Adaptative response of α - and β -glucosidase kinetics along the villi of rat self-filling jejunal blind loops

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SUMMARY Four and 12 days after the construction of self-filling jejunal blind loops in the rat, the apparent K_m - and V_{max} -values of lactase/ β -glucosidase and neutral α -glucosidase were determined by *in-situ* quantitative enzyme histochemistry, and changes in the villus-crypt architecture of the mucosa were examined by microdissection. The results were compared with corresponding data from sham-operated controls. The kinetic data were obtained from the base and the transition zone between medium and apical villus third by the use of a microdensitometric technique. The apparent V_{max} of lactase/ β -glucosidase is significantly smaller than in the control rats at both measuring sites of the villi and even decreases from day 4 to day 12. The apparent V_{max} of neutral α -glucosidase is not affected, and thus the same increase in enzyme activity along the villi as in the controls is observed. The apparent K_m of this enzyme, however, is already significantly increased on day 4 at both villus positions in the blind loops. A pronounced increase in villus surface area is detected in the blind loops as a result of an increase in crypt cell proliferation. The results indicate that enzymatic adaptation in the self-filling blind loops of rat jejunum exhibits different patterns for brush border α - and β -glucosidases and is at least in part accomplished independently of the pronounced mucosal transformation occurring in this experimental condition.

Hyperplasia occurs in both villus and crypt compartments of the mucosa in self-filling jejunal blind loops.¹⁻⁵ In both mucosal homogenates^{3,6} and in brush border preparations⁷⁻¹⁰ decreases in the specific activity of disaccharidases and peptide hydrolases have been described in this form of intestinal reaction. One assumes thereby a decrease in the functional capacity of the individual enterocytes when the rate of crypt cell proliferation is increased.^{1,5,11,12} Until now, however, there have been no reports on kinetic investigations of brush border enzymes at different stages of enterocyte maturation along the villus in this condition.

In the present study, therefore, a quantitative histochemical method is applied, which makes it possible to obtain with tissue sections apparent K_m - and V_{max} -values for brush border neutral α -glucosidase and lactase/ β -glucosidase at different villus sites.¹³⁻¹⁶ The temporal course of the functional adaptation of these brush border membrane enzymes in self-filling, jejunal blind loops is

recorded four and 12 days after surgery, and is related to the extent of the morphological transformation of the crypts and villi, quantified by microdissection.^{17,18} Finally, a comparison is made with corresponding data from control rats that have undergone sham operations. In this way an attempt is made to gain some insight into the mechanisms of adaptation of the small intestinal mucosa in a blind-loop syndrome, and into possible correlations between its functional and morphological characteristics.

Methods

SURGICAL PROCEDURE AND POSTOPERATIVE CARE Forty female Wistar rats, body weight 170-220 g, were maintained under standard conditions (temperature $22 \pm 2^\circ\text{C}$, 12 hour light cycle, Altromin standard pellet diet, tap water *ad libitum*) until 12 hours before operation, when they were starved. They were anaesthetised with ether and underwent either sham operation ($n=20$) or construction of self-filling jejunal blind loops ($n=20$). For this purpose, the intestine was first transected 15 cm

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below the ligament of Treitz; next, the proximal orifice was closed with sutures, and then the distal opening was joined to the jejunum, 5–6 cm above the closure, by side-to-end anastomosis.⁵ For the first two days after the operation all animals received only a solution consisting of a mixture of equal parts of 150 mM NaCl and 300 mM glucose; thereafter, pellet diet was again offered *ad libitum*. After four and 12 days each 10 animals with loops and 10 controls were killed and thus four experimental groups ensued. In each group four rats chosen at random had received 1 mg/kg body weight vincristine intraperitoneally one hour before death. The blind loops and corresponding jejunal segments from the sham-operated controls were excised for quantitative histochemical studies of enzyme kinetics, morphometry (samples from all 10 animals of each experimental group), and evaluation of the total number of cells in metaphase arrest per crypt in the four injected animals of each group. Taking into account the great number of single absorbance measurements necessary to perform the *in-situ* determinations of apparent K_m - and V_{max} -values, intestinal samples from only five random selected animals per group (excluding animals injected with vincristine) were investigated quantitative histochemically.

QUANTITATIVE HISTOCHEMICAL STUDY OF ENZYME KINETICS

The principles and details of the enzyme assay procedures on unfixed cryostat tissue sections (10 μ m thickness) have been reported.^{13–16} Briefly, eight different substrate concentrations were used for each enzyme at pH 6.0: for the detection of neutral α -glucosidase saturation kinetics, the range was between 0.1 mM and 8.88 mM 2-naphthyl- α -D-glucoside and for lactase/ β -glucosidase between 0.03 mM and 2.24 mM 5Br-4Cl-3indoxyl- β -D-fucoside. Hexazonium-p-rosaniline and a Fe^{II}/Fe^{III} -redox-system served as additional reagents for the development of the azo-dye and indigo-dye deposits in the brush border membrane respectively. The tissue sections were incubated for 10 minutes (neutral α -glucosidase) and 20 minutes (lactase/ β -glucosidase) at 37°C in a moist chamber. The coloured reaction products had their absorbance maxima at 480 nm (azo-dye) and 640 nm (indigo) respectively. Absorbance measurements were performed on a Leitz MPV2 microdensitometer using measuring fields of 4×4 μ m in the brush border region, firstly, above the crypt mouth and, secondly, at the transition zone between medium and apical third of the villus; these will henceforth be called 'basal' and 'apical' measuring positions. Uncoloured tissue served as blank (100% transmission). Ten

single measurements per measuring position and substrate concentration were made on each of two tissue sections from each animal. The apical and basal saturation curves thus each consisted of 160 single absorbance measurements for every experimental condition and animal. The calculations of the *in-situ* apparent K_m - and V_{max} -values were made from these data separately for each animal and villus position both parametrically and non-parametrically,¹⁹ using weighted regression analysis (wra)^{20, 21} and direct linear plotting (dlp).²² Thus two apparent K_m - and two apparent V_{max} -values resulted from each saturation kinetic consisting of 160 original absorbance data. By this procedure 80 values were calculated for each apparent kinetic constant. To test the influences of the experimental condition, villus position, time, and type of plot on the K_m - and V_{max} -values factorial analysis of variance²³ were performed with these data, which represent 'means' of one animal and villus position. For this reason, only one residual sum of squares (between rats) was calculated.

MICRODISSECTION AND MORPHOMETRY

Methodological details concerning the analysis of the changes in small intestinal mucosa will be presented in a special study on the time course of jejunal adaptation to the construction of self-filling blind loops (H Menge *et al*, unpublished observations). Briefly said, the intestinal samples destined for microdissection as described by Clarke¹⁷ had been fixed in ethanol/glacial acetic acid (3:1, v/v) for each 24 hours, then rinsed with and stored in 75% ethanol. They were afterwards stained with the Feulgen reagent before the individual villi were liberated under a stereomicroscope. From each sample 10 random isolated villi were chosen for the determination of their height, width, and breadth both at the base and at the apex. From these values the surface area of an individual villus was calculated.¹⁸ Subsequently, the individual crypts were dissected, stained with Feulgen reagent, and their lengths recorded. The total numbers of cells in metaphase arrest per crypt one hour after intraperitoneal injection of vincristine, henceforth called mitoses/crypt, were detected in intestinal samples that had been prepared in the same manner.

The means of the morphometric data for the individual animals were compared using factorial analysis of variance²³ to test the influence of surgical treatment and time after operation.

Results

IN SITU APPARENT KINETIC CONSTANTS *Neutral α -glucosidases*

Table 1 presents the means and their standard deviations of the histochemically detected Vmax (absorbance units) and Km (mM) values of the neutral α -glucosidase calculated from the data of each animal at the apical and basal villus positions and for the experimental conditions on days 4 and 12 evaluated by weighted regression analysis and direct linear plotting. Factorial analysis of variance performed with the data from each animal (Table 2) supplies statistical evidence for the following findings: the control animals show a pronounced increase in apparent Vmax and substrate affinity (decrease in Km) from the basal to the apical villus position. This functional pattern is equally detected on days 4 and 12 after sham-operation. In comparison with the controls no significant changes in the apparent Vmax occur in the self-filling blind loops. This holds true for both villus positions, and therefore the normal Vmax-gradient is sustained. The apparent Km, however, is increased at the 5% level of confidence on the villi of the blind loops. This finding appears to be more pronounced at the apical measuring positions, but there is no significant (Table 2) two factor interaction of position $\times$ treatment that could supply statistical evidence for this trend. The alterations in the patterns of kinetic parameters for α -glucosidase data along the villi of self-filling blind loops are already established on day 4; time, alone or in combination with other effects, fails to have any significant influence.

Lactase/ β -glucosidase

The control animals showed an increase in the apparent Vmax-values of about 50% from the villus base to the transition zone between medium and apical villus third (Table 3), which is highly significant (Table 4). The Km-values (Table 3) do not

Table 1 Apparent kinetic data of neutral α -glucosidase (mean $\pm$ SD), Vmax (AU), Km (mM)

	Controls		Blind loops	
	Apical	Basal	Apical	Basal
Day 4				
wra { Vmax	1.66 $\pm$ 0.268	1.15 $\pm$ 0.113	1.74 $\pm$ 0.323	1.11 $\pm$ 0.152
Km	0.38 $\pm$ 0.106	0.52 $\pm$ 0.163	0.52 $\pm$ 0.216	0.64 $\pm$ 0.181
dip { Vmax	1.62 $\pm$ 0.272	1.15 $\pm$ 0.216	1.71 $\pm$ 0.338	1.02 $\pm$ 0.177
Km	0.39 $\pm$ 0.089	0.58 $\pm$ 0.157	0.49 $\pm$ 0.140	0.55 $\pm$ 0.160
Day 12				
wra { Vmax	1.47 $\pm$ 0.251	1.16 $\pm$ 0.209	1.54 $\pm$ 0.177	1.22 $\pm$ 0.082
Km	0.40 $\pm$ 0.185	0.62 $\pm$ 0.197	0.58 $\pm$ 0.157	0.68 $\pm$ 0.138
dip { Vmax	1.40 $\pm$ 0.221	1.10 $\pm$ 0.226	1.47 $\pm$ 0.140	1.09 $\pm$ 0.100
Km	0.40 $\pm$ 0.196	0.61 $\pm$ 0.174	0.55 $\pm$ 0.174	0.63 $\pm$ 0.260

wra, dip = Apparent kinetic data determined by weighted regression analysis and direct linear plotting respectively.

Table 2 Factorial analysis of variance (α -glucosidase)

Effect	DF	Vmax		Km	
		ssq	msq	ssq	msq
Total	79	7.7233		2.6033	
Treatment	1	0.0092	0.0092	0.1737	0.1737*
Position	1	4.0586	4.0586‡	0.3767	0.3767†
Time	1	0.1582	0.1582	0.0472	0.0472
Plot	1	0.0715	0.0715	0.0070	0.0070
Position $\times$ treatment	1	0.0465	0.0465	0.0468	0.0468
Treatment $\times$ time	1	0.0116	0.0116	0.0020	0.0020
Treatment $\times$ plot	1	0.0091	0.0091	0.0198	0.0198
Position $\times$ time	1	0.2997	0.2997*	0.0029	0.0029
Position $\times$ plot	1	0.0015	0.0015	0.0009	0.0009
Time $\times$ plot	1	0.0122	0.0122	0.0005	0.0005
Treatment $\times$ position $\times$ time	1	0.0857	0.0857	----	----
Treatment $\times$ time $\times$ plot	1	----	----	0.0041	0.0041
Position $\times$ time $\times$ plot	1	0.0679	0.0679	0.0002	0.0002
Plot $\times$ treatment $\times$ position	1	0.0209	0.0209	0.0047	0.0047
Treatment $\times$ position $\times$ time $\times$ plot	1	----	----	0.0077	0.0077
Residual	64	3.0074	0.0470	1.9129	0.0299

* $p < 5\%$. † $p < 1\%$. ‡ $p < 0.1\%$.
---- = Negative values calculated.

display any systematic changes along the normal villi, which is at least in part due to the different results obtained by the two types of calculation procedures leading to a significant effect in the plot line of the factorial analysis of variance (Table 4). Moreover, the time after sham-operation exerts a significant effect on the Vmax of lactase/ β -glucosidase. This parameter decreases at both villus positions from day 4 to day 12. On the villi of the self-filling blind loops the Vmax is appreciably decreased (Table 3 and 4). This effect is most

Table 3 Apparent kinetic data of lactase/ β -glucosidase (mean $\pm$ SD), Vmax (AU), Km (mM)

	Controls		Blind loops	
	Apical	Basal	Apical	Basal
Day 4				
wra { Vmax	0.93 $\pm$ 0.161	0.34 $\pm$ 0.073	0.47 $\pm$ 0.272	0.09 $\pm$ 0.119
Km	0.19 $\pm$ 0.113	0.14 $\pm$ 0.086	0.16 $\pm$ 0.085	0.61 $\pm$ 0.920
dip { Vmax	0.91 $\pm$ 0.093	0.40 $\pm$ 0.100	0.45 $\pm$ 0.242	0.12 $\pm$ 0.206
Km	0.19 $\pm$ 0.104	0.41 $\pm$ 0.114	0.15 $\pm$ 0.125	0.55 $\pm$ 0.521
Day 12				
wra { Vmax	0.68 $\pm$ 0.151	0.29 $\pm$ 0.112	0.16 $\pm$ 0.240	0.01 $\pm$ 0.029
Km	0.21 $\pm$ 0.022	0.22 $\pm$ 0.127	—	—
dip { Vmax	0.72 $\pm$ 0.176	0.26 $\pm$ 0.118	0.15 $\pm$ 0.239	0.01 $\pm$ 0.024
Km	0.25 $\pm$ 0.049	0.17 $\pm$ 0.090	—	—

wra, dip = Apparent kinetic data determined by weighted regression analysis and direct linear plotting respectively.

Table 4 Factorial analysis of variance (β -glucosidase)

Effect	DF	Vmax		Km	
		ssq	msq	ssq	msq
Total	79	8.5081		0.5364	
Treatment	1	2.9193	2.9193‡		
Position	1	2.7387	2.7387‡	0.0058	0.0058
Time	1	0.6399	0.6399†	0.0049	0.0049
Plot	1	0.0011	0.0011	0.0436	0.0436*
Position $\times$ treatment	1	0.2863	0.2863†		
Treatment $\times$ time	1	----	----		
Treatment $\times$ plot	1	0.0010	0.0010		
Position $\times$ time	1	0.1393	0.1393*	0.0389	0.0389*
Position $\times$ plot	1	0.0023	0.0023	0.0234	0.0234
Time $\times$ plot	1	0.0011	0.0011	0.0482	0.0482*
Treatment $\times$ position $\times$ time	1	0.3609	0.3609†		
Treatment $\times$ time $\times$ plot	1	0.3495	0.3495†		
Position $\times$ time $\times$ plot	1	0.0125	0.0125	0.0886	0.0886†
Plot $\times$ treatment $\times$ position	1	0.0009	0.0009		
Treatment $\times$ position $\times$ time $\times$ plot	1	----	----		
Residual	64	1.7408	0.0272		

* $p < 5\%$. † $p < 1\%$. ‡ $p < 0.1\%$.

---- = Negative values calculated.

pronounced basally, as is reflected by a significant two-factor interaction between position $\times$ treatment. The reduction in Vmax is even more dramatic 12 days after construction of the blind loops, as witnessed by the great number of zero absorbance values in most animals. Because of this, apparent Km-values could not be calculated in that experimental group (Table 3). There is a trend towards an increase in Km at the basal measuring position of the villi in self-filling blind loops (Table 3). This observation, however, is derived from the data of only three animals, and their variance is rather large. Significant differences in the apparent Km-values between the controls and the animals with blind loops could not be assured statistically either at the villus base or at the apical villus position.

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The data depicted in Table 5 represent the results from the morphological analysis performed on the plain of the villus/crypt-architecture at the same time after operation as the histochemical investigations. These results are part of a more detailed analysis of the time course of the changes in mucosal architecture and enterocyte kinetics after construction of self-filling jejunal blind loops that will be reported elsewhere (H Menge *et al*, unpublished observations). The parameters given in the present work have been selected especially for comparison with the enzymatic data obtained on the level of

Table 5 Morphometric data (mean, number of animals in parentheses)

Treatment controls	S F B	
Day 4		
24.3 (4)	41.6 (4)	m/c
138.4 (10)	224.7 (10)	cl
356.1 (10)	435.3 (10)	vh
0.26 (10)	0.43 (10)	vs
Day 12		
25.0 (4)	43.6 (4)	m/c
142.4 (10)	290.8 (10)	cl
338.7 (10)	349.5 (10)	vh
0.29 (10)	0.59 (10)	vs

m/c = mitoses per crypt, cl = crypt length (μm), vh = villus height (μm), vs = villus surface (mm^2).

single villi. As witnessed by the results of factorial analysis of variance the number of mitoses per crypt is affected only by the surgical procedure, whereas the other parameters are additionally influenced by the time after operation. In general, crypt length, villus height, and villus surface are all significantly increased in the loops. Moreover, the time course of the changes in crypt length and villus height differs in the loops and the controls, as shown by the significant influence of the two-factor interaction treatment $\times$ time: an increase in crypt length until day 12 is shown only for the loops; during this time villus height slightly decreases in the controls, but is considerably diminished in the loops reaching the initial values of the controls. The villus surface, however, is increased until day 12 in both experimental groups, and this occurs on a higher level in the loops than in the controls. In the loops the villus height does not increase to the same extent as the villus surface; this is mainly due to an increase in villus breadth (values not shown in the Table) especially from day four to day 12.

Discussion

The present study makes it clear that different adaptative patterns of α - and β -glucosidases occur in

Table 6 Factorial analysis of variance

Parameter	Treatment	Time	Treatment $\times$ time
Mitoses/crypt	‡	—	—
Crypt length	‡	†	†
Villus height	†	†	*
Villus surface	‡	†	—

* $p < 5\%$. † $p < 1\%$. ‡ $p < 0.1\%$.

the brush border membrane after the construction of self-filling jejunal blind loops. It has only been possible, however, to reach this conclusion through kinetic investigations at the level of the individual enterocytes on consideration of the functional gradients of operational K_m - and V_{max} -values of neutral α -glucosidase and lactase/ β -glucosidase, which normally occur along the villi of the sham-operated controls. Parallel to the hyperproliferative transformation of the mucosa of self-filling blind loops,¹⁻⁵ which is already present after four days, there is a progressive reduction in the apparent V_{max} of lactase/ β -glucosidase accompanying further increases in villus surface but not in villus height on day 12. The reduction of V_{max} is especially evident at the base of the villi. The decrease in lactase activity agrees with reports on the reduction in brush border enzyme activities in mucosal homogenates^{3,6} as well as in brush border membrane preparations.⁷⁻¹⁰ It also agrees with histochemical findings for leucine-aminopeptidase reported for another example of a hyperproliferative transformation of the jejunal mucosa – namely, that arising from transposition of the jejunum to the colon of the rat.²⁴ In that study, which did not include any kinetic analysis, the specific activity of leucine-aminopeptidase was reduced both at the base and the apex of the villi of the transported intestine, but the distribution of the enzyme along the villus was unchanged.

In contrast with these findings, there is merely an increase in the apparent K_m of neutral α -glucosidase in the blind loop mucosa, without any decrease in V_{max} at the level of the individual enterocytes. This decrease in substrate affinity is complete after four days and does not change further between day four and day 12. It occurs, therefore, independently of the progressive enlargement of the villus surface area. In the controls that have undergone sham operations there is an increase in substrate affinity along with the increase in V_{max} from the base to the transition zone between the middle and upper third of the villi. This change is maintained in the mucosa of the blind loops. Thus the less differentiated enterocytes at the base of the villus have not only less brush border enzyme protein but, in addition, some enzymes have apparently not yet reached full functional maturity as judged by their apparent K_m -values. We have previously made similar observations in various control series in which quantitative histochemical investigations of α -glucosidase kinetics were performed (S Gutschmidt *et al*, unpublished observations). It therefore appears possible that in self-filling blind loops the increased proliferative rate in the crypts,¹ resulting in an increased turnover of

enterocytes, may be the cause of decreased substrate affinity in the brush border membrane of less differentiated enterocytes. This hypothesis is supported by the opposite finding of increased substrate affinity of neutral α -glucosidase in hypoproliferative atrophic jejunal mucosa in rats (S Gutschmidt *et al*, unpublished results).

The finding of an unchanged V_{max} for neutral α -glucosidase at the level of the individual cell of the blind loop mucosa might be expected to lead to an overall increase in activity in the total homogenate in view of the enlarged surface area of the villus. A decrease, however, in the specific activity of this enzyme in the mucosa of self-filling blind loops has been widely reported.⁷⁻¹⁰ The reason for this discrepancy is not yet clear. The cited authors did not carry out any kinetic investigations and possibly did not work with optimal substrate concentrations, in view of the altered K_m . Furthermore, it is not known whether the non-enzyme protein content of the mucosa, on which the specific activity clearly depends, also changes in the experimental blind loops. Löhrs *et al*,¹ for instance, reported a hypertrophic change of the muscularis propria during this condition. A relative preponderance of non-enzyme protein in the brush border could result from the effect of bacterial proteinases, which enhance the degradation of glycoproteins¹⁰ and may act like elastase⁹ on the disaccharidases located in and on the outside of the brush border membrane.^{25,26} It remains to be clarified whether the enzyme activity in the area of the villus tips declines disproportionately under the influence of the accumulated chyme and thereby leads on balance to a decrease in substrate hydrolysis in investigations on brush border membrane preparations, in which up to now the activity pattern along the villus has not been taken into consideration.

Even animals that have undergone sham operation develop a discrete transformation of their villi. Nevertheless, these are not accompanied by significant changes in parameters of enzyme kinetics. A closer analysis of the morphometrically measurable changes at various times after real as well as sham operations will be reported elsewhere (H Menge *et al*, unpublished observations).

In summary, one must settle for the time being for a description of altered patterns of enzyme kinetics in two brush border membrane disaccharidases in self-filling jejunal blind loops in rats. When cell proliferation is increased, lactase/ β -glucosidase appears to be especially susceptible to factors that decrease its activity. Neutral α -glucosidase merely shows a decreased substrate affinity that could possibly be the precursor to a subsequent decrease of V_{max} . The enzymatic response presented in

these series is very different from the decrease in apparent $V_{\max}$ of α -glucosidase accompanied by an increase in lactase/ β -glucosidase activity obtained in the ileal remnants after proximal intestinal resection (S Gutschmidt *et al*, unpublished observations). This might be due largely to differences between jejunum and ileum, but also because of the nature of the hyperproliferative response in the different experimental conditions.

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