different concentration of adrenaline (0.001-10 μM) before and after propranolol (10 μM) treatment in colon (A) and rectum (B) of adult rats.

Figures (D) and (E) show the dose-response curves showing the comparison of effect of adrenaline (0.01 μM- 10 μM) on contractile tension (% of initial) in colon (D) and rectum (E) of adult rats before and after propranolol (10 μM) treatment. No change in contractile tension to adrenaline was observed before and after propranolol treatment in colon (D) ($p>0.05$, two-way ANOVA) whereas in case of rectum contractile response to adrenaline was enhanced after propranolol treatment ($p<0.05$, two-way ANOVA). Data points indicate mean ± SEM values (n=4-5).

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Figures (C) and (F) show dose-response curves for the effect of adrenaline (0.01 µM- 10 µM) on contractile tension (% of initial) in colon(C) and rectum (F) of neonate rats before and after propranolol (10 µM) incubation. There was increase in contractile tension to adrenaline after propranolol treatment in both colon and rectum (C & F) of neonate ($p<0.05$, two-way ANOVA). Data points indicate mean ± SEM values (n=4-5).

On the other hand, as mentioned earlier, the relaxation induced by adrenoceptor agonists also involved α-receptor mediated mechanism. Similar relaxation could also be induced by adrenaline in human isolated taenia coli and blocked by α and β-receptor antagonist, hydergine and pronethalol respectively [17]. Likewise, in rat gastric fundus and mice colon blockade of noradrenaline and adrenaline induced relaxation by prazosin (an α-blocker) along with propranolol was reported [13]. In present study labetalol blocked the adrenaline-induced response in adult rectum but not in colon demonstrating the involvement of some other pathway in adrenaline mediated relaxation of colon. Similar speculations were also made earlier for longitudinal colonic muscle of rat [15].

However, in case of neonate, pre-incubation with propranolol and labetalol blocked adrenaline-relaxation in both colon and rectum (Fig 2 C, F & Fig 3 C, F). Hence, it suggests the role of both α-and β-receptors in adrenaline mediated relaxation in neonate large gut.

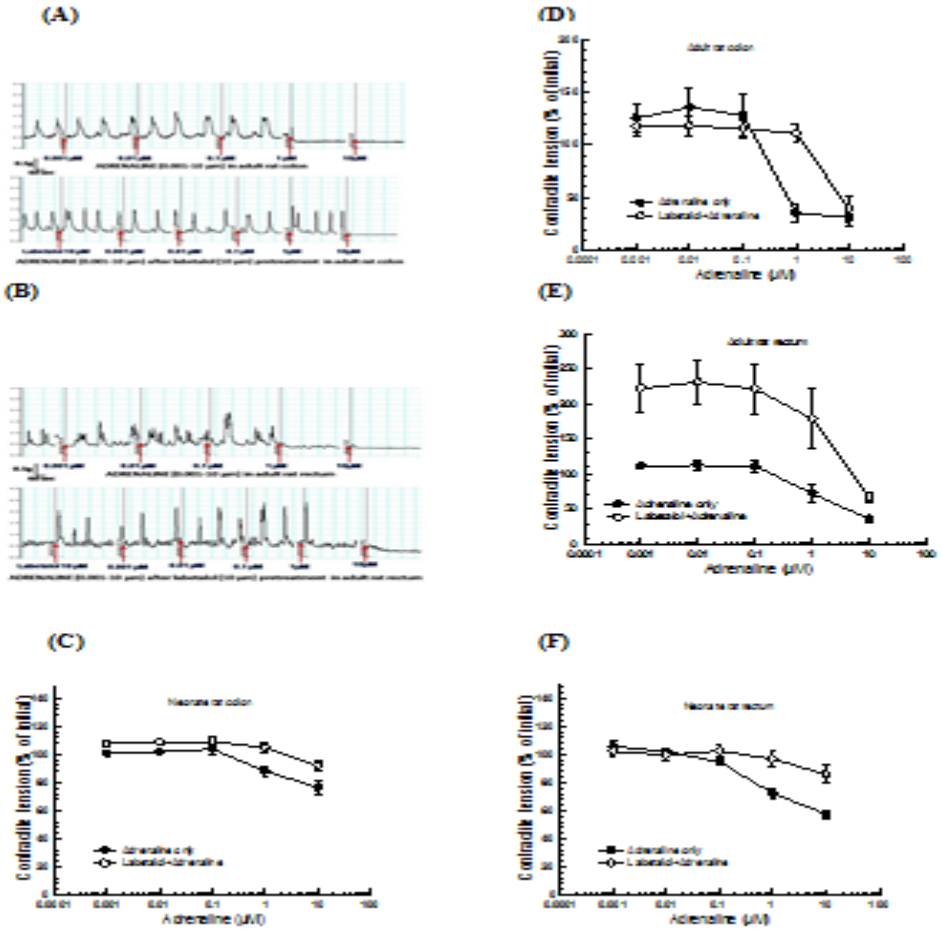

Fig. 3. Figures (A) and (B) showing original representative recording of contractile response at different concentration of adrenaline (0.001-10 µM) before and after labetalol (10 µM) treatment in colon (A) and rectum (B) of adult rats.

Figures (D) and (E) showing dose-response curves for the effect of adrenaline (0.01 µM- 10 µM) on contractile tension (% of initial) in colon (D) and rectum (E) of adult rats before and after labetalol (10 µM) application. No change in contractile tension to adrenaline was observed before and after labetalol treatment in colon (D) ($p>0.05$, two-way ANOVA) whereas in case of rectum (E) contractile response to adrenaline was more pronounced after labetalol treatment ($p<0.05$, two-way ANOVA). Data points indicate mean ± SEM values (n=4-5).

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Figures (C) and (F) shows the effect of adrenaline (0.01 µM- 10 µM) on contractile tension (% of initial) in colon (C) and rectum (F) of neonate rats before and after labetalol (10 µM). Contractile tension to adrenaline was elevated after labetalol treatment in both colon (C) and rectum (F) ($p<0.05$, two-way ANOVA). Data points indicate mean ± SEM values (n=6).

Thus, present investigation indicated the involvement of α and β-receptor mediated relaxation of colon and rectum of neonate but only rectum of adult. The differences in response to propranolol and labetalol between colon and rectum of adult and neonate may be due to differences in their developmental stage. The lower sensitivity seen in neonate colon may be due to underdeveloped α and β-receptors.

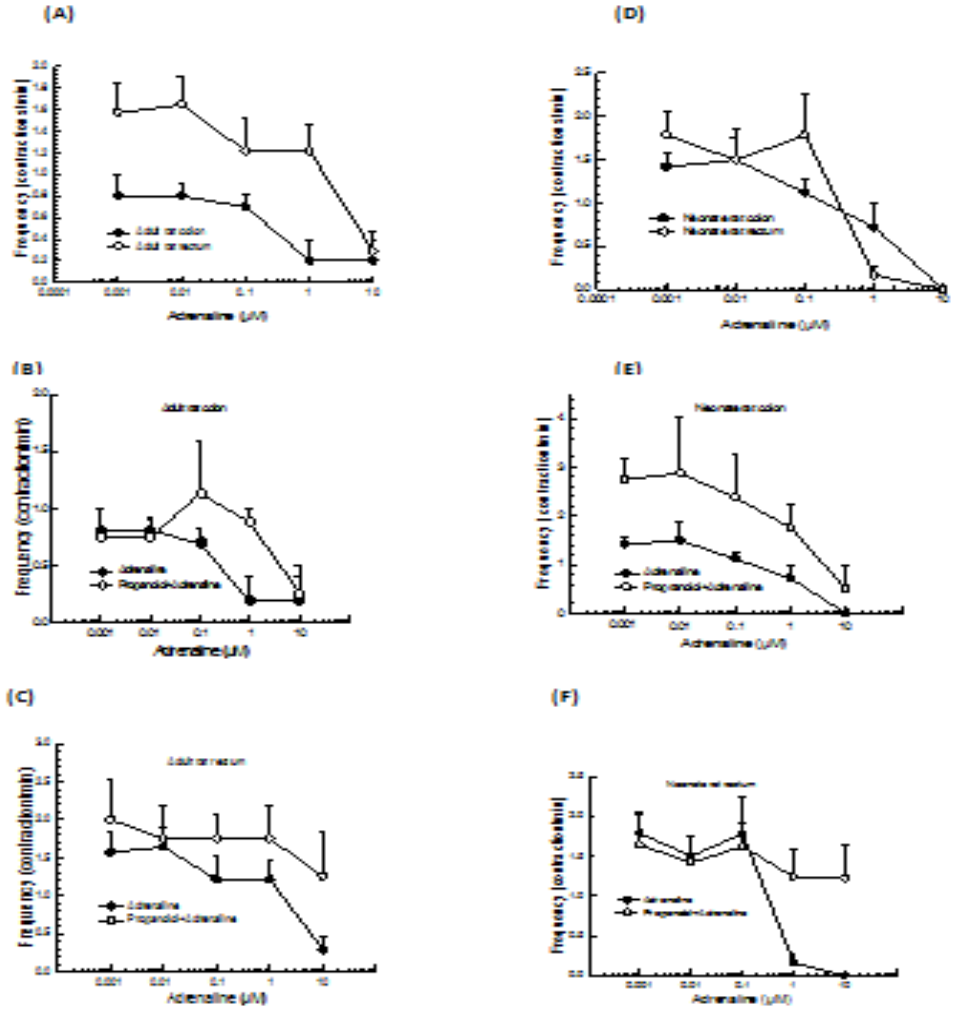

Fig. 4: Dose-response curves showing the effects of adrenaline (0.01 µM- 10 µM) on contractile frequency (contraction/min) in colon and rectum of adult (A) and neonate (D) rats. Contractile frequency decreased as concentration of adrenaline increased and it was more in adult colon than rectum ($p<0.05$, two-way ANOVA) but there was no difference between neonate colon and rectum ($p>0.05$, two-way ANOVA), Data points indicate mean ± SEM values (n=5-7).

Figures (B) and (C) show dose-response curves for the comparison of effect of adrenaline (0.01 µM- 10 µM) on contractile frequency (contraction/min) in colon (B) and rectum (C) of adult rats before and after propranolol (10 µM) incubation. No change in contractile frequency to adrenaline before and after propranolol treatment was observed in both colon and rectum of adult rats ($p>0.05$, two way ANOVA) Data points indicate mean ± SEM values (n=4).

Figures (E) and (F**)** show dose-response curves for the comparison of effect of adrenaline (0.01 µM- 10 µM) on contractile frequency (contraction/min) in colon (E) and rectum (F) of neonate rats before and after propranolol (10 µM) incubation. There was increase in contractile frequency to adrenaline after propranolol treatment in both colon and rectum of neonate rats ($p<0.05$, two-way ANOVA). Data points indicate mean ± SEM values (n=4).

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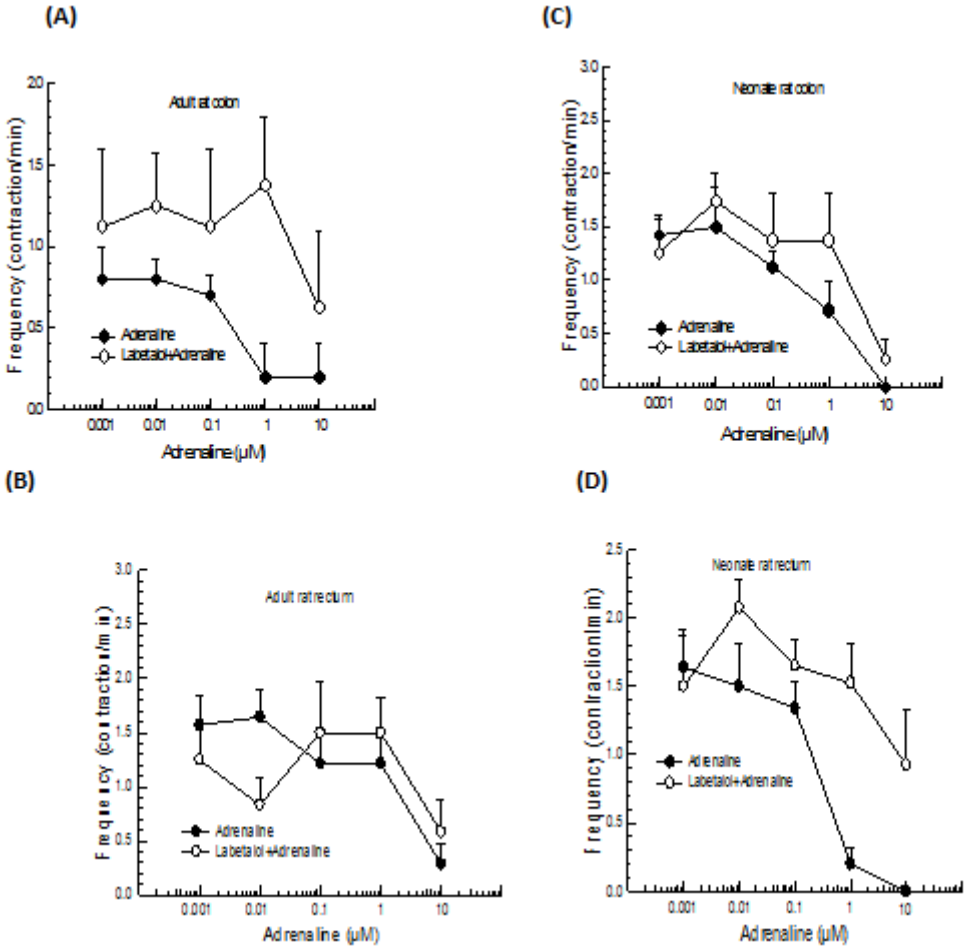

Fig.5. (A) and (B) showing dose-response of effect of adrenaline (0.01 µM-10 µM) on contractile frequency (contraction/min) in colon (A) and rectum (B) of adult rats before and after labetalol (10 µM) treatment. No change in contractile frequency to adrenaline before and after labetalol treatment was seen in both colon and rectum of adult rats (p>0.05, two way ANOVA). Data points indicate mean ± SEM values (n=4-7)

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Figures (C) and (D) show curve effect of adrenaline (0.01 µM- 10 µM) on contractile frequency (contraction/min) in colon (C) and rectum (D) of neonate rats before and after labetalol (10 µM) application. There was no change in contractile frequency to adrenaline after labetalol treatment in colon ($p>0.05$, two-way ANOVA) whereas contractile frequency increased in rectum ($p<0.05$, two-way ANOVA). Data points indicate mean ± SEM values (n=5-6).

As far as the contractile frequency is concerned, adrenaline treatment decreased frequency of contraction in colon and rectum of both adult and neonate. However, there was more decrease in adult rectum than colon (Fig. 4 A). No such difference was observed between neonate colon and rectum (Fig. 4 D). Study in longitudinal muscle of rat showed no change in contractile frequency to norepinephrine [16]. Further, propranolol and labetalol pre-application failed to inhibit adrenaline-induced decrease in contractile frequency in both colon and rectum of adult rats (Fig 4 B, C & Fig. 5 A, B) suggesting non involvement of α- and β-receptor in reducing contractile frequency in adult colon and rectum. However, this was not the case with neonate. Pre-incubation with propranolol inhibited adrenaline-induced decrease in contractile frequency in both colon and rectum of neonate rats (Fig. 4 C, F). This indicated the role of β-receptor in neonate. Whereas, pre-treatment with labetalol showed the similar effects only in rectum but not in colon (Fig. 5 C, D). This suggested that blocking of α-receptor by labetalol has antagonizing effect on the frequency of neonate colon.

Thus, it may be concluded that adrenergic mechanism exists right from the birth as evident by its effect on gut contractile activity of neonate rats. Both contractile tension as well as frequency in colon and rectum of adult and neonate rats appeared to be regulated by adrenergic system. Further, sensitivity to adrenaline induced contractile activity may vary between colon and rectum of adult and neonate rats with

colon being more sensitive to adrenaline than adult rectum whereas rectum is more sensitive than colon in neonate. The differences in the sensitivity and responses to adrenaline in adult and neonate signified the changes in receptors or other signaling molecules during the process of development from neonate to adulthood.

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