BIOGERONTOLOGY

Effect of Vilon and Epithalon on Activity of Enzymes in Epithelial and Subepithelial Layers in Small Intestine of Old Rats

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Per os administration of Vilon (Lys-Glu) or Epithalon (Ala-Glu-Asp-Gly) to aged Wistar rats for 1 month significantly increased activity of membrane enzymes maltase and alkaline phosphatase in epithelial layer of the small intestine. In addition, Vilon significantly increased activity of cytosolic glycyl-L-leucine dipeptidase in the stromal and seromuscular layers of the small intestine in comparison with the control rats not treated with this agent. These findings suggest improvement of trophic and barrier functions of the small intestine and corroborate the hypothesis on the existence of not only epithelial, but also subepithelial enzymatic barrier supporting the enzyme system in the small intestine, especially in aged animals.

Key Words: *small intestine; enzyme activity; Vilon; Epithalon; aging; rats*

It is hypothesized that enzyme systems of subepithelial layers of the small intestine (SI), *i.e.* stromal layer (SL), seromuscular layer (SML), and epithelial layer (EL), serve as a barrier preventing penetration of peptides and peptide-related compounds into internal milieu of the organism [12]. The enzyme systems of SI subepithelial layers significantly increase their transformation potency. Numerous experiments demonstrated the involvement of enzyme systems of SL and SML in the response to extreme factors such as hunger, refeeding, surgery, *etc*. It was shown that SI acts as perfect and reliable barrier due to cooperation of enzyme systems, especially peptidase enzymes located in the epithelial and subepithelial layers [3,8,10]. Some age-related peculiarities in the distribution of enzyme systems in various layers of SI and their sensitivity to stress were demonstrated [6,7,11]. Peptidase activity in various layers of SI and its sensitivity to stress are higher in young animals. Young and middle-age animals are more tolerant to protein deprivation compared to aged animals.

We previously demonstrated beneficial effects of peptide preparations Vilon and Epithalon on activity of some digestive enzymes in aged rats: treatment with these preparations increased activity of digestive enzymes to a level characteristic of young animals [14]. These data prompted us to study the effects of Vilon and Epithalon on the trophic and barrier functions of SI in aged animals. It is known that in aged animals these functions are impaired [6].

MATERIALS AND METHODS

Experiments were carried out on 18-month-old male Wistar rats (n=24) maintained under standard vivarium conditions with food and water *ad libitum*. The control rats (n=8) weighing 432±25 g at the start of

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experiments were fed a standard diet for 1 month. The rats of experimental groups daily received 100 μg (1 tablet) Vilon (Lys-Glu) or Epithalon (Ala-Glu-Asp-Gly). The body weight of experimental rats before treatment with Vilon and Epithalon was 444±23 g (n=7) and 474±26 g (n=8), correspondingly. The rats were weighted before and after the experiment. Isolation of SI and separation of EL, SL, and SML layers were performed as described elsewhere [3,4]. All procedures were carried out on the cold.

Activity of the following membrane-bound enzymes was estimated in the homogenates of each SI layer (middle portion): saccharase (EC 3.2.1.48), maltase (EC 3.2.1.20), alkaline phosphatase (EC 3.1.3.1), aminopeptidase M (EC 3.4.11.2), and predominantly cytosolic glycyl-L-leucine dipeptidase (EC 3.4.13.2). Saccharase activity served as a criterion of separation purity of SI layers, since in rat SI this enzyme is absent in SL and SML. Enzyme activities were estimated in the linear zone by the methods described previously [4]. The data are presented both in micromoles of hydrolysis products formed over 1 min per 1 g protein and in percentage of total activity in all layers. Protein content was measured by the method of Lowry [15].

The data were processed statistically using Student's t test.

RESULTS

The weight of control and experimental rats did not significantly differ throughout the experiment.

Saccharase activity, a marker enzyme of enterocyte brush border membranes, was detected only in EL, and its level was similar in rats of all groups (Table 1).

In control rats, activity of maltase, a typical membrane-bound enzyme, was maximum in EL and minimum in subepithelial layers (Table 1). Vilon given *per os* for 1 month increased maltase activity in EL by 66% in comparison with the control, while maltase activity in SL and SML was below the control (Table 1).

Epithalon given *per os* for 1 month produced a more pronounced increase (by 129%) in maltase activity in EL and 1.5-2-fold reduced this activity in SL and SML in comparison with Vilon-treated rats.

Activity of alkaline phosphatase (a membrane-bound enzyme) and disaccharidases were maximum in EL. By contrast, activity of alkaline phosphatase in SL

TABLE 1. Effect of Vilon and Epithalon on Activity of Enzymes (μM/min 1 g protein) in Various Small Intestine Layers in Old Rats

Enzyme, layer	Control		Vilon		Epithalon	
	abs.	%*	abs.	%*	abs.	%*
Saccharase						
EL	149±14	100	156±57	100	167±29	100
SL	0	0	0	0	0	0
SML	0	0	0	0	0	0
Maltase						
EL	471±78	95	783±83^{+}	97	1080±193^{+}	98
SL	18±4	3.5	18±4	2.3	14±3	1.2
SML	8±1	1.5	6±2	0.7	10±3	0.8
Alkaline phosphatase						
EL	64±8	62	80±5	62	120±17*	74
SL	17±4	16.5	28.9	21.7	22±6	13.6
SML	22±3	21.4	21±4	16.3	20±4	12.4
Aminopeptidase M						
EL	94±17	88	69±11	82	72±11	87
SL	6±2	5.5	7.0±0.9	8.4	5.0±0.7	6
SML	7±2	6.5	8±2	9.6	6±1	7
Glycyl-L-leucine Dipeptidase						
EL	606±125	52	593±119	40	486±127	40
SL	284±37	24	484±26*	33	348±39	29
SML	279±26	24	397±39*	27	377±53	31

Note. *Percent of total activity. ^{+}p<0.05 compared to the control.

and SML was lower in both control and experimental animals (Table 1). Vilon had no effect on activity of this enzyme in various layers of SI. At the same time Epithalon 2-fold increased alkaline phosphatase activity in EL in comparison with the control value. In other layers we detected no changes in activity of this enzyme under the effect of Epithalon.

Distribution of membrane aminopeptidase M activity in EL, SL, and SML of SI was similar to that of other membrane-bound brush border enzymes. Vilon and Epithalon administered *per os* did not change aminopeptidase M activity in examined SI layers (Table 1).

Distribution of glycyl–L-leucine dipeptidase activity in SI layers differed from that of the above enzymes. More than half of total activity of this enzyme was detected in SL and SML. It is noteworthy that 1-month treatment with Vilon increased activity of this dipeptidase in SL and SML by 70 and 40%, correspondingly. Epithalon increased activity of enzyme in these layers by 22 and 35%.

Thus, administration of Vilon and Epithalon to aged rats for 1 month changed the enzyme status of EL, SL, and SML of SI. Both Vilon and Epithalon increased maltase activity in EL, moreover Vilon increased alkaline phosphatase activity. These data agree with previous observations. It is widely known that aging is accompanied by degenerative and atrophic changes in structural elements of SI mucosa, *e. g.* inhibition of some intestinal membrane-bound enzymes, which perform membrane hydrolysis of basic alimentary substances [2,13,14]. We previously found that *per os* administration of Vilon to 11-month-old male and female rats for 2 weeks increased activity of membrane-bound saccharase, maltase, alkaline phosphatase, and dipeptidases in various subdivisions of SI. It is noteworthy that Vilon exerted more favorite effect on old rats that on young ones [14]. In addition to the increase of maltase and alkaline phosphatase in EL induced by Vilon and Epithalon, there was a pronounced increase (especially by Vilon) in activity of glycyl-L-leucine dipeptidase in SL and SML in comparison with that in old controls.

It is a common knowledge that structural and functional characteristics of SI change pronouncedly with age. They manifested in altered enzyme profile of mucous membrane frequently accompanied by decreased activity of digestive enzymes [2,6,13]. At the same time, Vilon and Epithalon enhanced activity of maltase and alkaline phosphatase, the important membrane digestive enzymes in EL of SI, which is another indication of beneficial effects of these drugs on the synthesis of enzyme proteins. The increase in activity of SL glycyl-L-leucine dipeptidase by Vilon attests to improvement of the barrier functions of SI by the drug. The important role of peptidase systems of subepithelial layers of SI, which are the barriers against foreign substances (especially against the protein-like agents), was demonstrated during the effect of stressor factors, surgical intervention in SI, and protein deprivation [8-10]. These data are extremely important in the study of aging and searching for the drugs with geroprotective features [1,5]. Therefore, the effect of Vilon and Epithalon on activity of the enzymes not only in EL, but also in the subepithelial layers involved in trophic and barrier functions, is one of the mechanisms of geroprotective action of these peptides.

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