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Comparative effects of osmotic and secretory diarrhoea on brush- border disaccharide hydrolases in rat

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Summary

The effects of diarrhoea on the activities of brush-border disaccharidases namely lactase (EC 3.2.1.23), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.48) of Sprague-Dawley strain albino rats were induced in the rats with mannitol while secretory diarrhoea was induced with Salmonella typhimurium after an initial treatment with streptomycin. The activities of the enzymes were significantly reduced by diarrhoea. The extent of reduction in enzyme activity varied in the different segment of the small intestine in all the groups. The jejuno-ileal region had more changes in enzyme activities than in the duodenum. Higher activity levels were observed for maltase than for lactase. In the osmotic diarrhoea model, lactase activity was significantly lowered ($P< 0.05$) in the experimental group from day 5 to 10. Maltase activity on the other hand was significantly lowered ($P< 0.001$) at the peak of diarrhoea. Sucrase activity was also lowered significantly ($P< 0.025$) in the experimental animals within the first 10 days of diarrhoeal induction. In the secretory diarrhoea model, lactase activity was similar in all the experimental groups except for the streptomycin-salmonella-treated groups and control ($P< 0.05$). Higher lactase activity levels were observed in the secretory diarrhoea model compared to level in the osmotic diarrhoea model. Maltase activity levels were also lowered significantly ($P< 0.05$) in the experimental animals. Streptomycin had no effect on the activity of maltase.

Résumé

Les effects de la diarrhée sur les activités des enzymes hydrolases dans l'intestin grêle(lactase, maltase et sucrase) étaient étudiés chez les rats. L'osmose était induite chez ces rats par le mannitol alorsque la sécretion de la diarrhée était induite par le salmonella typhirumum après l'initiation du traitement à la streptomycine. Les activités de ces enzymes étaient significativement réduite par la diarrhée. L'ampleur de la réduction d'activités variait dans different segment de l'intestin grêle chez tous les groupes de rats. La région jéjuno-iléale avait plus de changements en activités enzymatiques que le duodénum. De plus grande activités étaient observées au maltase qu'au lactase. Dans le modéle de la diarrhée osmotiques, l'activité du lactase était significativement faible ($P<0.005$) dans le groupe expérimentale du 5 ième au 10 ième jour. L'activité du maltase était significativement faible ($P<0.01$) au peak de la diarrhée. L'activité du sucrase était aussi significativement faible($P<0.025$) aux groupes expérimentaux dans le 10 ième jour de diarrhée induite. Dans le modéle de la diarrhée sécretoire, l'activité du lactase était similaire aux groupes expérimentaux a l'exception de ceux traités au streptomycine- salmonella. et leur controle($P<0.05$). Des taux d'activités de lactase plus elevés étaient observés dans ce modele compare au modéle osmotique. Les taux d activites du maltase étaient aussi faible ($P<0.05$) aux groupes expérimentaux et la streptomycine n'avait pas d'influence sur l'activité du maltase.

Keywords: *Salmonella typhimurium, osmotic diarrhoea, hydrolases.*

Introduction

Diarrhoea is increasingly being recognized as a nutritional disease and this has pointed to the importance of the nutritional needs of the sick child and is spurring new research efforts into the dietary management of diarrhoea [1]. Diarrhoea is still responsible for over 30 percent of all deaths in children less than 5 years of age [2]. There is more documented evidence of the adverse effect of diarrhoea disease on growth [3]. More specifically, carbohydrate intolerance has been reported to be common immediately following an episode of diarrhoea [4,5]. The duration of disaccharidase deficiency in infants with severe small intestinal mucosal injury following various disarrhoeal syndromes have also been reported to be variable [6,7]. The reduced capacity of the small intestine to absorb monosaccharides is another cause of carbohydrate intolerance in diarrhoea diseases but only a minority of infants with disaccharidase intolerance have monosaccharide malabsorption [8,9].

Secondary carbohydrate intolerance is also not always associated with proven mucosal damage as assessed by light microscopy. A few children were found to have disaccharidase deficiency despite a histologically normal small intestinal mucosa [10]. It has been argued that since carbohydrate intolerance occurs during the acute stages of diarrhoea, and diarrhoea itself has adverse

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effect on mucosal integrity, dietary carbohydrate may have a deleterious effect by aggravating diarrhoeal symptoms and prolonging mucosal injury. This then contributes to a vicious cycle of mucosal injury and carbohydrate malabsorption. The aim of this investigation is therefore to study the effects of two distinct models of diarrhoea on brush-border disaccharidases using the animal model.

Materials and methods

Adult albino rats (male and female) of Sprague-Dawley strain were obtained from the animal house of the College of Medicine, University of Lagos. The animals were mated in the metabolic laboratory of the Department of Biochemistry of the College. Animals were handled according to the National Research Council Guide [11]. Young rats were suckled for 18-20 days, weaned and fed on rat chow (Pfizer product) and water until they were 30 days old. All animals were housed in steel cages that had elevated wire mesh floors to prevent coprophagia. Twelve hours of light and darkness were provided alternatively in the room to minimize stress [12]. The temperature in the room was maintained at 25±1° C. Analytical grade reagents were purchased from Sigma Chemical Co., St. Louis, MO. Tom Cat Catheter was obtained from Monoject, St. Louis, MO. Centrifuge used was from LKB, Cambridge, England (Model 4050). The spectrophotometer used was Pye Unicam SP-450. *Salmonella typhimurium* was obtained from the Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos.

Diarrhoea induction

Osmotic diarrhoea was induced in the experimental rats according to the method of Pergolizzi *et al* [13]. 28 rats weighing about 180g. each were divided into two groups of 14 rats, one serving as the control group. The weight difference between the two groups was ±2.0g. Force-feeding each rat in the experimental group with a 1300 milliosmolar (20%) solution of mannitol daily between 9.00am and 10.00am induced diarrhoea.

Secretory diarrhoea was induced using *Salmonella typhimurium*. The method of Bornhoff and Miller [14] was adopted. This method employed streptomycin to suppress the intestinal microflora of the rats to make the infecting agent more invasive.

40 rats were divided into four groups of 10 rats each. The average weight of rats used in each group was 187±2.0g. A saline suspension containing approximately 10^9 S. typhimurium per cm^3 was made from an overnight culture on bismuth sulphite agar. From this suspension was prepared a series of 10 fold dilutions and the bacterial populations of spore formers were determined by counting the colonies using a colony counter (Gallenkamp, England). The dilution used contained 10^3 S. typhimurium per cm^3. Innocular were introduced into the stomach as described previously [15].

Group 1 was treated with salmonella, group 2 was treated with streptomycin, group 3 was treated with streptomycin and salmonella while group 4 served as the control.

Preparation of intestinal homogenates

The method of Pergolizzi et al [13] was employed. Abdominal cavity was opened by a midline incision and the intestine was cut at the pyloric spinchter and the ileocecal junction. A 10cm segment representing the jejunum was taken from 30-40cm segment distal to the pylorus and another 10cm segment representing the ileum was taken from 30-40cm distal to the ileocecal valve. No tension was applied to the intestinal segment at the time of measurement. The entire content of the small intestinal digest was collected in a test tube and frozen at –20°C immediately until analyzed for products of digestion.

The intestinal segments were cut open longitudinally and the mucosa was scrapped off with a microscope cover slip [13,16], and mechanically homogenized for 2 minutes in a tissue grinder with Teflon pestle (Fisher Scientific, Pittsburg, PA) after adding four parts of distilled water. The homogenate was centrifuged at 3000 r.p.m. for 10 minutes in order to remove layer of cell debris and thereafter frozen at –20°C.

Determination of disaccharidases activities

The disaccharidases, which include lactase (EC 3.2.1.23), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.48), were assayed as outlined by Dahlqvist [16]. Mucosa homogenate was diluted 10 fold with 0.89/100cm^3. NaCl solution and 100cm^3 aliquot was placed in a conical test tube and incubated at 37°C in a water bath for 5 minutes. 100cm^3 of substrates containing 0.056M of the appropriate disaccharide dissolve in 0.1M sodium maleate buffer; pH 6.0 was added and mixed. The mixture was incubated for exactly 60 minutes. The glucose liberated was quantified using the glucose oxidase method of Raabo and Terkildsen [17]. All samples assayed were analysed for protein content according to Lowry et al [18] as modified by Ohnishi and Borr [19] using bovine serum albumin as standard.

Results

The effect of osmotic diarrhoea on the activities of the disaccharidases lactase, maltase and sucrase are shown

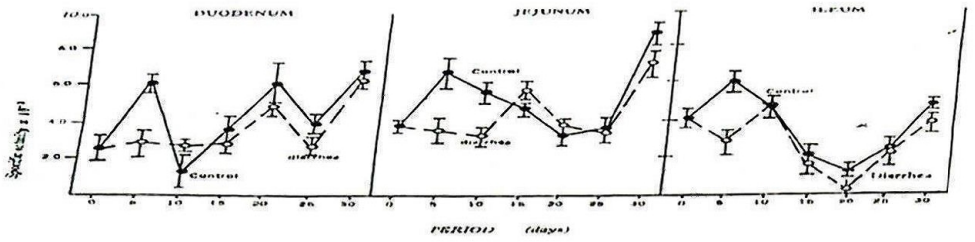

Fig. 1: Effect of mannitol induced osmotic diarrhoea on lactase activity in rats. Lactase activity is expressed as micromole of Sucrose hydrolysed per milligram protein per hour. Each point represents mean ± S.D. of control (○), and rats with diarrhoea (●).

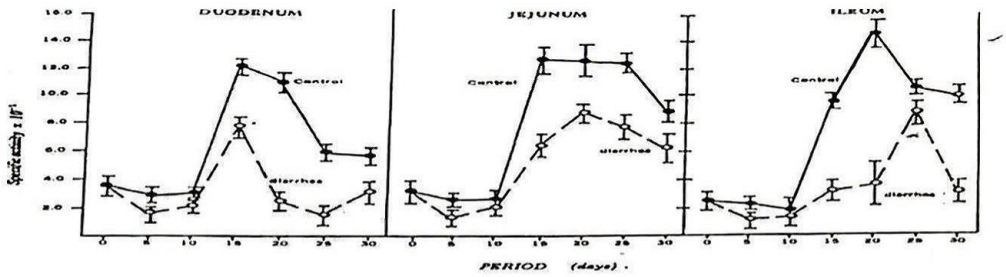

Fig. 2: Effect of mannitol-induced osmotic diarrhoea on maltase activity in rats. Maltase activity is expressed as micromole of Sucrose hydrolysed per milligram protein per hour. Each point represents mean ± S.D. of control (○), and rats with diarrhoea (●).

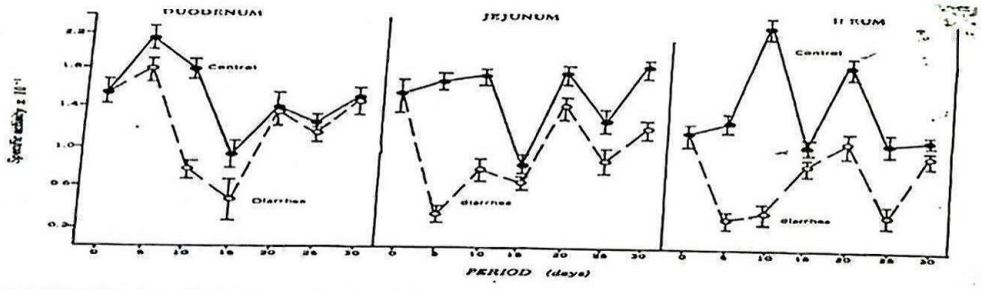

Fig. 3: Effect of mannitol-induced osmotic diarrhoea on sucrase activity in rats. Sucrase activity is expressed as micromole of Sucrose hydrolysed per milligram protein per hour. Each point represents mean ± S.D. of control (○), and rats with diarrhoea (●).

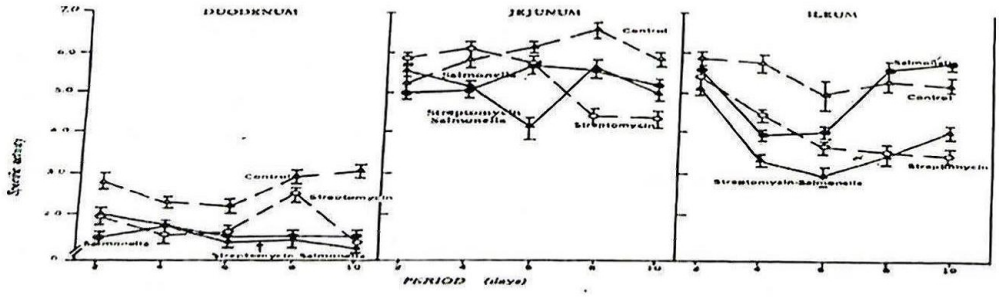

Fig. 4: Effect of Salmonella-induced secretory diarrhoea on lactase activity in rats. Sucrase activity is expressed as micromole of Sucrose hydrolysed per milligram protein per hour. Each point represents mean ± S.D. of four animals. Salmonella treated (●), Streptomycin treated (▪), Streptomycin-Salmonellas treated (*) and control rats (Δ).

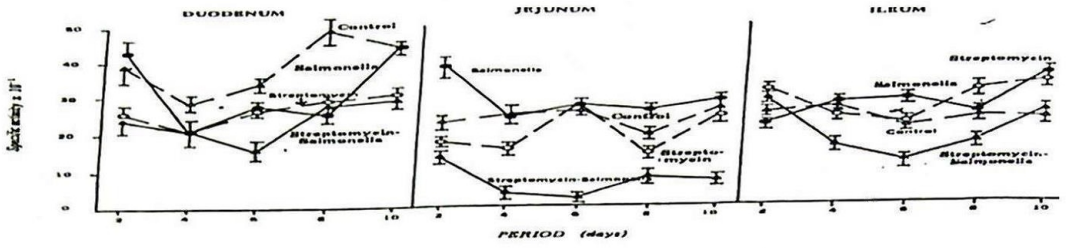

Fig. 5:

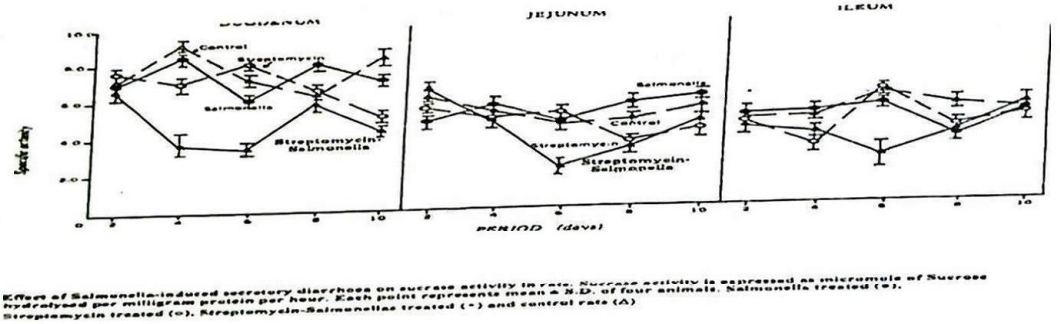

Fig. 6:

in figures 1-3 respectively. The activities of the enzymes were significantly reduced by diarrhoea. The extent of reduction in enzyme activities varied in the different segments of the small intestine in all the groups. There are more changes in the activity levels of enzymes in the jejuno-ileal region than in the duodenum. Higher activity levels were observed for maltase than for lactase.

In the osmotic diarrhoea model, lactase activity was significantly lowered ($P<0.01$) in the experimental group from day 5 to 10. The maltase activity on the other hand was significantly lowered ($P<0.001$) at the peak of diarrhoea (Fig.2). It was observed that sucrase activity was lowered significantly ($P<0.025$_ in the experimental animals within the first 10 days of diarrhoea induction. The reduction in sucrase activity was more pronounced in the ileum than in the duodenum (Fig.3). Sucrase activity of the control and experimental groups at the peak of diarrhoea were similar.

Figures 4-6 show the effect of secretory diarrhoea on disaccharidase activity in the rats. Lactase activity was similar in all the experimental groups except for streptomycin-salmonella-treated group and control ($P<0.05$). Higher lactase activity levels were observed in the secretory diarrhoea model compared to levels in osmotic diarrhoea model. The jejunum contain higher lactase activity than the duodenum and ileum.

Discussion

The reduction in the activities of the disaccharidases studied is consistent with other reports in the literature [7,8,21]. This observation is not unexpected since biochemical studies have shown that the brush border carbohydrases are attached to the outside surface of the intestinal brush-border membranes and can be removed by mere treating with a detergent like 1% Triton X-100 [22]. Also, disaccharidases are primarily found in the apical brush-border membrane of mature epithelial cells but are nearly absent in the crypt cells [23] thus it would be expected that even mild or transient diarrhoea would lead to transient carbohydrate maldigestion as a result of reduced carbohydrases activities.

It was observed in this study that osmotic diarrhoea resulted in a greater reduction in disaccharidases activities than secretory diarrhoea. It is pertinent to note that while enzyme levels have been reported to be decreased, normal

or increased in situations of malnutrition [24-26] workers have consistently reported reduced activities in mucosal disaccharidases as a result of diarrhoea. There is however the contention that the presence of inflammatory cells in the mucosa of diarrhoeal infected subjects may have erroneously decreased the observation of dissacharidases activities by increasing the amount of measured protein [27]. Thus this factor has to be taken into consideration whenever little or no reduction in disaccharidase activity is reported. Studies before now have been on either of the two forms of diarrhoea, which have different aetiologies. This report represents a comparative study of the effects of both osmotic and secretory diarrhoea.

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