Effect of Oral Insulin on the Development of Small Intestine Carbohydrate Hydrolysis and Absorption in Suckling Rats

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Abstract: The study is conducted to reveal the effect of oral insulin on the development of carbohydrate hydrolysis and transport in the small intestine in suckling rats. Oral insulin administration (5 U/kg/day) from the 1st to the 21st day of postnatal life resulted in induction of the carbohydrate assimilation in the small intestine of growing rats. Oral insulin increased intestinal maltase, sucrase and lactase activity as well as absorption of free and hydrolysis-dependent glucose from maltose in the small intestine of suckling rats. The effect was age-dependent and was clearly expressed during the second half of the milk nutrition period. After weaning oral insulin effect on the intestinal carbohydrate digestion and absorption was absent. These data show oral insulin keeps its biological activity in the intestinal cavity and they suggest involvement of milk insulin in the hydrolysis and absorption of carbohydrates in the small intestine.

Keywords: Oral Insulin, Disaccharidases, Glucose Absorption, Small Intestine, Suckling Rats

1. Introduction

Newborn rats as well as many other immature mammal offspring are totally dependent on their mother milk and this relationship is maintained for two weeks. At the time of weaning which takes place at the end of the third week after birth the breast milk lactose is replaced by solid food containing starch, maltose, sucrose and other poly- and disaccharides [1-5].

These diet changes correspond to shifts in the intestinal carbohydrate assimilation. During breast-feeding lactase activity as well as free and comprised in lactose glucose absorption are well defined. At the same period the intestinal maltase and sucrase activity as well as hydrolysis-dependent transport glucose from maltose or sucrose are low. In contrast, at the time of weaning the assimilation of lactose is decreasing and the assimilation of maltose and sucrose is increasing [1, 4, 6-11].

Hormonal control plays an important role in the development of intestinal enzyme activities [1, 4, 7, 10-13]. The level of corticosterone [14], thyroxin [15] and insulin [16] increases in the rat serum together with abrupt shifts in the structure and function of the small intestine at the time of weaning [1-8]. Moreover, insulin level is elevating as in the suckling blood and in their mother breast milk [16]. Oral administration of insulin causes an increase of the pancreatic α-amylase activity which takes part in the initial stage of carbohydrate hydrolysis in small intestine in suckling rats [16, 17]. It is not yet clearly known whether oral insulin has any effect in both final hydrolysis and transport of carbohydrates in the small intestine of sucklings.

2. Objective

To study the effect of oral insulin on the activity of carbohydrate hydrolysis and transport in the small intestine in suckling rats.

3. Materials and Methods

3.1. Animals and Treatment

Wistar rats were used and fed standard laboratory diet and

tap water ad libitum. To obtain offspring four females and one male (170-180 g) were kept during 2 days in plastic cages (50 x 30 x 28 cm³). When obvious signs of pregnancy were seen, the females were placed into individual cages (35 x 28 x 28 cm³). Newborns from different litters (at least 3-4) were mixed and placed in litters of eight. Sucklings were kept with their nursing mother by the end of experiments. The air temperature in the room where rats were housed was 18-26°C and relative humidity was 40-60%.

Insulin (Novo Nordisk, Denmark) was administered orally (5 U/kg b. w. /day) from the first to the 21st day of postnatal life. Control animals were treated by the same volume of solvent. To determine the activities of intestinal disaccharidases animals were sacrificed 1, 7, 14, 21, and 28 days after birth. To determine the glucose transport rate the small intestine perfusion (*in situ*) was performed 10, 20, and 30 days after birth.

3.2. Sampling Procedure

After rat decapitation the abdominal cavity was quickly dissected, and the small intestine was removed and carefully separated from the mesentery. Then small intestine was washed with 10 ml of cold saline, dried with filter paper, weighed, and homogenized in saline (100 mg of tissue per 1 ml of saline) with the aid of glass-teflon homogenizer (200-300 g/min) per minute. All steps were performed in cold condition. Obtained homogenate was used for determination of disaccharidase activity.

To identify the rate of intestinal glucose absorption rats were anesthetized by intraperitoneal injection of nembutal (3.5 mg/100 g b. w.). Abdomen was opened by midline incision and a 10 cm loop of jenium starting near the Treitz ligament was defined by two incisions. The defined loop of small intestine was cannulated and washed with 10 ml of warm saline. Then the intestine was lowered into the peritoneal cavity and skin wound edges were sutured. The small intestine segment was perfused with 27.6 mM preheated to 37°C solution of glucose or maltose. The perfusate was collected into cooled by ice tubes for 15 minutes and then was

used for biochemical analysis. The volume of liquid in the isolated segment was about 0.4 ml. The rate of perfusion (0.3 ml/min) was physiological for growing rats [18]. The weight of perfused segment of small intestine was determined.

3.3. Biochemical Analyzes

Activity of enteral maltase (EC 3.2.1.20), succrase (EC 3.2.1.48) and lactase (EC 3.2.1.23) was determined by Dahlqvist [19]. The protein content in small intestine tissues was determined by Lowry et al. [20].

Free glucose absorption rate was determined by the difference of glucose concentration in the initial and final solution by glucose oxidase method [19]. The transport rate of glucose comprised in maltose was determined by both anthrone [21] and glucose oxidase [19] methods in the initial and final perfusate solution.

3.4. Statistics

All results are presented as mean ± S. E. Difference between mean of experimental and control groups were evaluated by unpaired Student's test with P <0.05 considered as significant.

4. Results

The body weight increased rapidly in two groups. There was no significant difference in body weight between the control and experimental groups up to 21st day after birth. But there was some tendency to increase the rate body weight in the experimental groups compared with control groups. In 28-day-old rats treated with insulin body weight was significantly higher than that in control group rats (Table 1).

The weight of the small intestine also increased with age in both groups. However, the weight of small intestine of 14- and 21-day-old rats exposed to daily oral insulin treatment significantly increased compared with control group rats (Table 1).

Table 1. Effect of oral insulin on the body and small intestine weight in growing rats (M ± m; n = 5-6).

Animal groups	Age (days)				
	1	**7**	**14**	**21**	**28**
Control Experiment P		Body weight (g)			
		14.4 ± 0.6	24.7 ± 1.2	32.4 ± 1.8	41.1 ± 2.6
	5.3 ± 0.2	16.2 ± 0.9	26.7 ± 1.5	37.2 ± 2.2	49.0 ± 3.0
		>0.5	>0.25	>0.1	<0.05
Control Experiment P		Small intestine weight (mg)			
		456.1 ± 19.2	678.6 ± 43.4	913.2 ± 64.5	1511.4 ± 97.3
	196.2 ± 11.0	511.1 ± 23.9	933.0 ± 65.4	1233.1 ± 97.6	1732.2 ± 102.1
		>0.1	<0.01	<0.01	>0.1

In the sucklings treated with insulin the development of enteral maltase activity was the same as in control. The enzyme activity was low in the intestine during the first

postnatal week, but later its activity increased, reaching maximal levels by the end of the fourth week. The level of enzymatic specific activity was significantly increased in

insulin-treated groups beginning from the 14th postnatal day compared with that of the control (Table 2).

The specific activity of sucrase was very low on the 7th postnatal day, increased on the 14th postnatal day, and reached maximal level on the day 28th after birth in both groups, but it was significantly higher in insulin treated groups than in control groups.

Even the development pattern of lactase activity was the

same in the rats of both groups. The lactase activity was maximal during the first week after birth, and then began to decline, reaching adult values by the end of the fourth week. However, the average lactase activity level on the 14th, 21st, and 28th postnatal day was significantly increased in insulin-treated groups compared with that of the control groups (Table 2).

Table 2. Effect of oral insulin on the activity disaccharidases (mmol/min/g protein) in small intestine in growing rats (M ± m; n = 5-6).

Animal groups	Age (days)				
	1	7	14	21	28
Control Experiment P			Maltase		
Control		56.1 ± 4.5	84.6 ± 6.6	111.9 ± 12.2	250.7 ± 19.9
Experiment	74.2 ± 6.1	59.6 ± 5.9	295.9 ± 22.4	313.3 ± 10.3	336.8 ± 32.5
P		>0.5	<0.001	<0.001	<0.05
			Sucrase		
Control			2.3 ± 0.2	14.1 ± 0.6	25.9 ± 2.1
Experiment	0.8 ± 0.1	1.0 ± 0.1 1.1 ± 0.2	12.0 ± 1.4	21.1 ± 1.4	35.3 ± 3.0
P		>0.5	<0.001	<0.001	<0.02
			Lactase		
Control			65.3 ± 2.3	54.0 ± 2.0	20.4 ± 1.1
Experiment		69.9 ± 2.7	101.8 ± 3.7	100.7 ± 3.2	63.0 ± 3.8
P	70.7 ± 1.9		<0.001	<0.001	<0.001
Control Experiment P		73.9 ± 4.2 >0.5			

Tendency of age-dependent changes in the absorption of free glucose was identical in both groups (Table 3). However, the rate of glucose transport was significantly increased in the 10-day-old rats as compared with control rats.

Development of the rate hydrolysis-dependent absorption

glucose from maltose solution in experimental group rats was the same as in control group, but the level of glucose absorption was significantly increased in insulin-treated rats on the 10th and 20th postnatal days (Table 3).

Table 3. Effect of oral insulin on the rate of glucose absorption (mmol/min/g tissue) in small intestine in growing rats (M ± m; n = 5-6).

Animal groups	Age (days)		
	10	20 20	30
	Free glucose		
Control	7.2 ± 0.5	5.0 ± 0.3	3.4 ± 0.2
Experiment	8.8 ± 0.5	5.3 ± 0.4	4.0 ± 0.3
P	<0.05	>0.5	>0.1
	Glucose comprised in maltose		
Control		6.2 ± 0.4	7.6 ± 0.5
Experiment	4.6 ± 0.3 6.3 ± 0.5 <0.01	8.4 ± 0.7	8.9 ± 0.7
P		<0.02	>0.1

Thus, the results show that the chronic oral administration of insulin to suckling rats leads to increase of activity of hydrolysis and transport of carbohydrates in the small intestine. It is expressed in age-dependent increase of carbohydrase (maltase, sucrose, and lactase) activity and free and hydrolysis-dependent glucose absorption in the small intestine in growing rats.

5. Discussion

Results show that oral insulin affects on the carbohydrate assimilation in the small intestine during milk nutrition period

in suckling rats. Oral administration of insulin caused age-dependent induction of carbohydrate hydrolysis and absorption activity in growing rat. Effect of oral insulin was absent in the early age (7-day-old rats) and well expressed in the middle and end of the milk nutrition period (10-21-day-old-rats). The oral insulin effect on intestinal digestion and absorption was disappeared after weaning (28-30-day-old-rats). It should be noted that the oral hormone effect on the intestinal carbohydrate hydrolysis and absorption is most pronounced when insulin level elevates in the suckling serum and breast milk [16].

It is known hormonal control plays major role in the early

postnatal ontogeny of the intestine. Administration of glucocorticoids or thyroids at the critical stage in maturation results in precocious increasing of intestinal α-glycosidase activity and hydrolysis-dependent glucose absorption from maltose and/or saccrose [1, 4, 7, 11-13, 23]. However, glucocorticoids have no effect on lactase activity and free glucose transport [11, 25]. Obtained and literature data show insulin has the same effect as cortisone and/or thyroxin on the intestinal functions which are low at birth, then increase [22-32]. However, our current and earlier results show insulin unlike hydrocortisone or thyroxin [25, 26] leads to increase of intestinal carbohydrate hydrolysis and transport which are high at birth, then decrease (intestinal lactase and free glucose transport (Table 2 and 3)). The same results concerning the lactase activity and free glucose transport have been obtained in suckling rats after subcutaneous administration of insulin [22-26]. Indeed, insulin and/or corticosteroids have no effect on the developmental lactase decline [1, 4. 11, 22, 27]. The natural decline in intestinal lactase activity as well as absorption of free glucose and glucose from lactose controlled mainly by thyroid hormones [12, 13, 25, 26]. These results indicate possible participation of oral insulin, including milk insulin, in the prevention of precocious decrease in lactase activity which might cause lactose intolerance in sucklings.

The increase of serum insulin level in sucklings after intragastric administration insulin as part of an infant formula [16, 17] and the presence of insulin receptors in developing mucosa in suckling rats [24, 28] also show the high biological role of oral hormone for small intestine development. The revealed an increase in the small intestine mass in the insulin-treated rats confirm the findings of other authors that milk insulin and insulin-like factors have trophic effect on the intestinal mucous [29-31] in growing rats.

The data show oral insulin keeps its biological activity and suggest involvement of breast milk insulin in the regulation of intestinal carbohydrate hydrolysis and transport in suckling rats. This suggestion is confirmed by the findings that the intestinal mucosa is completely permeable to insulin [16] and insulin has gastrointestinal stability, i.e. it does not split in the gastrointestinal tract cavity [32].

Maternal milk contains a number of factors that can enhance development of the immature of gastrointestinal tract [33]. Of these, insulin is present in maternal milk at levels three to fourfold higher than in maternal blood [34]. Except for participation in hydrolysis and transport of carbohydrates, it has been shown oral insulin, including milk insulin, to accelerate a number of other gastrointestinal functions [16, 17, 23, 24, 28- 31, 33-35].

Taken together, the present results suggested that oral insulin plays a crucial role in the development of intestinal digestion and absorption of carbohydrates. Oral insulin increases activities of α-glucosidases (maltase and saccrase) and β-galactosidases (lactase) as well as transport of free and hydrolysis-dependent glucose from maltose in the small intestine. Therefore, the exogenous insulin, including milk-borne insulin, might stimulate the intestinal adaptation to adult food and participate in the precocious decrease in lactase

activity.

6. Conclusion

Orally administered insulin leads to a sharp induction of the hydrolysis and transport of carbohydrates in the small intestine in suckling rats. This is manifested in increase of intestinal activity of maltase, sacrase and lactase as well as in absorption of free and hydrolyse-dependent glucose from maltose in the small intestine of sucking rats. That suggests the participation of oral insulin, including milk insulin, in carbohydrate assimilation in the small intestine.

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