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Perfusion of the Functioning Portion of the Small Intestine of the Rats: Parameters of Perfusion and Dependence of the Velocity of Absorption of Glucose on the Velocity of Perfusion

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Abstract

Objectives and method: To research the parameters of perfusion of the functioning portion of the rats′ small intestine in the chronic experiments under physiological condition without operation trauma, pain, narcosis and atrophy of the small intestine. To determine the volumes of the outflowing perfusate and the dependence of the velocity of glucose absorption in the functioning portion of the rats′ small intestine on the rate of its perfusion.

Results: At perfusion of the functioning portion of the small intestine of rats for each 5 min it is possible to collect 50-70% of the perfusion solution introduced into the researching area. The volume of outflowing perfusate is largely determined by peristalsis, by the degree of satiety of the animal and by its functional state. In the range of the changing of the rate of perfusion from 0.37 to 2.61 ml/min the velocity of absorption of glucose from its 25 mmol/l solution changes from 7.63 ± 0.38 to 10.33 ± 1.90 mcmol/min and concentration of unabsorbed marker PEG changes from 102.89 ± 0.87 to 98.28 ± 1.85% correspondingly. The minimal velocity of glucose absorption and minimal concentration of PEG were detected under the velocity of perfusion 5.39 ± 0.36 ml/min: 1.12 ± 2.28 mcmol/min and 95.07 ± 2.93% correspondingly. In the range of the rate of perfusion from 0.37 ± 0.01 to 1.43 ± 0.04 ml/min there were detected absorption of water from the perfusate. Dilution of the perfusate starts only under the rate of perfusion 2.61 ± 0.04 ml/min and increases under the 5.39 ± 0.36 ml/min.

Conclusions: The volume of outflowing from the functioning portion of the rats' small intestine perfusate is largely determined by peristalsis, by the degree of satiety of the animal and its functional state. The velocity of glucose absorption from its 25 mmol/l solution in the functioning portion of the rats′ small intestine is not very sensitive to the rate of the perfusion in the range from 0.37 ± 0.01 to 2.61 ± 0.04 ml/min: it changes on 26% at the changing of the rate of perfusion on 86%.

Keywords: Perfusion; Small intestine; Physiological condition; Velocity; Glucose; Absorption

Introduction

The significance and informativeness of experimental data is largely determined by the method of investigation with which they were obtained. Each method *in vitro*, *in situ* and *in vivo* has its own advantages and limitations [1-3]. Thus, methods of research using preparations *in vitro* and in acute experiments on an anesthetized animal *in situ*, provide information on basic, fundamental processes occurring in the body under extreme conditions or even soon after its death. Experiments *in vivo* allow investigating the chronic processes occurring in a certain long period of time, which makes it possible to neutralize the effect on the final result of individual animal characteristics, seasonal and magnetic changes in the environment, the effects of anesthesia, operational trauma, and stress. The closer to the physiological, real conditions of existence of the animal will be the method of investigation, the more reliable the result of the experiment will be. However, the result will be difficult to verify - because it will be influenced by a huge number of regulatory factors involved at the level of the whole living organism. Apparently, at the present time there is no ideal method of investigation and each of the existing methods *in vitro*, *in situ* and *in vivo* allows us to highlight a certain range of problems and answer questions within their capabilities. One of the methods for studying of digestive and transport processes *in vivo* is the original method of formation and perfusion of the functioning portion of the small intestine of rats directly included in the digestive tract [2-6]. This method allows us to investigate the processes of hydrolysis and transport of nutrients in the absence of operational trauma, anesthesia and stress by perfusion of the intestinal portion of the intestine with appropriate perfusion solutions. The aim of the present study was to study such perfusion parameters of the functioning portion of the small intestine as the volume of the perfusate outflowing from the examined portion, and the effect of the velocity of perfusion on the velocity of glucose uptake from the perfusion solution.

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 used rats with functioning part of the small intestine. The functioning portion was prepared according the method described by us previously [2,5,6]. 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 glucose 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 glucose was determined using method described by Scott TA et al. [7] colorimetrically on photoelectron colorimeter – CFC-2MP, λ=625 nm. The concentration of PEG was determined based on modified method colorimetrically on CFC-2MP, λ=465 nm [8]. All experiments were conducted in accordance with scientific/practical recommendations regarding animal care and work with them and in compliance with the positions of "European convention about defense of the vertebrates used for experimental and scientific aims" [9]. The statistical processing of the obtained data was conducted using "Primer Biostatistics" software.

Results and Discussion

At perfusion of the functioning portion of the small intestine of rats for each 5 min it is possible to collect from 0.4 to 2.9 ml of perfusate flowing out of the outgoing fistula. The volume of flowing perfusate is largely determined by peristalsis: the samples of perfusate collected during one experiment during 60 minutes of perfusion may vary considerably in volume. Also the degree of satiety of the animal effects on the volume of perfusate - in an insufficiently starved (less than 24 hours) animal together with the perfusate will be obtained the fractions of the chyme of different consistency are derived - from liquid masses to solid fragments. As well as the functional state of the animal - in nervous and maladjusted rats spasm of the intestine can occur, while the perfusion solution can enter the functioning portion of the intestinal tract for 20-25 minutes, stretching the gut and sides of the animal, however, the perfusate is not released from the fistula.

Usually in the experiment for 5 minutes of perfusion at a perfusion speed of 0.6 ml/min from the outgoing fistula of the functioning portion of the small intestine, it is possible to collect 1.5-2 ml of flowing perfusate: this is approximately 50-70% of the perfusion solution introduced into the researching area. Since the functioning portion of the small intestine is not closed, the residual volume of the perfusion solution appears to enter the lower parts of the bowel. A certain amount of fluid is absorbed in the small intestine, which is confirmed by concentrating the non-absorbable marker - PEG (Table 1). Interestingly, the concentration of perfusate occurs in the range of physiological rates of fluid movement through the small intestine - from 0.3 to 0.79 ml/min and even at a perfusion rate of 1.43 ml/min, which exactly does not refer to physiological parameters. Nevertheless, water absorption at this value of the perfusion rate is only a little bit less than at a perfusion rate of 0.37 ml/min (102.35 ± 1.57% vs. 102.89 ± 0.87%, respectively). It should be noted that the data and direction of water flow from the 25 mmol/l solution of glucose in the functioning portion of the rats′ small intestine is very close to the corresponding parameter, received in our previous experiments [10] – it is the evidence of adequacy of experimental method. Dilution of the perfusion solution begins at a perfusion rate of 2.61 ml/min (PEG concentration is 98.28 ± 1.85%) and increases with an increase in the perfusion rate by half (to 5.39 ml/min) - the concentration of PEG reduces to 95.07 ± 2.93% (Table 1).

Velocity of perfusion, ml/min	Absorption of glucose, mmol/min	Concentration of PEG, %	Velocity of glucose absorption without counting of water, mcmol/min	Velocity of glucose absorption with counting of water, mcmol/min	Number of experiments
0.37 ± 0.01	20.49 ± 2.04	102.89 ± 0.87	7.58 ± 0.42	7.63 ± 0.38	3
0.65 ± 0.03	14.16 ± 1.55	105.09 ± 1.16	9.20 ± 1.37	9.54 ± 1.12	4
0.79 ± 0.01	10.59 ± 0.50	101.31 ± 0.37	8.37 ± 1.41	8.51 ± 1.27	3
1.43 ± 0.04	8.56 ± 0.57	102.35 ± 1.57	12.24 ± 0.86	12.78 ± 0.66	6
2.61 ± 0.04	4.32 ± 0.81	98.28 ± 1.85	11.27 ± 2.10	10.33 ± 1.90	6
5.39 ± 0.36	1.43 ± 0.39	95.07 ± 2.93	7.71 ± 2.80	1.12 ± 2.28	3

Table 1: Dependence of absorption of glucose and water from 25 mmol/l solution of glucose on the velocity of perfusion of the functioning part of the rat′ small intestine.

The physiological value of the rate of passage of the chyme in the small intestine is 0.5 - 0.6 ml/min [1,11,12], although some authors use the perfusion rates of the isolated portion of the small intestine of the rat 0.25-0.3 ml/min [13,14]. However, in our experiments, when trying to set a specific perfusion rate, it was found that the peristaltic pump during the experiment significantly changes the perfusion rate at a given and fixed 0.5 ml/min. Several types of peristaltic pumps (chromatographic pump, apparatus for regional heparinization and lymphography (piston), Programmable gradient pump PPM Microtechna, Peristaltic miniflow pump type 304 Zalimp were tested, but all of them during the experiment changed the preset perfusion rate.

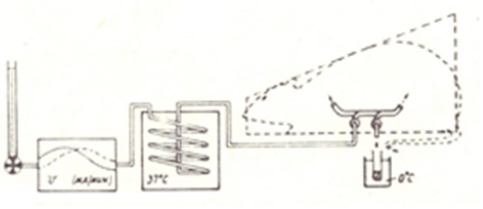

Figure 1: Scheme of the perfusion of the functioning portion of the small intestine of the rats.

Therefore, a burette calibrated to an accuracy of 0.1 ml was connected to the inlet tube of the Zalimp peristaltic pump using a flexible polyethylene hose (Figure 1). When the pump was turned on, the fluid level in the burette decreased with the time of perfusion, which made it possible to measure the loss of the perfusion solution per unit time.

Thus, it was found that the perfusion rate of the functioning portion of the small intestine of rats varies from 0.4 to 0.8 ml/min at a given rate of 0.5 ml/min. This led to the need to determine the effect of the perfusion rate of the functioning portion of the intestine on the rate of glucose uptake from the perfusion solution. To do this, it was determined at various preset perfusion rates: from 0.39 ml/min to 5.75 ml/min, monitoring them by decreasing the perfusion solution in the burette.

It turned out that the dependence of uptake of glucose from the 25 mmol/l solution on the perfusion rate of the site is described by the hyperbolic curve and in the perfusion rate range from 0.39 to 0.8 ml/min the glucose absorption rate determined by the formula "absorption velocity=perfusion rate x difference of the initial and the final concentration" is practically a constant value, i.e., the rate of glucose uptake at a perfusion rate of 0.4 and 0.7 ml/min is practically equal (Figure 2).

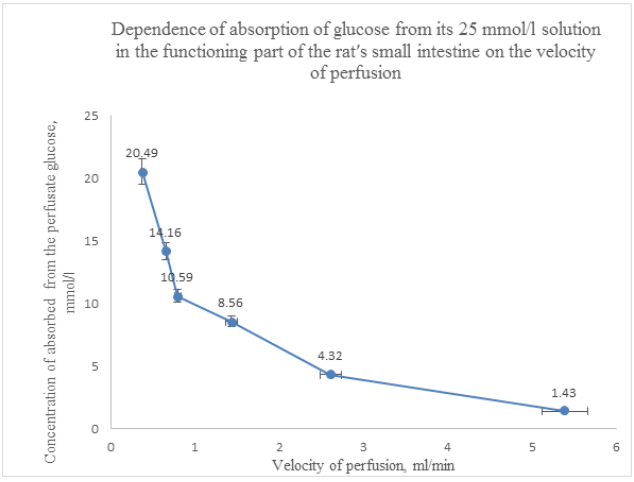

Figure 2: Dependence of absorption of glucose from its 25 mmol/l solution in the functioning portion of the rats′ small intestine on the velocity of perfusion.

However, when we include into the calculation the results of water flows we had to change the formula of calculations of the velocity of absorption into:

$V_{abs} = V_{perf} \times (C_0 - C_{final} \times [PEG_0]/[PEG_{final}])$,

Where,

V_{abs} - velocity of absorption,

V_{perf} – velocity of perfusion,

C_0 – initial concentration of glucose in the perfusate solution,

C_{final} – final concentration of glucose in the outflowing perfusate,

$[PEG_0]$ – initial concentration of PEG in the perfusate (100%),

$[PEG_{final}]$ – final concentration of PEG in the outflowing perfusate.

After the calculation of the velocity of absorption of glucose according this formula, we received two curves - without counting of water flows in the small intestine and with them. Mainly these curves are approximately the same, but in the last point (it corresponds to the highest velocity of perfusion) they are very different (Figure 3). It means that dilution of perfusate as a result of non-physiological, high velocity of perfusion, results to the decreasing the velocity of absorption of glucose from the perfusate in the functioning portion of the small intestine. This result is in line with expectations, but unexpected was the fact of the very close parameters of the velocity of glucose absorption under the range of velocity of perfusion from 0.37 to 2.61 ml/min: from 7.63 to 10.33 mcmol/min correspondingly (Table 1). Apparently, under the real physiological conditions in the living organism there are some mechanisms for the adaptation of the processes of nutrients absorption to the different conditions of the flowing of the chyme along the intestine.

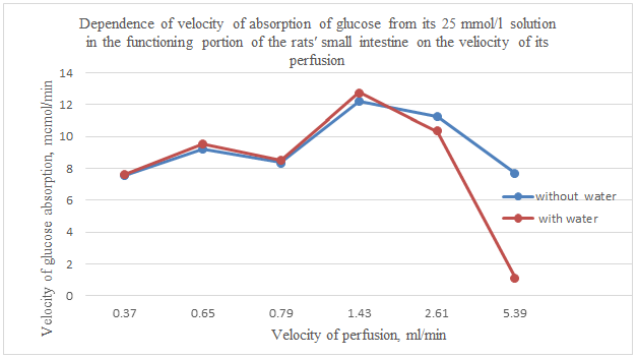

Figure 3: Dependence of velocity of absorption of glucose from its 25 mmol/l solution in the functioning part of the rats′ small intestine on the velocity of its perfusion.

Conclusion

The volume of outflowing from the functioning portion of the rats' small intestine perfusate is largely determined by peristalsis, by the degree of satiety of the animal and its functional state. The velocity of glucose absorption from its 25 mmol/l solution in the functioning portion of the rats′ small intestine is not very sensitive to the rate of the perfusion in the range from 0.37 ± 0.01 to 2.61 ± 0.04 ml/min: it changes on 26% at the changing of the rate of perfusion on 86%. Obviously, in the living organism there are mechanisms for the adaptation of processes of absorption of nutrients to the changings in the rate of the passage of intestinal fluids.

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