The value of in vivo electrophysiological measurements for monitoring functional adaptation after massive small bowel resection in the rat

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Abstract

The process of functional adaptation after extensive small bowel resection is complex and imprecisely understood. In vivo electrophysiological measurements for monitoring the functional adaptive process after massive small bowel resection in Brown-Norway rats were evaluated. Rats underwent either a sham operation (SH) or a 90% small bowel resection (SB). Standard rat chow was fed in unlimited quantities. At three or 10 weeks after operation, jejunal and ileal transepithelial potential differences (PD, mV) were determined. Electrogenic ion transport in the villus was measured after glucose (sodium coupled active glucose absorption; PD-glu) and in the crypt, after theophylline infusion (theophylline stimulated chloride secretion; PD-theo). Biopsies were taken simultaneously. Each experimental group consisted of three to five animals. At three weeks the PD-theo and PD-glu in SB rats were significantly lower than in SH rats in both jejunal and ileal segments. At 10 weeks PD-theo and PD-glu were significantly diminished in the jejunal segment of the SB rats compared with the SH rats. The values of PD-theo and PD-glu in the ileal segments were, however, no longer different between the two groups. Three and 10 weeks after operation the length of the villi in the SB group was increased significantly compared with the SH controls. These results indicate that in the early phase of adaptation in vivo electrophysiological variables do not correlate with histological changes in the SB rats. This might be due to cell immaturity resulting from an increased rate of cell turnover or lack of intercellular tight junctions. This hypothesis is supported by a recovery of PD responses in the ileum 10 weeks after resection.

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and functional response to various internal and external stimuli to maintain equilibrium in the gastrointestinal tract.⁴ After intestinal resection, hyperplastic changes caused by increased cell turnover in the proliferative zone of the crypt are generally seen in the enteric remnant.⁵ There is evidence that the unchanged number of cells per unit length may indicate immature enterocytes,⁵⁻⁷ but increased absorptive capacity per unit length has been reported as well.^{8,9} These divergent results may be ascribed to chronological differences in the studies performed. Urban and Michel postulated that morphological growth precedes functional components of intestinal adaptation after resection.¹⁰ The exact interrelation between histological changes and function, possibly regulated by different factors that may be sequentially interrelated, is unknown. The purpose of this study was to determine the value of in vivo electrophysiological measurements for monitoring functional intestinal adaptation. Electrophysiological responses are reliable indicators of small bowel function.¹¹ We developed an in vivo technique, measuring transepithelial potential differences (PD) evoked by sodium coupled active glucose absorption, which is an index of villus function, and cyclic adenosine monophosphate (cAMP) mediated chloride secretion, predominantly reflecting crypt cell function. Moreover, histological specimens were collected to explore the interrelations between morphological and functional changes during the adaptation phases studied.

Material and methods

ANIMALS

Male rats of the inbred Brown-Norway strain (Harlan CPB, Zeist, The Netherlands) were used. The animals weighed 250-400 g and were bred under specific pathogen free conditions. They underwent either a sham operation (SH), a 90% small bowel resection (SB), or were control animals (IS). During the experimental period, all animals were kept under standard laboratory conditions (12 hours light and 12 hours dark) and were given free access to water and standard rat chow (AM-II; Hope Farms, Woerden, The Netherlands). The experimental protocols adhered to the rules laid down in The Dutch Animal Experimentation Act (1977) and the published *Guidelines on the protection of experimental animals* by the Council of the EC (1986). Specific protocols were approved by the committee on animal research of the Erasmus University, Rotterdam.

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Extensive small bowel resection triggers complex adaptive changes.^{1,2} All resected patients need intravenous infusion to avoid the development of malnutrition while oral feeding is attempted.³ The considerably reduced absorptive surface can result in the short bowel syndrome, which is defined as incapability to thrive through oral nutrition. On the other hand, the remaining bowel can adapt sufficiently to provide essential nutritional support. Manipulating the outcome is difficult as the process of functional adaptation after extensive small bowel resection is not fully understood. Intestinal adaptation has been described as the proliferative, morphological,

Figure 1: Overview of the set up for electrophysiological measurements.

OPERATIVE PROCEDURE

The rats were anaesthetised with ether and a midline laparotomy was performed. The SB rats were created by near total small bowel resection – that is, 2.5 cm distally from the ligament of Trietz to 2.5 cm proximally from the ileocecal valve. A sham operation was performed by transection midway between the ileum and jejunum, without removal of bowel mass. After each procedure gastrointestinal continuity was restored end to end, with Ethicon 7-0. After the operation, all animals were given 1 ml depomycin

20/20 (10% v/v in phosphate buffered saline (PBS); Gist-Brocades, Animal Health bv, De Bilt) subcutaneously. Half of the animals in each group underwent electrophysiological monitoring and were killed three weeks after the operation (SH₁, n=5 and SB₁, n=7); the other animals (SH₂, n=5 and SB₂, n=5) were treated similarly 10 weeks after the operation. A third group of animals (IS, n=3) were not subjected to any operation. In these rats the superior mesenteric artery was clamped two hours before starting the electrophysiological measurement to provoke

Figure 2: Weight curves.

ischaemic intestinal injury. The intestinal segment selected for measurement was anatomically identical in all rats.

TECHNIQUES

Growth assessment

The animals were weighed three times a week after the operation.

Electrophysiology

Rats were anaesthetised with ether and a midline laparotomy was performed to expose the bowel for the electrophysiological measurement. This method is a modification of the technique developed by Meyssen *et al* to monitor function of the small bowel in dogs.¹² Well defined jejunal and ileal segment was chosen as the measurement area, in which a continuous flow of test solution was maintained with canulae. Several iso-osmolar test solutions were flushed through the segment to determine intraluminal transepithelial PD with reference to a subcutaneous Ag/AgCl₂ electrode (37°C, 8 ml/min). The standard solution consisted of: NaCl (110 mM); HEPES (5 mM); KCl (4 mM); Na₂SO₄ (10 mM). In the solution containing theophylline part of the mannitol was iso-osmotically replaced by 5 mM theophylline and in the solution containing glucose, by 30 mM α -D-glucose. Before starting the measurement preperfusion was performed for six minutes to equilibrate luminal content with the standard perfusion solution. Then the following solutions were infused for five minutes each: standard, theophylline, standard, glucose, standard. The standard solution was used to assess the basal PDs, which reflect physiologically active ion transport. Infusion of theophylline solution (5 mM) evoked chloride (Cl⁻) secretion, predominantly a crypt function, resulting in an intraluminal negative PD (PD-theo). A glucose solution (30 mM) evoked sodium coupled glucose absorption, reflecting villus function, also resulting in an intraluminal negative PD (PD-glu). Figure 1 shows an overview of the technique.

Histology

Full thickness biopsies were collected just before the electrophysiological measurements, immediately fixed in 3.6% buffered formalin, then

dehydrated and embedded in paraffin. Sections of 4–5 μ m were stained with haematoxylin azophloxin safran. By standardised projection of sections on a screen through a light microscope, crypt and villus length could easily be measured. Villus height was measured by subtracting crypt length – that is, the shortest distance between the bottom of intestinal villi and the lamina muscularis mucosae, from mucosal height. For each rat, 10 measurements were made and the average was used in the comparative study.

Metabolic variables

During the sixth postoperative week, rats were kept for four days in metabolic cages provided with a system to collect faeces and urine and to measure food and water consumption.

Statistical analysis

Statistical analysis of data between experimental groups was performed with the Student's *t* test. We preferred to express our data as the difference between SH and SB at each time point, because the SH group tended to show a decreased PD response, apparently as a consequence of the operation. Differences with *p* values <0.05 were considered to be significant.

Results

WEIGHT

Figure 2 shows mean weight change curves during the period after the operation for SB and SH animals. The SH animals had a small postoperative weight loss followed by weight gain to 110% of the preoperative weight. In the SB group the follow up showed a postoperative weight loss to 70%–80% followed by a gradual recovery to 90% of the preoperative weight. None of these rats had returned to their weight before operation by 10 weeks after operation. Two SB₁ rats died during the experimental period due to clinical short bowel syndrome.

ELECTROPHYSIOLOGY

Figures 3 and 4 show all electrophysiological data from the SH and SB rats.

Jejunal electrophysiological measurements

At three weeks, PD-glu and PD-theo in the jejunal segment of SB₁ rats were significantly reduced compared with SH₁ rats (PD-glu *p*<0.02; PD-theo *p*<0.05). At 10 weeks the stimulated PD responses in SB₁ rats were significantly lower in the jejunal segment compared with SH₂ rats (PD-glu *p*<0.01; PD-theo *p*<0.05). In IS rats, no response was found to either of the test solutions (results not shown).

Ileal electrophysiological measurements

At three weeks, stimulated PD responses in the ileal segment of rats from the SB₁ group were significantly less for both PD-glu (*p*<0.01) and PD-theo (*p*<0.05) compared with those in

Figure 3: Jejenum: electrophysiological and histological findings. SH, sham operated; SB, short bowel; PD, potential difference; Glu, glucose; THEO, theophylline; values in mean (SD); p values calculated by Student's *t* test.

group SH₁. At 10 weeks no significant differences in PD responses were found in either of the groups. In IS rats, again, no response was found to either of the test solutions (results not shown).

HISTOLOGY

Figures 3 and 4 show histological data.

Jejunal findings

At three weeks postoperatively, jejunal villus length was significantly enlarged in SB₁ rats compared with SH₁ rats. Also, jejunal villus length was significantly enlarged in SB₂ rats 10 weeks after operation compared with SH₂ rats. No differences in crypt length were found in either group.

Ileal findings

Three weeks after the operation, ileal villus length was significantly increased in SB₁ animals compared with SH₁ animals ($p < 0.05$). Ten weeks after the operation, no significant difference was found in ileal villus length between the

experimental groups. Again, no differences in crypt length were found in either of the groups.

METABOLIC VARIABLES

Figure 5 shows that six weeks after the operation there were no significant differences between SH and SB animals for food intake, water consumption, and production of urine and faeces.

Discussion

After extensive loss of intestine both functional and morphological adaptive changes occur. Functional adaptation is determined by numerous indices, including the absorption per segment, intestinal transit time, the presence of the ileocaecal valve, intestinal contents or specific activities of brush border enzymes, and bacterial colonisation of the remaining small bowel. Increased exposure to luminal nutrients and pancreaticobiliary secretions, trophic effects of enteric hormones, and neurovascular effects on the remaining intestine are important factors that stimulate mucosal growth. Although factors regulating mucosal morphology and function of the

Figure 4: Ileum:
electrophysiological and
histological findings.
Abbreviations as for Fig 3.

gut may be sensitively interrelated the overall process is still unravelled. The complexity of the adaptive events is indicated by region related differences in mechanisms controlling mucosal growth and function as well as the existence of dissociation between mucosal growth and components of functional adaptation.¹⁰

In our experiments we performed nearly total small bowel resection to obtain a sublethal small bowel syndrome model. In our view the occurrence of malnutrition, which is a characteristic of our sublethal model, may be a major trigger for intestinal adaptation. We set out to evaluate the interrelations between the mechanisms of active transport of electrolytes and mucosal growth in jejunal as well as ileal segments in the follow up of intestinal adaptation. The metabolic studies six weeks after operation showed that at that time differences between sham operated and resected animals were not caused by major metabolic changes. Electrophysiology, a reliable tool for functional assessment of the mechanisms of active transport of electrolytes,² might be useful to monitor the functional adaptation process. The electrophysiological results show that the methodology developed is technically feasible in rats. It has been shown that clamping the superior mesenteric artery for two hours results in ischaemic intestinal injury in which the mechanisms of active transport of electrolytes have been damaged.¹³ As expected, ischaemic control

animals did not develop a transepithelial PD in response to any of the solutions. By contrast, transepithelial PD values obtained in sham operated and resected animals show that active transepithelial transport of electrolytes by an isolated bowel segment can be evaluated quantitatively by *in situ* electrophysiological monitoring. Previous studies have already shown that *in vitro* preparations of intestine can be used to study electrogenic Na^+ absorption and electrogenic Cl^- secretion.¹⁴ From both *in vitro* and *in vivo* studies, it is generally assumed that villus epithelial cells are responsible for electrolyte coupled absorptive processes.^{14,15} Crypt cells on the other hand, are thought to be mainly responsible for secretion of ions and water.^{14,16} Barry *et al* showed that electrical potential differences are fundamentally similar between *in vivo* and *in vitro* results.¹⁷ The *in vivo* technique is preferred, to avoid ischaemia, disruption of neural, lymphatic, and blood supply, and to have better sources of endogenous metabolites. The technique described in this study, however, should be refined to develop a non-invasive measuring system, as recently done for large animals.¹² Such a refinement would have great value in the investigation of adaptive mechanisms by determining the effect of composition of the diet,¹⁸ hormonal supplementation,^{19,20} or manipulation of the polyamine metabolism.²¹ Previous experiments showed that timing of functional adapta-

Figure 5: Metabolic state six weeks after operation.

tion varies. These variations may be related to the amount of tissue resected, postoperative time points studied, the specific region of the enteric remnant explored, and the nature of the transport activities tested. For example, Urban and Michel found decreased duodenal and ileal transport of sodium, chloride, water, and galactose in rats two weeks after a 70% small bowel resection.¹⁰ By four weeks after resection, increased duodenal sodium, chloride, water, and ileal galactose transport were found. There was increased morphological growth both two and four weeks after resection. Another study evaluated carbohydrate absorption two, six, and 12 weeks after an 80% resection.²² Both in jejunal and ileal remnants, a progressive rise, first seen by six weeks, was evident. The present experiments show that three weeks after resection, both the glucose induced (reflecting Na^+ coupled glucose transport by the villus epithelium) and the theophylline provoked PD response (reflecting active Cl^- secretion by the crypt epithelium) in the ileum were significantly reduced as compared with sham operation. At that time, a considerable increase in villus length was found in resected rats but no enlargement of the crypts. Similar results were found for the jejunum three weeks after resection. We assume that three weeks after resection the enteric remnant exhibits functional immaturity as a consequence of increased turnover of cells. Such immaturity has already been postulated^{5-7,10} and may explain the reduction in Na^+ glucose coupled transport, a characteristic feature of mature villus cells. The parallel reduction in the theophylline induced PD is more difficult to interpret, considering the prominent role of immature intestinal crypt cells in active Cl^- secretion. This finding cannot be explained by a hyperactivation of the Cl^- secretory pores by endogenous secretagogues in the SB group, because the basal PD was not significantly different between SH and SB rats. A possible factor expected to result in impairment of the electrical response to both glucose and theophylline in the early adaptive phase after small bowel resection might be the absence, or a functional alteration, of intercellular tight junctions during this hyperplastic period. Madara highlighted the important role of tight junctions in the function of intestinal barriers as well as the maintenance of polarity of the epithelial cells, a prerequisite for vectorial

transcellular transport.²³ The analysis of possible changes in occluding junction structure and function will be the subject of a separate investigation.

In the ileum, a different picture emerges 10 weeks after resection. Ileal PD measurements in resected rats were then no longer different from those in sham operated rats. Also, villus length was no longer significantly increased in resected animals. By contrast, by 10 weeks after resection, evoked jejunal PD responses remained lower compared with values 10 weeks after the sham operation. This finding, coupled with still significantly enlarged jejunal villus length after resection, implies that, for the specific transport capacities tested, functional adaptation of the ileum precedes functional adaptation of jejunum after a 90% bowel resection in rats.

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