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Absorption of Glycine in the Small Intestine of Rats Under Physiological Condition

Storchilo OV*

Odessa National Medical University, 2-Valyhovsky lane, Odessa, 65 082, Ukraine

***Corresponding author:** Storchilo O.V, Odessa National Medical University, 2-Valyhovsky lane, Odessa, 65 082, Ukraine, Tel: +38 (048)7255937; Fax: 063 308 50 29; E-mail: alena-61@mail.ru

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Abstract

Objectives and method: To determine the velocity of glycine absorption in the chronic experiments under physiological condition with no operation trauma, pain, narcosis and atrophy of the small intestine using and original method of surgical formation of the functioning fragment of the small intestine with 'living fistulas' in the presence of chime, all gastrointestinal secrets and natural innervations.

Results: The glycine absorption velocity increases during one hour of perfusion. Absolute parameters of absorptive activity of the small intestine in the chronic experiments *in vivo* are higher than in the isolated loop of the rats' small intestine. We observed no dissolution of perfusate with gastrointestinal fluids in the small intestine functioning part indicating that absorption of water in this fragment of small intestine prevails.

Conclusions: Formation and perfusion of the functioning fragment of the rats' small intestine is an adequate approach to the investigating the activity of the small intestine under physiological condition. It allows detecting the impact of the regulatory activity of chime (its exogenic and endogenic components).

Keywords: Glycine; Absorption; Perfusion; Small intestine; Physiological condition; *In vivo*

Introduction

The investigation of absorption, i.e. transportation of the low weight nutrients or products of the intestinal or membrane hydrolysis of the food biopolymers from the alimentary canal into the blood is one of the actual directions of the nutrition physiology. The substrate regulation (food substrates, bile, pancreatic secret, hormones) plays a leading role among the basic factors influencing hydrolysis and transport processes in a small intestine [1-7]. That is why it's important to take a methodical approach to researching parameters of absorption in a small intestine. One of the main principles in the organization of living systems is hierarchy. Every level in this hierarchy from molecule to organism has unique characteristics, not stemming directly from the properties of the lower level. It was reflected in the hierarchy of the research levels, each having its advantages and limits. It is assumed that, in many respects, reduced system satisfactorily reproduces the behavior of the entire system of the organisms under normal conditions. Nevertheless real activity of the small intestine, and most importantly the processes of its regulation, can be studied using non-injured systems only. Furthermore the regulatory characteristics of the small intestine, apparently, can be researched in the chronic experiments *in vivo* only. That is why the method of using an isolated loop of the rat's small intestine in the chronic experiments was created [8]. In comparison with the acute experiments *in situ* this method allows getting results from the same animal repeatedly without pain, narcosis and operation trauma. However the main problem of this method is an isolation of the investigated intestine fragment from the alimentary canal. As the result it does not get the endogenic factors and exogenic substrates and experience fast atrophy with the decreasing of the digestive and absorptive ability [4,8,9]. It triggered the search for a new ways and under the leadership of academic Ugolev A.M. we created the method of formation and investigation of a functioning fragment of a rat's small intestine in the chronic experiments under physiological condition *in vivo* [10-13]. This fragment of small intestine is directly involved in the digestive system and, consequently, preserves innervations, blood circulation, natural streams of physiological secrets, and passage of chime. All experiments were conducted 4-5 days after the surgery to eliminate an impact of surgical trauma, pain and narcosis on their outcome. Therefore, this method has all advantages of the isolated loop method and lacks its shortcomings.

One of the most investigated substrate in the research of alimentary canal absorption is glycine. There is a lot of data collected about its transportation *in vitro* and *in situ* experiments [7,14] and *in vivo* on the isolated loop of the rat's small intestine [4,8]. Therefore, we proceeded with using glycine in research of absorption in the small intestine under physiological condition without its atrophy, in the presence of chime passage, normal blood circulation, innervations, and natural streams of physiological secrets.

Materials and Methods

The experiments were performed on male rats of Vistar breed weighted 170-180 g that were held out on the standard ration of vivarium and were not fed for 18-24 hours prior to the experiment. There were five rats in the group with functioning part of the small intestine and four rats in the group with the isolated loop of the small intestine. During the surgery we formed two "V"-shaped contacts by connecting two anastomoses using "end to end" principle and placing the free ends of intestine in the side of animal ("living" fistulas),

secured with standard metallic fistulas (Figure 1). Including "living" fistulas, the length of the investigated area was 10 cm. The isolated loop was prepared according to method described by Ugolev and Zaripov [8]. 4-5 days after the operation the animals were perfused by peristaltic pump "Zalimp"(Poland). Velocity of perfusion was 0.6 ml/min. For the perfusion we used 25 mmol/l solution of glycine on the Ringer solution (pH=7.4, to of the perfusion solution=37°C). We added an unabsorbed marker polyethylene glycol (PEG – 400) to the perfusion solution to control possible dilution of perfusion solution with the liquids of digestive tract (saliva, gastric, intestinal and pancreatic juices, bile or reflux from the next part of intestine). The concentration of glycine was determined using method described in ref. [15] colorimetrically on photoelectrocolorimeter – CFC-2MP, λ=540 nm. The concentration of PEG was determined based on modified method [16] colorimetrically on CFC-2MP, λ=465 nm. All experiments were conducted in accordance with scientific/practical recommendations regarding animal care and work with them [17] and in compliance with the positions of "European convention about defense of the vertebrates used for experimental and scientific aims". The statistical processing of the obtained data was conducted using "Primer Biostatistics" software.

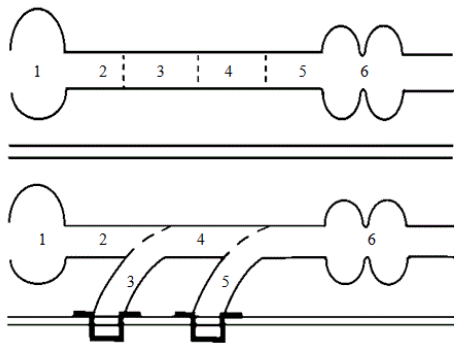

Figure 1: Scheme of the formation of the functioning fragment of the rats′ small intestine. 1-stomach, 2-5-small intestine, 3,5 - "living" fistulas, 6-large intestine.

Results

The research of the free glycine absorption in the functioning part of the small intestine of adult rats under physiological condition showed that rate of this process during first 60 minutes of perfusion had a trend to an increase (Figure 2). At the end of perfusion (after 55-60 minutes) velocity of absorption was for significantly higher, than in the first few minutes ($p<0.004$: 5.01 ± 0.64 against 2.12 ± 0.33 mcmol/l·min, n=5). These results cannot be explained by the dissolution of perfusate by the digestive fluids: we detected absorption of water during perfusion (Figure 2).

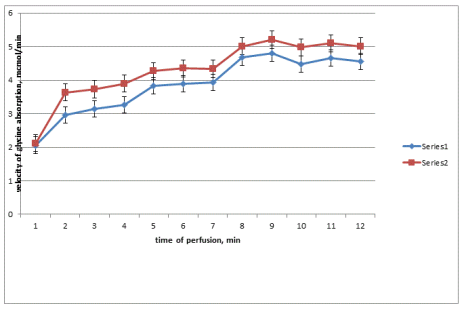

Figure 2: Dynamics of glycine absorption from its 25 mmol/l solution in the functioning part of the rats′ small intestine (mcmol/min, n=5).

Absorption rate of free glycine on 1 cm of functioning fragment of the small intestine obtained in this experiment was 0.431 mcmol/l /sec (including water absorption). It is necessary to mention that in our previous experiments [10] it was 0.522 mcmol/l /sec when using 20 mmol/l solution of free glycine in the functioning fragment of the small intestine 8 cm long. Considering nonlinear dependence between rate of absorption of substrate from perfuse solution and difference in length of functioning fragment in the past and present experiments, it is safe to assume that the base indexes of absorption of free glycine stayed within marked range that testifies to an adequacy of the approach and quality of its execution.

Discussions

Increase of the absorption rate of free glycine during perfusion shows the switching of absorption mechanisms. It comports with the phenomenon of the fast substrate adaptations, founded by academic A.M.Ugolev [18] – presumably, the penetration of substrate through the wall of a small intestine stimulates its transport activity. It is important that the "quality" of the free glycine absorption in our experiments *in vivo* was approximately 0.431 mcmol/min/cm – compare to the corresponding data [4,19], where the rate of the glycine absorption from the same concentration solution (25 mmol/l) in the isolated loop 22 cm long was 0.173 mcmol/min/cm – 2.5 times lower than in our experiments on the functioning fragment despite of the significant difference in the length of the investigated fragment. These are important results demonstrating the dependence of the outcome on the length of the investigated loop used in the experiments [4]: the longer is the loop the more effective is absorption of substrate. Usage of 22 cm isolated loop in the experiments of Gromova L.V. is justified by the low activity of the researched fragment. In the experiments with the functioning part of the small intestine we did not have to use such a long fragment because even 10 cm of the functioning fragment absorbed more substrate than 22 cm of the isolated loop [4,19]. It's also essential to compare the difference in the velocity of perfusion in our experiments and on the isolated loop [4]. In our experiments we always use 0.6 ml/min as the most physiological condition [2,9]. Gromova and Gruzdkov used 0.25-0.3 ml/min especially for experiments with glycine because of the relatively low rate of the free glycine absorption compare to glucose [19]. Obviously two times slower perfusion allows for a higher absorptive ability of the investigated intestine loop. However, regardless of the differences between our experiments and experiments mentioned above with

isolated loop an absorptive ability of the functioning fragment of the small intestine is much higher than isolated loop.

Therefore, the rate of the absorptive activity of the small intestine under physiological condition is in fact higher than in the experiments with the isolated loop. It proves the role of endogenic factors and exogenic substrates (natural gastrointestinal fluids, native food components and different levels of the regulation) in the functioning of a small intestine. Absence of these components leads to the atrophy of the small intestine and even regular daily one-hour 'feeding' of the isolated loop with glucose or glutamate solution cannot significantly change this situation [4,19]. This data leads to conclusion that use of the functioning fragment of the rats' small intestine to research its absorption rate is more physiological than the previous methods, including isolated loop.

The interesting results were received when comparing the mass of mucosa and muscle tissue in the functioning fragment and isolated loop of the small intestine: 1 cm of the functioning fragment contains 2.25 times more mucosa than isolated loop and 1.78 times more muscle (Table 1). It's worth noting that our result for isolated loop mucosa mass matches exactly the results of Gromova L.V. and Gruzdkov A. A. [19]: 44.0 ± 9.6 in our experiments with no additional loading and 46.6 ± 6.5 in their experiments after regular daily one-hour loading with glucose. As expected mass of 1 cm of functioning fragment was 1.75 times greater than the mass of isotopic intact intestine fragment, mainly due to the difference in the muscle amount (4 times) rather than mucosa (1.3 times). Similarly mass of 1 cm of isolated loop was 1.2 times (16 %) less than the mass of isotopic intact intestine fragment – owing to 2 times difference in muscle first and then – to mucosa (1.75 times) (Table 1). These results confirm that isolation of the loop of small intestine from normal digestion leads to the explainable hypotrophy of mucosa and unexpected hypertrophy of muscle. It means that surgery leads to increasing of the muscle tissue (possibly because of the partial replacing with the connective tissue as a result of operation). It comports to the data obtained by Ugolev et al. [20,21].

S.no	Type of the fragment	Total mass of the 1 cm of intestine	Mass of mucosa of the 1 cm of intestine	Mass of muscle of the 1 cm of intestine	n
1	Functioning	160.0 ± 22.0*	99.7 ± 10.5*	60.0 ± 12.0	3
2	Isolated	78.0 ± 10.0	44.0 ± 9.6***	33.7 ± 4.1****	4
3	Intact, isotopic to functioning	91.0 ± 14.0**	75.8 ± 9.4	15.5 ± 5.0**	4
4	Intact, isotopic to isolated	93.0 ± 8.0	77.0 ± 6.9	16.3 ± 2.4	4

Table 1: Mass of 1 cm of the humid tissue of the investigated fragment of the rats small intestine in 30 days later of the operation. Note: *p 1-2=0.013, **p 1-3=0.039, ***p 2-4=0.045, ****p2-4=0.011.

Conclusions

The method of the formation and perfusion of the functioning fragment of the rats' small intestine provides an adequate approach to the investigation of the functional activity of the small intestine under physiological condition. It allows detecting the effects of the regulatory activity of chime (its exogenic and endogenic components). The obtained data shows that higher velocity of the glycine absorption in the functioning fragment of the rat's small intestine comes from stimulating effect of chime on the transport system of separate enterocyte as well as the substrate regulation of the cellular pool.

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