RESEARCH ARTICLE

IN VITRO STUDY OF CONTRACTILE RESPONSES OF BOWEL WITH HIRSCHSPRUNG’S DISEASE

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Background: Hirschsprung’s disease (HD) is a congenital disorder in neonates which results from the absence of parasympathetic ganglion cells in the myenteric and submucosal plexus of the rectum, possibly extending to the colon.

Aims & Objective: To assess the contractile status of intestinal tissue of HD for better understanding of motility problems.

Materials and Methods: Circular muscle strips were prepared from freshly excised specimens of HD obtained from pediatric surgery operation theatre. The in vitro contractile activity of the tissue was assessed in an organ bath filled with physiological solution.

Results: Spontaneous contractions were recorded in 66% cases. Dose-dependent increase in response with the application of histamine and acetylcholine (0.1–100 µM) was observed. Responses of histamine were significantly (P < 0.05) blocked by pre-application of H 1 blocker pheniramine (80%). Pre-application of atropine significantly (P < 0.05) blocked (62%) acetylcholine responses.

Conclusion: Large number of cases showed spontaneous contractions indicating functionally normal tissue.

INTRODUCTION

Hirschsprung’s disease (HD) is a congenital multifactorial developmental disorder of the enteric nervous system characterized by the absence of ganglion cells in the hindgut with variable distal bowel involvement. This is a rare disease, which occurs with an incidence of 1 into 5000 live births as a consequence of premature arrest of craniocaudal migration of neural crest-derived neuroblasts in the hindgut, and it is therefore regarded as a neurocristopathy.[1,2] However, the outcome of the surgery of these conditions often remains unsatisfactory. This may partly be due to nonavailability of functional studies in terms of contractility of intestinal smooth muscles, because most of the studies so far conducted on these disorders were focused on histology and immunohistochemistry. The majority of children with HD after corrective surgery develop defecation disorders, such as constipation, fecal incontinence, and/or enterocolitis.[3] The basic tools that may help in evaluating the functional status of smooth muscle in gastrointestinal tract is by recording the spontaneous and chemically evoked contractions in in vitro preparations. Therefore, this study is aimed to explore the functional aspect of HD by recording the spontaneous and chemically evoked contractions in in vitro preparations. This study may help us to evaluate the functional alterations and the mechanisms involved in this disease, and thereby may help us in formulation of strategies for better surgical management.

MATERIALS AND METHODS

The study was performed on the excised specimen, of the patient of HD, collected from the operation theater of Department of Pediatric Surgery, Sir Sunderlal Hospital, Banaras Hindu University, Varanasi. The specimens were bubbled with 100% oxygen (O 2) and collected in a wide-mouth bottle containing ice-cold (4–6°C) Krebs–Ringer solution. 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 for handling human tissues.

The specimens were transferred to a Petri dish containing ice-cold (4–6°C) oxygenated Krebs–Ringer solution and thoroughly cleaned with freshly
prepared cold Ringer solution. After removing the adventitious layer, circular muscular strips (2- to 3-mm wide and 15- to 20-mm long) were prepared from apparently healthy looking part of specimen.

The excised strips were mounted in an organ bath filled with Krebs–Ringer solution (15 ml) at 30 ± 2°C and continuously bubbled with 100% O₂. One end of the strip was tied to the curved end of the oxygen tube with the help of thread and the other end with fine force transducer (MLT 0210; AD Instruments, Australia). Initial tension of 0.5 g was applied on the muscle strip. After 30–45 min of equilibration, isometric muscle contractions were recorded using bridge amplifier and displayed onto personal computer with the help of Power lab data acquisition system and software Chart5 for windows (AD Instruments).

Spontaneous and chemically evoked contractions were observed in stabilized preparation. After the recording, the strips were removed from their ends (force transducer and glass tube ends). The parts of tissue that 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 dabbed on a bloating 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), histamine (Hist), atropine sulfate (Atrp) (Sd-Fine-Chem, Mumbai, India), and pheniramine maleate (Phn; Aventis Pharma, Mumbai, India) were used for contractile study. The stock solutions of these chemicals were prepared with distilled water in the strength of 10 mmol/L. Final strengths (0.1–100 µM) were prepared by diluting these solutions with Krebs–Ringer solution just before the experiment.

Statistical Analysis: The amplitude of contractions was converted to tension (gram) with the help of the Chart5 software and then the tension thus 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 ± SEM. The statistical significance of differences between mean values was determined using paired t-test and one-way analysis of variance (ANOVA), as applicable. The p-value < 0.05 was considered significant.

RESULTS

Contractile study was carried out with a total of 20 muscle strips obtained from 9 individuals with HD to evaluate spontaneous contractions as well as the effect of the various drugs.

Spontaneous Contractions: Spontaneous contractions (without application of chemical) were observed in 6 individuals with HD.

Response of Histamine: The effect of Hist with four different concentrations (0.1, 1, 10, and 100 µM) was observed in intestinal tissue obtained from four individuals with HD. After stabilization for 30 min, different concentrations (0.1–100 µM) of Hist were applied in tissue and maximum height was noted. There was concentration-dependent increase in the maximum height (Figure 1), and the response was found to be significantly different (P< 0.05, one-way ANOVA).

Effects of Pheniramine on Histamine-Induced Contractions: The effects of Phn on Hist-induced contractions were studied in intestinal tissue obtained from six individuals with HD. Maximum height achieved by Hist (100 µM) before and after application of Phn was noted. Responses of Hist (100 µM) before Phn were considered as 100%, and the effects of Hist after application of Phn were noted. It was found that Phn blocked nearly 80% of Hist responses (Figure 2).

Response of Acetylcholine: The effects of Ach with four different concentrations (0.1, 1, 10, and 100 µM) were observed in intestinal tissue obtained from five individuals with HD. After stabilization for 30 min, different concentrations (0.1–100 µM) of Ach were applied in tissue and maximum height was noted. There was concentration-dependent increase in the maximum height (Figure 3), and the response was found to be significantly different (P< 0.05, one-way ANOVA).

Effects of Atropine on Acetylcholine-Induced Contractions: In five individuals with HD, Ach-induced contractions were studied before and after application of Atrp. Maximum height achieved by Ach (100 µM) before and after the application of Atrp was noted. Responses of Ach (100 µM) before the application of Atrp were considered as 100%, and the effects of Ach after the application of Atrp were noted. It was found that Atrp blocked nearly 62% of Ach responses (Figure 4).
Figure 1: (A) Original recordings of responses to different concentrations (0.1–100 μM) of Hist obtained from the muscular strip excised from the individuals with HD before and after the application of different concentrations (0.1, 1, 10, and 100 μM) of Hist. Arrows indicate the point of application of the drug. Vertical and horizontal calibrations represent the tension (g) and time (min), respectively. Hist = histamine. (B) Dose–response curve: mean ± SEM values of Hist-induced contractions (g/g) with the different concentrations of Hist (*P < 0.05, one-way ANOVA, N = 4).

Figure 2: (A) Original recordings of responses of Hist (100 μM) before and after addition of Phn in segments obtained from the individuals with HD. Arrows indicate the point of application of the drug. Vertical and horizontal calibrations represent the tension (g) and time (min), respectively. Hist = histamine; Phn = pheniramine. (B) Histogram showing mean ± SEM values of the responses (contraction g/g%) to Hist (100 μM) before and after the application of Phn. Responses of Hist after the application of Phn were found to decrease significantly (*P < 0.05, paired t-test, N = 6). Hist = histamine; Phn = pheniramine.

Figure 3: (A) Original recordings of responses to different concentrations (0.1–100 μM) of Ach obtained from the muscular strip excised from the individuals with HD before and after the application of different concentrations (0.1, 1, 10, and 100 μM) of Ach. Arrows indicate the point of application of the drug. Vertical and horizontal calibrations represent the tension (g) and time (min), respectively. Ach = acetylcholine. (B) Dose–response curve: mean ± SEM values of Ach-induced contractions (g/g) with the different concentrations of Hist (*P < 0.05, one-way ANOVA, N = 4).

Figure 4: (A) Original recordings of responses of Ach (100 μM) before and after the addition of Atrp in segments obtained from individuals with HD. Arrows indicate the point of application of the drug. Vertical and horizontal calibrations represent the tension (g) and time (min), respectively. Ach = acetylcholine; Atrp = atropine. (B) Histogram showing mean ± SEM values of the responses (contraction g/g%) to Ach (100 μM) before and after the application of Atrp (*P < 0.05, paired t-test). Ach = acetylcholine; Atrp = atropine.
DISCUSSION

In this study, the contractility of intestinal tissue was evaluated to know the severity and extent of contractile impairment.

Spontaneous contraction is one of the important parameters to understand the functional status of the intestine. The origin of spontaneous contraction is believed to be associated with the activity of interstitial cells of Cajal (ICCs). The spontaneous contractions in the intestinal smooth muscles are elicited by slow waves that are rhythmic oscillations in membrane potential. ICCs, distributed in specific locations within the tunica muscularis of the gastrointestinal tract, serve as electrical pacemakers and mediators of enteric neurotransmission. They exhibit oscillations in their intracellular Ca2+ concentration, which may be responsible for changes in membrane potential. The propagation of slow waves is thought to be via the gap junctions to adjacent smooth muscle cells.

In this study, 66% of individuals with HD showed spontaneous contractions. However, the contractions present were not much strong. Earlier studies on HD showed the altered distribution of ICCs in HD. After the application of Hist and Ach, a dose-dependent increase in the response was observed, indicating the stimulatory effect of these agonists on ICCs.

An earlier study on pouch colon in this laboratory showed lack of spontaneous contractions with the presence of abnormal neural elements in histopathological observations.

In this study, H1-receptor blocker Phn blocked 80%, and not 100%, of Hist-induced responses. It is possible that other type of Hist receptors (H2, H3, or H4) may be implicated in the Hist-induced contractions and responsible for remaining 20% of contractions. Involvement of H1 receptor in normal intestinal smooth muscle contraction has been documented in animal as well as in human elsewhere. Atrp block 62% of Ach-evoked responses suggesting the action of Ach mediated through muscarinic receptor.

CONCLUSION

The effects of some drugs were observed in this study; however, further detailed studies on contractile function, electrophysiology, immunohistochemistry, and biochemical assays involving more number of cases are required for better understanding and management of these problems.

REFERENCES


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