

A Study of Contractile Status of Proximal and Distal Segment of Neonatal Rectum in Anorectal Malformations

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Abstract

Objectives: The present study was undertaken to assess the contractile status of proximal & distal segments of high type of anorectal malformations (ARM) from neonates, for better understanding of colorectal motility problems in these patients.

Methods: Circular muscle strips were prepared from freshly excised specimens of high ARM (n= 17) obtained directly from paediatric surgery operation theatre. The in vitro contractile activity of the tissue was assessed initially without any chemical interventions and thereafter with application of acetylcholine, in an organ bath filled with physiological solution. The isometric contractile activity was recorded with the help of force transducer and computerised data acquisition system.

Result: Spontaneous contractions were recorded only in the proximal segment of 18 % of ARM cases. Acetylcholine (0.1-100 μ M) evoked contractions were significantly ($p < 0.05$) greater in proximal segment as compared to distal segment

Conclusion: Largely absence of spontaneous activity in ARM cases indicated that the tissue is functionally abnormal. Further, as compared to the proximal segment, the distal segment of ARM was badly affected as evidenced by severely impaired contractility in this segment. Thus, the result of this study may help formulation of better surgical management strategies.

Keywords: contractile, neonatal, rectum, anorectal & malformations.

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Introduction

Anorectal malformation (ARM) is common congenital problem in neonates in which the rectum ends either in a blind pouch that does not connect with the anus or may open in the urethra, bladder, base of the penis scrotum in male and in vagina in girls by forming fistula.[1]

Management of this condition is surgical. Despite the development of several

surgical techniques motility related disorders like constipation; incontinence and soiling as common sequelae after surgical repair of ARM.[2]

Anorectal malformation histological studies, showed the immaturity of the enteric nervous system and absence or reduced number of interstitial cells of Cajal[3&4]. It also has been seen that the

pelvic floor and the smooth muscle of the terminal rectum in ARM remained maldeveloped.[5] Further, studies on fetal rats showed that there were abnormal innervations of neural plexus in anorectum in ARM.[6]

. Earlier, in this laboratory, the functional studies on pouch colon also demonstrated alteration in contractility of colonic smooth muscle aimed to explore the functional aspect by recording the spontaneous and chemically evoked contractions of muscle strips from proximal and distal segment of ARM using in vitro preparations. Thus, this study may help understanding the functional alterations in anorectal malformation and the mechanisms involved, and thereby may provide a guideline for formulating better surgical management strategies. [7] Present study aimed to explore the functional aspect by recording the spontaneous and chemically evoked contractions of muscle strips from proximal and distal segment of ARM using in vitro preparations.

Materials & Methods

Collection of Samples

This study was carried out on the excised specimen from 17 neonatal patients of anorectal malformation. The specimens were collected immediately after excision from the Department of Paediatric Surgery operation theatre B.H.U., Varanasi in a wide mouth bottle containing pre-oxygenated ice-cold (4°C-6°C) Krebs-Ringer solution having the following composition (in mmol/L): NaCl, 119; KCl, 4.7; CaCl₂.2H₂O, 2.5; KH₂PO₄, 1.2; MgSO₄.7H₂O, 1.2; NaHCO₃, 5; and glucose, 11. They were quickly transferred to the laboratory in the Department of Physiology for contractile studies. All the experiments were conducted as per the guidelines laid down by the ethical

committee of the Institute of medical sciences, Banaras Hindu University.

Dissection and Preparation of muscle strips the excised specimens were transferred to a petri dish containing ice-cold (4°C-6°C) Krebs-Ringer solution continuously bubbled with 100% O₂ Mounting and recording of contractile responses. Each specimen was thoroughly cleaned with freshly prepared cold Ringer solution. After removing the adventitious layer, 2 to 3 mm wide and 15 to 20 mm long, circular muscular strips were prepared from proximal and distal end of samples of ARM

Mounting and recording of contractile responses

The muscle strips were mounted in Krebs-Ringer filled organ bath (15ml) at 30 ± 2 °C and continuously bubbled with 100 % O₂. Once the preparation was stabilized, recordings were made to observe the spontaneous and chemically evoked contractions. After the recording, the strips were removed from their ends (force transducer and One end of the strip was tied by thread with curved end of the oxygen tube and the other end with fine force transducer (MLT 0210, AD Instrument, Australia). The tissue was left for 30-45 min for equilibration after application of Initial tension of 0.5 gm on muscle strip. Recording of Isometric muscle contractions were made through Bridge amplifier and displayed onto personal computer with the help of Power lab data acquisition system and software CHART-5 for windows (AD Instruments Australia). After the recording, the strips were removed from their ends (force transducer and glass tube ends). The parts of tissue which were lying beyond the point of attachment were not involved in contractions and therefore, were removed. The rest of the muscle strip was then lightly soaked on a blotting paper to

remove extra water and the weight of the strip was recorded to express the response in g/g of wet tissue.

Drugs and solution

Aqueous solutions of Acetylcholine Chloride (Ach), Atropine sulphate (Atrp), were used for contractile study. The stock solutions of these chemicals were prepared with distilled water in the strength of 10 mmol/L. Final strength (0.1-100 μ M) were made by diluting with Krebs Ringer solution just before the experimentation.

Statistical analysis

The amplitude of contractions was converted to tension (gram) with the help of the Chart-5 software and then the tension so developed was expressed as tension per unit mass (g/g wet tissue) using the tissue weight determined at the end of the experiments. The values were then

pooled to calculate mean \pm SEM. The statistical significance of differences between Mean values was determined by using paired or unpaired t-test and two-way ANOVA as applicable. P value < 0.05 was considered significant.

Observation And Results

Contractile study was carried out with a total of 50 rectal muscle strips obtained from 17 ARM cases to evaluate spontaneous as well as chemically evoked contractions.

Spontaneous contractions in ARM Cases

Spontaneous contractions in ARM Cases Weak spontaneous contractions (without application of chemical) were observed only in proximal segment of 2 cases of ARM. None of the distal segment showed any spontaneous contractions.



Figure 1: Recording of feeble spontaneous contractions seen in proximal segment of ARM in one of the sample

Characteristics of response produced by Acetylcholine

Responses induced by Ach at different conc. (0.1-100 μ M) were studied in Proximal and distal segment of ARM (n=4). There was dose dependent increase in response to Ach in proximal and distal segments. Responses of different conc. of Ach on proximal segment were higher as compare to distal segments. The difference of responses of Ach was statistically significant (*P < 0.05, two-way ANOVA; Fig 2)

Representative recordings of (A) proximal segment (B) distal segments of ARM before and after applying different concentrations (0.1, 1, 10 & 100 μ M) of Ach (Acetylcholine). Arrows indicate the point of application of drug. Vertical & horizontal calibrations represent the tension (g) and time (min.) respectively. (C) Dose response curve of proximal and distal segments of ARM. Mean \pm SEM values of Ach induced contractions (g/g) with the different concentrations of Ach. Responses were significantly different (*P < 0.05, two-way ANOVA, n=4)

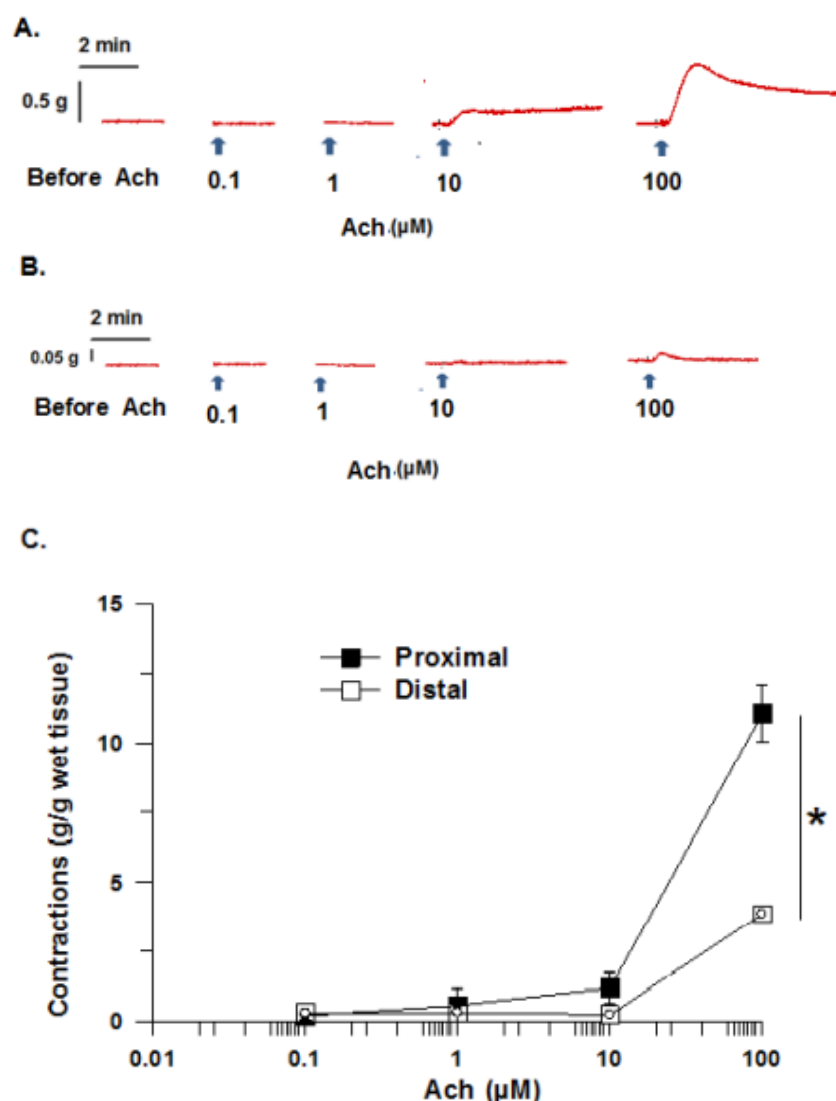


Fig. 2 Recording of response produced by Ach in proximal and distal segment of ARM

Acetylcholine induced contractions were blocked by atropine

Responses of Ach (100 μM) were significantly ($P < 0.05$, paired t test, $n=4$) blocked after application of Atrp (100 μM) in proximal as well as in the distal segments. In proximal segments, Ach produced 12 % of initial responses (88 % blockade) after application of Atrp. Whereas in the distal segment 28% of initial responses (72 % blockade) were produced (Fig 3).

Representative recordings to show the effect of Atrp (Atropine) on Ach induced contractions in (A) Proximal (B) distal segments of ARM. Arrows indicate the point of application of drug. Vertical & horizontal calibrations represent the tension (g) and time (min.) respectively. (C) Histogram showing Mean ± SEM values of % of the initial responses to Ach 100 μM before and after application of Atrp in proximal and distal segments of ARM. Responses of Ach after Atrp were

significantly decreased (*P < 0.05, paired t test, n=4)

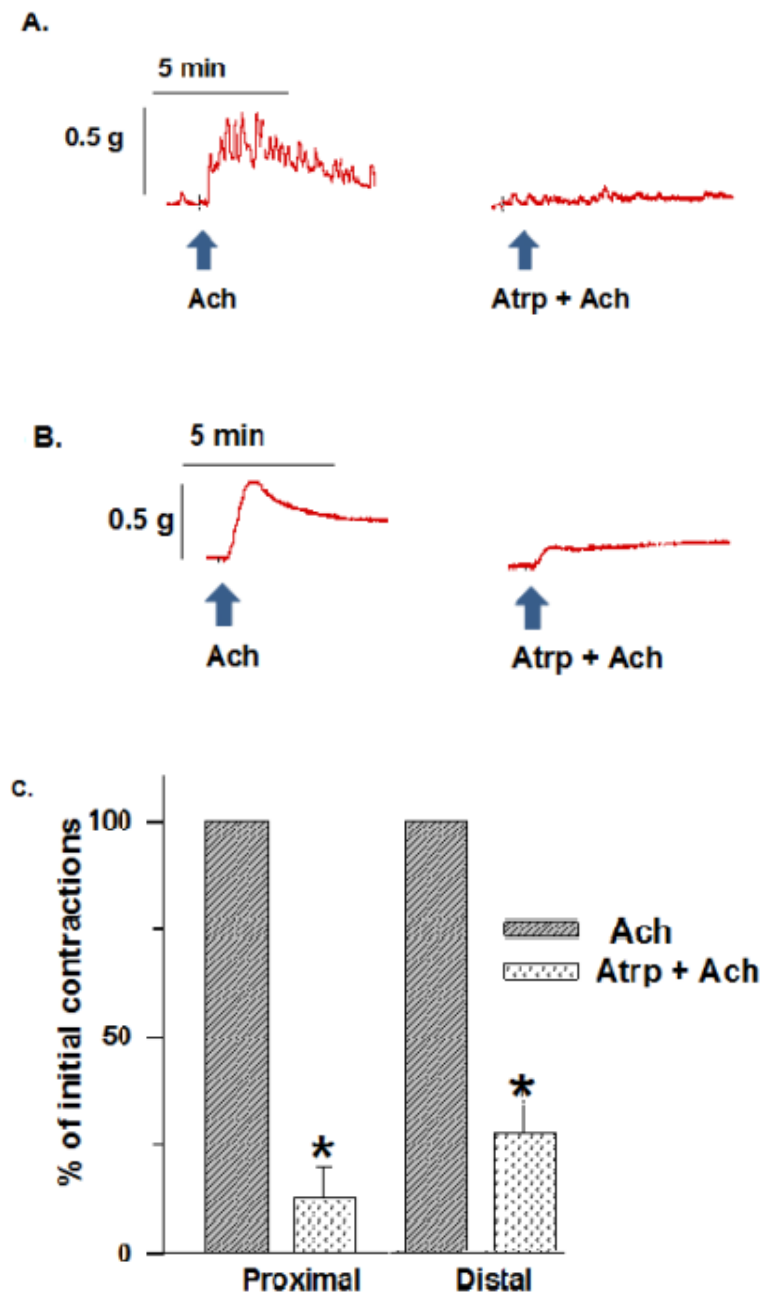


Fig. 3 Recording of Ach induced response, blocked by atropine in proximal and distal segment of ARM

Discussion

In the present investigation the contractility of rectal smooth muscle was evaluated by recording spontaneous as well as chemically (Ach) evoked

contractions from proximal and distal segments of rectal tissue of ARM to understand the severity and extent of contractile impairment. In the proximal segment, only 2 out of 17 cases (nearly

18%) of ARM and none of the distal segments showed spontaneous contractions. The origin of spontaneous contraction is believed to be related to activity of interstitial cells of Cajal (ICC). [8] Thus, the absence of spontaneous contractions in these tissues indicated abnormal functioning of ICC. Application of Ach is not inducing any contraction.

On examination of Ach induced contractile strength of proximal and distal segment, it was noted that there was dose dependent increase in the response. This agonistic effect is known to be mediated by their actions on muscarinic for Ach. In this study also it was observed that Atrp (muscarinic blocker) could largely block the response.[9]

Thus, the present investigation demonstrated that high type of ARM the rectal tissue is excitable by Acetylcholine. However, the tissue appeared to be functionally abnormal on account of absence of spontaneous contractions in most of the cases, with severe impairment of contractility in the distal segment.[10] These observations may have implications in surgical management of ARM problem in neonate, pertaining to the most controversial issue of preserving or excising the distal most part of the rectal tissue of high type of ARM.[11]

Conclusion

Largely absence of spontaneous activity in ARM cases indicated that the tissue is functionally abnormal. Further, as compared to the proximal segment, the distal segment of ARM was badly affected as evidenced by severely impaired contractility in this segment. Thus, the result of this study may help formulation of better surgical management strategies.

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