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Goblet cell number in the ileum of rats denervated during suckling and weaning

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ABSTRACT: The enteric nervous system plays a role on the stimulation of secretory cells of intestinal epithelia. We have demonstrated that ablation of ENS stimulates epithelial cell proliferation. As goblet cells are important constituents of the epithelial sheet, it is mandatory to investigate separately this cell type. The myenteric plexus of the ileum of rats in postnatal development was partially removed by the serosal application of benzalkonium chloride (BAC). Three groups of animals were used: those where BAC application was at 13 days and sacrifice was at 15 (13/28-day-old) or 23 days after treatment (13/36-day-old), and those where BAC was applied at 21 days and rats were killed 15 days after treatment (21/36-day-old) . The number of goblet cells in the ileum was estimated in sections stained by periodic acid - Schiff (PAS) histochemistry. In the 13/28 and 21/36 groups, the number of goblet cells was significantly higher after BAC treatment. These results suggest that the myenteric denervation may have an acute effect on the number of goblet cell in suckling and weanling rats, probably through submucous plexus.

Introduction

The intestinal mucosa is covered by a single layer of epithelial cells: enterocytes and goblet cells in the villus, and stem cells, proliferating cells, enteroendocrine cells, and Paneth cells in the crypt. The epithelium undergoes continuous renewal: cells are produced in the crypts, migrate into the villi, and are extruded at the villus tip (Cheng and Leblond, 1979).

Extensive networks of nerve fibres are found in the mucosa and muscle in the intestine. Most of these fibres arise from ganglion cells in the myenteric or submucous plexuses, whereas a relatively small proportion arise from extrinsic ganglia. A variety of functions may be subserved by intestinal innervation in the mucosa. Sensory nerve fibres are responsible for detecting chemical, osmotic and mechanical stimuli, while motor functions include contraction of the muscularis mucosae, secretions from endocrine and goblet cells, vascular tone, and movement of water, ions and nutrients across the epithelium (Cooke, 1986; Plaisancié *et al*., 1998).

Besides sensitive and motor functions, the intestinal innervation has an important trophic function on the mucosal sheet.

The presence of a rich innervation surrounding the base of the intestinal crypts (Keast, 1987) and the de-

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velopment of techniques of autonomic denervation has made possible the investigation of this function.

There are many experiments demonstrating that the autonomic denervation promotes changes in the kinetic parameters of intestinal epithelium (Lachat and Gonçalves, 1978; Klein and McKenzie, 1980; See *et al.*, 1990; Zucoloto *et al.*, 1997; Hernandes *et al.*,2000).

We have demonstrated that myenteric ablation by benzalkonium chloride at suckling and weanling rats promoted an increase on the height of the villus and on the crypts depth, besides exerting a trophic effect on cell proliferation at suckling and increasing the rate of migration at weaning (Hernandes *et al.*, 2000).

Other authors also reported a trophic action on cell proliferation after myenteric denervation in adult rats (Zucoloto *et al.*, 1988; See *et al.*, 1990; Holle, 1991; Hadzijahic *et al.*, 1993; Zucoloto *et al.*, 1997).

However a question remains: is this influence the same for every kind of cell composing the intestinal epithelium, or the denervation acts on each cell population in a different way?

To answer part of this question, we propose to evaluate the second largest cell population of the small intestinal epithelium surface, the goblet cell. The goblet cells produce a mucus gel that plays important physiological roles including lubrication, protection against colonization by pathogenic bacteria and their toxins, protection against luminal proteases arising from bacterial and mucosal cells, consisting of a diffusion barrier for small molecules (Forstner and Forstner, 1994). These cells store mucin, the major structural component in mucus, in apically located granules that are secreted continuously to maintain the mucin coat over the epithelium. The discharge of mucus may be mediated by enteric nervous system, among other factors (Plaisancié *et al.*, 1998).

The present study was then undertaken to investigate the influence of myenteric innervation on the number of goblet cells. For this purpose, suckling and weanling rats were chemically denervated by benzalkonium chloride, a cationic surfactant that destroys the myenteric neurons but not submucous neurons.

Material and Methods

Animal treatment

All procedures in this study that involve the use of animals are in accordance to ethical principles and were

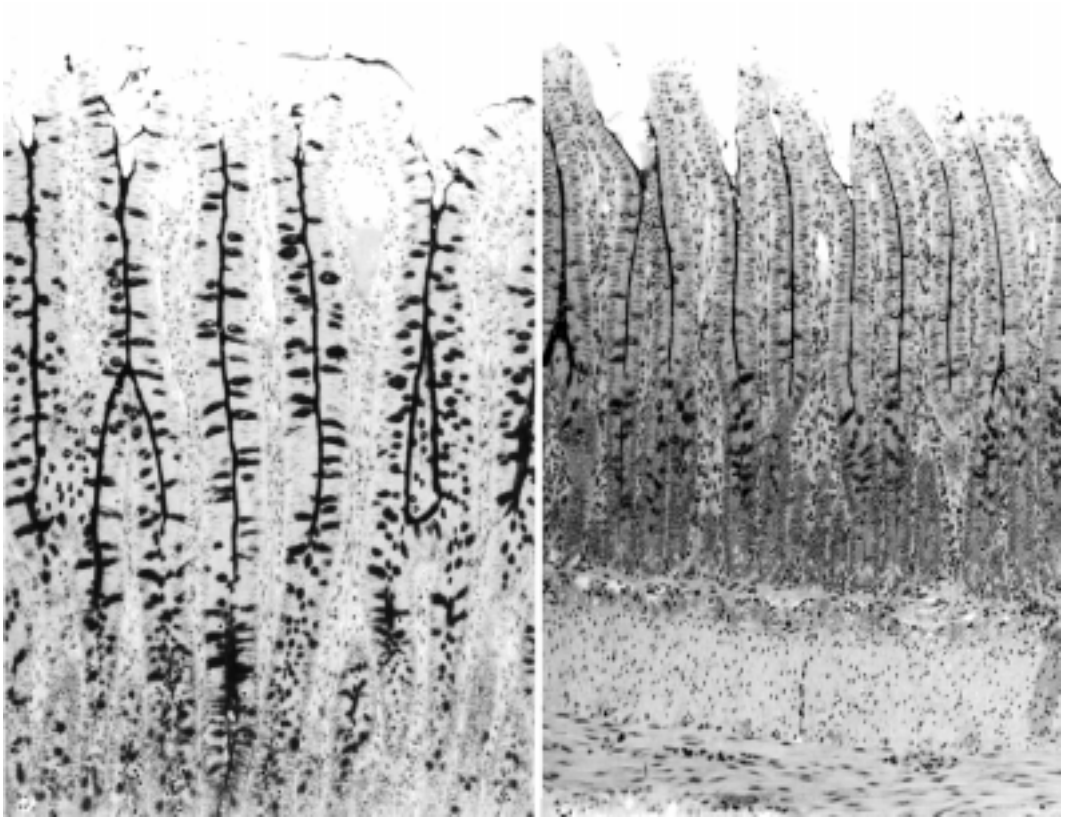

FIGURE 1. Villi from ileum of control and denervated rats sacrificed 15 days after surgery. PAS-staining (X 428). **A.** Denervated animal (13/28 group) showing a large number of goblet cells and visible thickening of intestinal mucosa. **B.** Control animal (13/28 group) with lower number of goblet cells.

approved by Ethical Committee of Animal Experimentation of the Institute of Biomedical Sciences of the University of São Paulo.

We used slides from the same rats of our previous report (Hernandes *et al.*, 2000). Briefly, after anesthesia, a midline abdominal incision was made in 13 and 21 day-old rats, and a segment of the ileum of 3-4 cm near the ileumcaecal junction was exteriorised from the peritoneal cavity. This segment was treated with serosal application of the cationic surfactant, 0.081% benzalkonium chloride solution (BAC) (Sigma, St Louis, MO, USA), every five minutes over thirty minutes in the treated rats, or with 0.9% saline in the control rats (Fox *et al.*, 1983). After treatment, the ileal segment was thoroughly rinsed with 0.9% saline and returned to the abdominal cavity. The midline incision was closed and the animals were allowed to recover. The 13 days old rats were placed with their mothers after recovery. The animals were maintained on a 12 h light/dark cycle. All groups were weaned at 21 days of age.

Three groups of rats (five rats/group) were studied: those that underwent surgery on day 13 of age were killed 15 days later (13/28 days old) or 23 days later (13/36 days old). A third group was operated on day 21 of age and was sacrified 15 days after surgery (21/36 days old).

Histological procedure

Under ether anesthesia, the animals were killed and cylindrical samples of ileum from treated and control segments were removed and opened along their long axes, carefully washed with saline and opened on a card. Fixation was performed immediately by immersion in Carnoy's solution for 6 h, and afterwards tissues were processesed for hydroxiethyl methacrylate (LKB-Technovit 7100; Kulzer, Wehrheim, Germany) embedding. Sections of 2 μm thickness were obtained and the slides were stained by histochemical technique of PAS to stain goblet cells.

Estimation of goblet cell in the villus

The number of goblet cells stained by PAS from one side of the villus and the total number of villus cells at the same side were counted in control and treated rats for each group. For each animal used, approximately 2,500 cells, including PAS-positive or not, were counted in longitudinal sections of intact villus. The number of goblet cells was expressed as the percentage of goblet cells stained by PAS divided by the total number of cells counted.

Statistical analysis

The mean number of goblet cells from control and denervated rats of each group was compared by Student's *t*-test for paired observations. The significance level was set at $P<0.05$.

Results

Myenteric neuron number

BAC treatment promoted decrease in the number

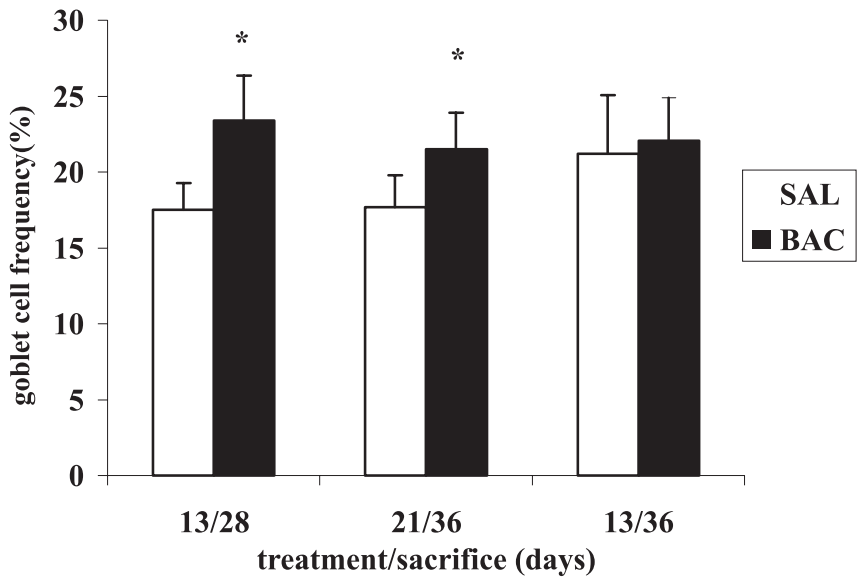

FIGURE 2. Number of ileum goblet cells (%) ± SD in saline and BAC-treated rats. N=5 animals/group, **P*<0.05 compared to controls (Student's t-test for paired observations).

of myenteric neurons as previously demonstrated (Hernandes *et al.*, 2000) (data not shown).

Microscopic observation

In the BAC- treated segments of ileum, microscopic observation showed evident increase in the number of goblet cells in the villus on 13/28 and 21/36 groups, compared to controls (Fig. 1).

Quantitative analysis

The goblet cell number in the villus was significantly increased in the denervated ileum from 13/28 and 21/36 groups (Fig. 2).

Discussion

Our results show that the number of goblet cells of the ileum increases significantly 15 days after the denervation promoted by BAC.

The animals operated during the lactation and weaning period – which were sacrificed fifteen days after the surgery (groups 13/28 and 21/36 respectively) – presented villi with a larger number of goblet cells, in relation to their respective controls. However, there was no statistical difference in the group of animals operated during the lactation stage that were sacrificed 23 days later (group 13/36), when compared to controls.

These results confirm the experiments in cell kinetics accomplished previously by us where mitotic index was significantly higher, cell migration rate was accelerated and an increase in villi height was observed 15 days after the denervation of suckling rats. Other parameters (faster cell migration in the villi and increase in crypts depth) were affected when denervation occurred at weaning period (Hernandes *et al.*, 2000). Cell kinetic parameters were not changed in the group treated for the longest time (23 days) (Hernandes *et al.*, 2000), and likewise the number of goblet cells was not altered in this group only. It is possible that other factors that regulate the populations of cells in the intestinal epithelium, as the extrinsic innervation, hormones, and growth factors, among others, might have acted in this group to maintain the steady-state.

In adult animals, the autonomic denervation reduced the number of goblet cells, which is the opposite of our results. A drastic reduction in the number of goblet cells, which remained concentrated in the inferior third of the villi, was observed after infra-diaphragmatic sectioning of the vagus nerve in adult mice. Three days after, there was a tendency to normality (Musso *et al.*, 1975). A reduction in the number of goblet cells in the ileum of vagotomized adult mice was also described (Lachat and Gonçalves, 1978).

After mesenteric and celiac ganglionectomy, the number of enterocytes, goblet cells and of enterochromaffin cells was determined in mini-pigs (Hanford) (Holle *et al.*, 1989). The results demonstrated that there was an increase (10-33%) in the thickness of the mucous membrane and a reduction (20-40%) in the number of goblet cells in the denervated animals. There were no significant changes in the number of enterochromaffin cells when compared to the treated and control animals (Holle *et al.*, 1989).

In a healthy condition, the goblet cells of the intestine and the mucous neck cells of the stomach express pS2, a trefoil member family. Recent studies demonstrated that the trefoil peptide can protect the gastrointestinal epithelium and promote the regeneration after injuries (Babyatsky *et al.*, 1996). On the other hand, the trefoil expression and secretion might be stimulated by neuropeptides as the vasoactive intestinal peptide (VIP) and by somatostatin, which are produced and liberated in great amounts in the inflammed intestine (Soeda *et al.*, 1992; Ogata and Podolski, 1997).

Recently it was demonstrated by us, that VIP-ergic submucous neurons of rats denervated with BAC, during lactation stage, present a larger area than the neurons that express VIP, in the control rats (Hernandes *et al.*, 1999).

In adult rats, See and collaborators have made a correlation between enlarged VIP-submucosal neurons, myenteric ablation and control of crypt cell proliferation. They suggest that submucosal plexus exerts stimulatory influence on crypts cell proliferation and the myenteric neurons normally inhibit mucosal cell division (See *et al.*, 1990).

These results point to a relationship between ENS and epithelium, and lead us to conclude that the enteric nervous system participates, with other neural and non-neural factors, on the control of the cellular populations in the intestinal epithelium. They also suggest that myenteric plexus could act over the epithelium through the submucous plexus, that have many VIP-secreting neurons.

Acknowledgements

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