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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Evaluation of Glucose Absorption Level in the Small Intestine of Different Rat Strains under Natural Conditions

A. A. Gruzdkov[a]*, Yu. V. Dmitrieva[a], A. S. Alekseeva[a], A. S. Polozov[a], and L. V. Gromova[a]

[a] *Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia*
**e-mail: gruzdkov@pavlov.infran.ru*

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Abstract—The peculiarities of carbohydrate metabolism were studied in seven rat strains under conditions maximally approximating natural ones. The glucose absorption level in the small intestine was evaluated using a method based on *ad libitum* drinking of concentrated glucose solutions by prefasted (18–20 h) rats. It was shown that in the steady-state regime the volume-normalized uptake rate of glucose solution (mL/min) was constant and inversely proportional to the glucose concentration in the solution, while the uptake rate of glucose itself (μmol/min) was independent of the substrate concentration in quite a wide range, being mainly determined by the absorptive capacity of the small intestine. A significant difference was revealed between the tested rat strains in terms of the rate of glucose absorption from its solution (200 g/L). In the daytime (10 AM–4 PM), the highest rates were observed in Sprague Dawley rats (116.7 ± 3.1 μmol/min) while the lowest—in Wistar Kyoto rats (35.6 ± 1.1 μmol/min). In the evening (4–10 PM), rates of glucose absorption in different rat strains were 1.3–2.2 times higher than in the daytime. Apparently, the increased absorptive capacity of the small intestine in the evening is due to enhanced SGLT1-mediated active glucose transport and reflects the peculiarities of carbohydrate metabolism regulation in different rat strains.

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Key words: different rat strains, glucose consumption and absorption, carbohydrate metabolism, diurnal rhythms.

INTRODUCTION

Laboratory rats are conventional objects for medical and physiological experiments. Different rat strains are often used as convenient experimental models in studying adaptational and compensatory processes in the digestive system under normal conditions and diverse pathologies and in testing the efficacy of various drugs and therapeutic modalities. To a large extent, this is due to the fact that during evolution rats and humans converged in terms of their feeding patterns and basic mechanisms of digestion. Therefore, results obtained on rats often allow their extrapolation to humans. For this reason, the use of different laboratory rat strains as experimental models is of great theoretical and practical relevance in studying the peculiarities of carbohydrate metabolism, specifically, in unraveling the mechanisms of glucose absorption in the small intestine as a key stage of assimilation of carbohydrate food components.

Most regularly, such studies are carried out under conditions of acute *in vivo* experiments on anesthetized animals [1, 2]. However, most fun-

damental principles underlying the performance of diverse systems in the integral organism can only be unraveled under conditions maximally approximating the physiological. The experimental model which optimally met these requirements was developed by A.M. Ugolev and B.Z. Zaripov [3], being based on perfusion of the isolated segment of the rat small intestine under conditions of chronic experiment: it was widely used in studying membrane hydrolysis and absorption of nutrients [4–6]. Nevertheless, even in chronic experiments the functional state of the digestive system (as well as of the whole organism) is far from being identical to its normal physiological status.

In this paper, we present the outcomes of a comparable evaluation of the glucose absorptive capacity of the small intestine in seven different rat strains using a novel experimental approach that provides for the first time an objective quantification of the glucose absorption level in the rat small intestine under natural conditions. With allowance for peculiarities of circadian rhythmicity in active glucose transport, the absorptive capacity of the rat small intestine was determined both in the daytime and in the evening, i.e. during the period of maximum feeding activity in these animals.

MATERIALS AND METHODS

Animals. Experiments were carried out on 7 rat strains available in the collection of the Pavlov Institute of Physiology and kept in standard conditions at the Laboratory Animal Husbandry Department (Leningrad Region, Koltushi/Pavlovo) under free access to water and food and 12 h/12 h dark/light regime.

The following rat strains were investigated:

— Sprague Dawley, a hybrid albino strain characterized by a high reproduction rate and low spontaneous tumor incidence;

— Wistar Kyoto, an outbred strain used as a normotensive control for the SHR strain;

— Long Evans, traditionally recommended for behavioral studies;

— SHR, a spontaneously hypertensive strain recommended for investigating the mechanisms of hereditary hypertension and testing anti-hypertensive drugs;

— Wistar, an outbred albino strain, referring to the species *Rattus norvegicus* (nowadays, one of the most demanded laboratory rat strains);

— Strains selected at the Pavlov Institute of Physiology for high (HT) and low (LT) CNS excitability thresholds.

Brief validation of the method. The glucose absorption level in the rat small intestine was evaluated by the rate of free (*ad libitum*) uptake (drinking) of concentrated glucose solution as described in our previous publication [7]. This method is based on current views that under natural conditions the amount and the rate of food uptake are determined both by nutritive demands of the organism and the capacity of the gastrointestinal tract to assimilate this food [8].

More than 40 years ago, in experiments by A.M. Ugolev and his associates [9], there had been demonstrated for the first time the temporal dynamics of free uptake of concentrated glucose solution in rats pre-fasted for 18–20 h. An important distinction of this dynamics (typical for all analogous experiments) was that, except a relatively short initial period (usually, 45–90 min), during the next several hours the mean rate of free uptake of glucose solution in rats remains practically invariable.

This fact finds explanation within the ileal brake concept, i.e. the regulatory mechanism of gastric emptying function and intestinal peristalsis [10–12] which performs in accordance with digestive and absorptive capacities of the small-intestinal mucosa. Due to this regulation, in a pre-fasted animal, relatively fast gastric filling is followed by a period, when consumption of glucose solution is maintained for several hours at a rate that corresponds to the absorptive capacity of the small intestine, preventing thereby its overloading. Thus, during the experiment and despite a permanent food demand, the animal drinks concentrated glucose solution exactly at such a rate at which it can be absorbed in the small intestine.

Results of our previous experiments and their mathematical analysis [7] showed that the rate of free uptake of glucose solution in rats is relatively constant in a broad temporal range (from 3 to 5 h) and hence can be considered as an objective quantitative criterion to evaluate the glucose absorptive capacity of the small intestine under normal natural conditions.

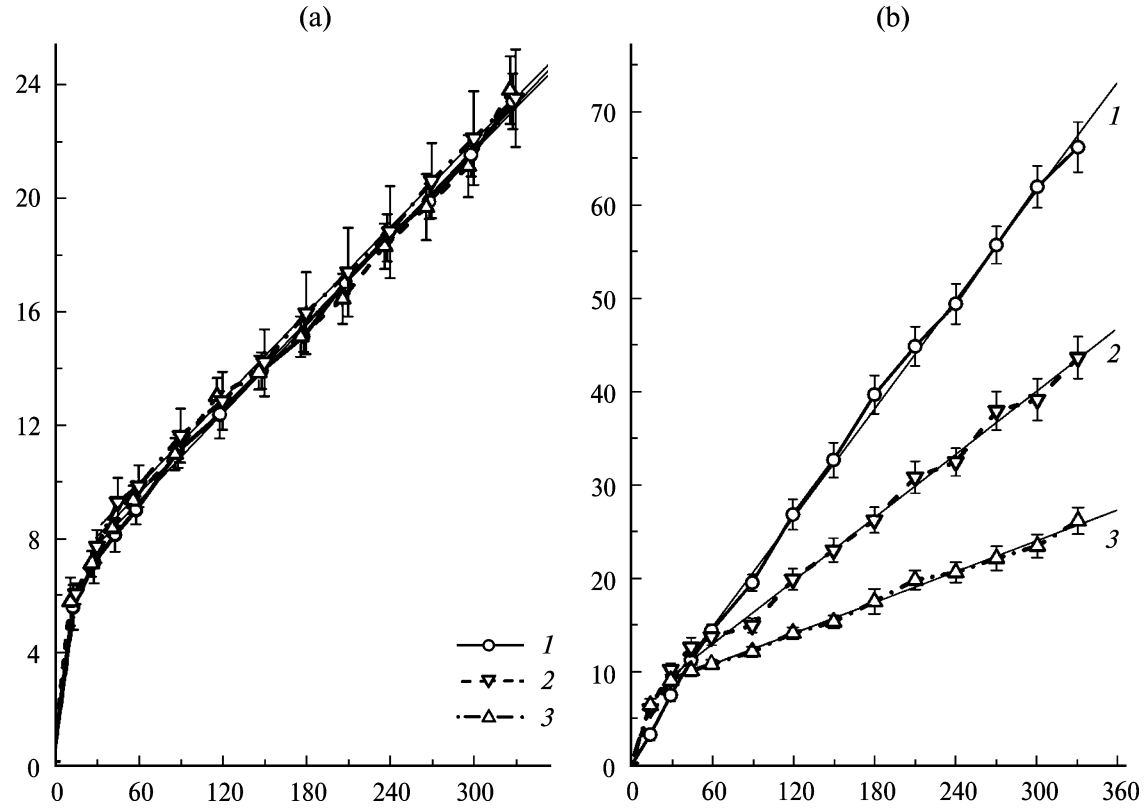

Fig. 1. Temporal dynamics of free uptake of solutions with different glucose concentration in Wistar rats. *Ordinate*: volume of glucose solution drunk (mL) (M ± m), *abscissa*: time from the onset of the experiment (min); (a) uptake of glucose solution (200 g/L) in tentative experiment by rats of three groups (1, 2 and 3); (b) uptake of glucose solutions (50, 100 and 200 g/L) by the same rats from groups 1, 2 and 3, respectively, after 7 days; *Thin solid lines*—outcomes of linear regression using Origin 6.1.

Experimental protocol. During experiments, each rat (pre-fasted for 18–20 h) was placed into an individual cage, sized 14 × 21 × 11 cm and equipped with two graduated nipple drinkers: one with water[1] and another with glucose solution (200 g/L).

When working with each of the tested rat groups, several tentative experiments were carried out first (at a 3–4-day interval), during which the temporal dynamics of glucose solution consumption in rats was investigated. To do this, 15, 30, 45 and 60 min after the onset of the experiment and then every 30 min for 5–6 h, volumes of the solution drunk by each animal were measured. Animals that did not drink glucose solution in any of the tentative experiments were expelled from further trials. Subsequently, each rat strain was involved in two major experiments (at a 3–4-day interval): one in the daytime (from 10 AM to 3 PM) and another in the evening (from 4 to 10 PM), both in the period of maximum feeding activity. In these experiments, the mean volume ($M \pm m$) of the solution drunk by animals of the given test group was calculated. Then, using linear regression and Origin 6.1 (OriginLab Corp., USA), the mean volume-normalized uptake rate of glucose solution (μL/min) was determined within the time interval that corresponded to a relative constancy of this parameter (from 60–90 to 330–360 min since the onset of the experiment). With allowance for the initial concentration of the solution (200 g/L), the obtained data were used to calculate the mean uptake rate of glucose itself (μmol/min). This rate objectively reflected the absorptive capacity of the small intestine in the tested rats under natural conditions. Additional experiments were also

[1] In the presence of a nipple drinker with glucose solution, in none of the experiments did animals drink water.

carried out on 27 adult Wistar rats, in which rates of glucose uptake from its solutions of different concentrations were compared. Using data obtained in three tentative experiments at an initial glucose concentration of 200 g/L, three groups of animals with close values of the mean uptake rate for this solution in each group were formed (N1 = 10; N2 = 9; N3 = 8). After 7 days, a repeated experiment was carried out on the uptake of glucose solutions with initial concentrations of 50, 100 and 200 g/L, respectively, by rats of the same groups.

RESULTS

Absorptive capacity of small intestine in free uptake of solutions with different glucose concentration. Figire 1a shows the results of the experiment on the uptake of glucose solution (200 g/L) in rats of three groups formed in accordance with the outcome of tentative trials. It is seen that within the time interval of 60–330 min the mean volume of the solution drunk by animals increased over time almost linearly (correlation coefficient $r >$ 0.99 in all groups). Regression slopes (thin solid lines in Fig. 1) reflect mean volume-normalized uptake rates of glucose solution. In groups 1, 2 and 3, these rates were 51.7 ± 0.8, 50.2 ± 1.7 and 50.5 ± 0.5 μL/min, respectively.

Uptake rates of glucose itself (given that its concentration in solution was 200 g/L) were close across all groups: 57.4 ± 0.9, 55.7 ± 1.9 and 56.1 ± 0.6, respectively.

In the experiment carried out on the same animals 7 days thereafter, mean volumes of solutions drunk by rats within the interval of 60–330 min also increased linearly over time (correlation coefficients *r* in all three groups were 0.998, 0.997 and 0.997, respectively) (Fig. 1b). Mean uptake rates for solutions with glucose concentrations of 50, 100 and 200 g/L were 194.5 ± 3.3, 113.6 ± 3.4 and 55.7 ± 1.6 μL/min, respectively, while those for glucose itself in groups 1, 2 and 3 were 54.0 ± 0.9, 63.1 ± 1.9 and 61.9 ± 1.7 μmol/min, respectively, i.e. differed only insignificantly.

Thus, as the glucose concentration in solution decreased from 200 to 100 g/L (i.e. two times), the rate of its absorption in the small intestine remained almost intact, while following a four-fold decrease (down to 50 g/L) it only diminished by 15%.

A comparison of the results of these experiments indicates that the mean rate of free uptake of concentrated glucose solution can be considered as an objective criterion for quantitative evaluation of the glucose absorption rate in the small intestine of intact animals.

Evaluation of absorptive capacity of small intestine in different rat strains. Figure 2 shows the results of experiments, in which the intestinal absorptive capacity was evaluated by recording the rate of free uptake of concentrated glucose solution (200 g/L) in rats of different strains under conditions that were close to natural. Experiments were carried out both in the daytime (from 10 AM to 3 PM) and in the evening (from 4 to 10 PM) on animals pre-fasted for 18–20 h.

— ***Sprague Dawley rats.*** 12 individuals, weighing 355.6 ± 8.9 g (before the evening experiment) and 377.7 ± 7.3 g (6 days thereafter, before the daytime experiment), were examined.

In the daytime experiment (Fig. 2a, line *1*), the mean volume of glucose solution drunk by rats in the range of 120–330 min increased almost linearly over time. The solution uptake rate was 106.1 ± 2.8 μL/min. In the evening time (during the onset of active feeding behavior), as soon as 60 min after the beginning of the experiment, the stationary regime of glucose solution uptake was established at a rate of 193.5 ± 5.3 μL/min (Fig. 2a, line *2*).

— ***Wistar Kyoto rats.*** In daytime experiments, 12 individuals (body weight 243.9 ± 6.7 g) were used but 6 of them, despite pre-fasting for 20 h, did not proceed to drink glucose solution. At the same time, in other 6 rats uptake rates of glucose solution were nearly constant throughout the experiment, being as small as 32.0±1.0 μL/min (Fig. 2b, line *1*). In the experiment carried out 4 days thereafter in the evening time, already 8 of 12 animals of this group drank the same solution after prefasting at a rate more than 2 times higher than in the daytime trial: 71.2 ± 1.5 μL/min (Fig. 2b, line *2*).

These results indicate that Wistar Kyoto rats, unlike their Sprague Dawley peers, even against the backdrop of fasting, are by far less prone to uptake glucose solution and may have a lowest absorptive capacity of the small intestine.

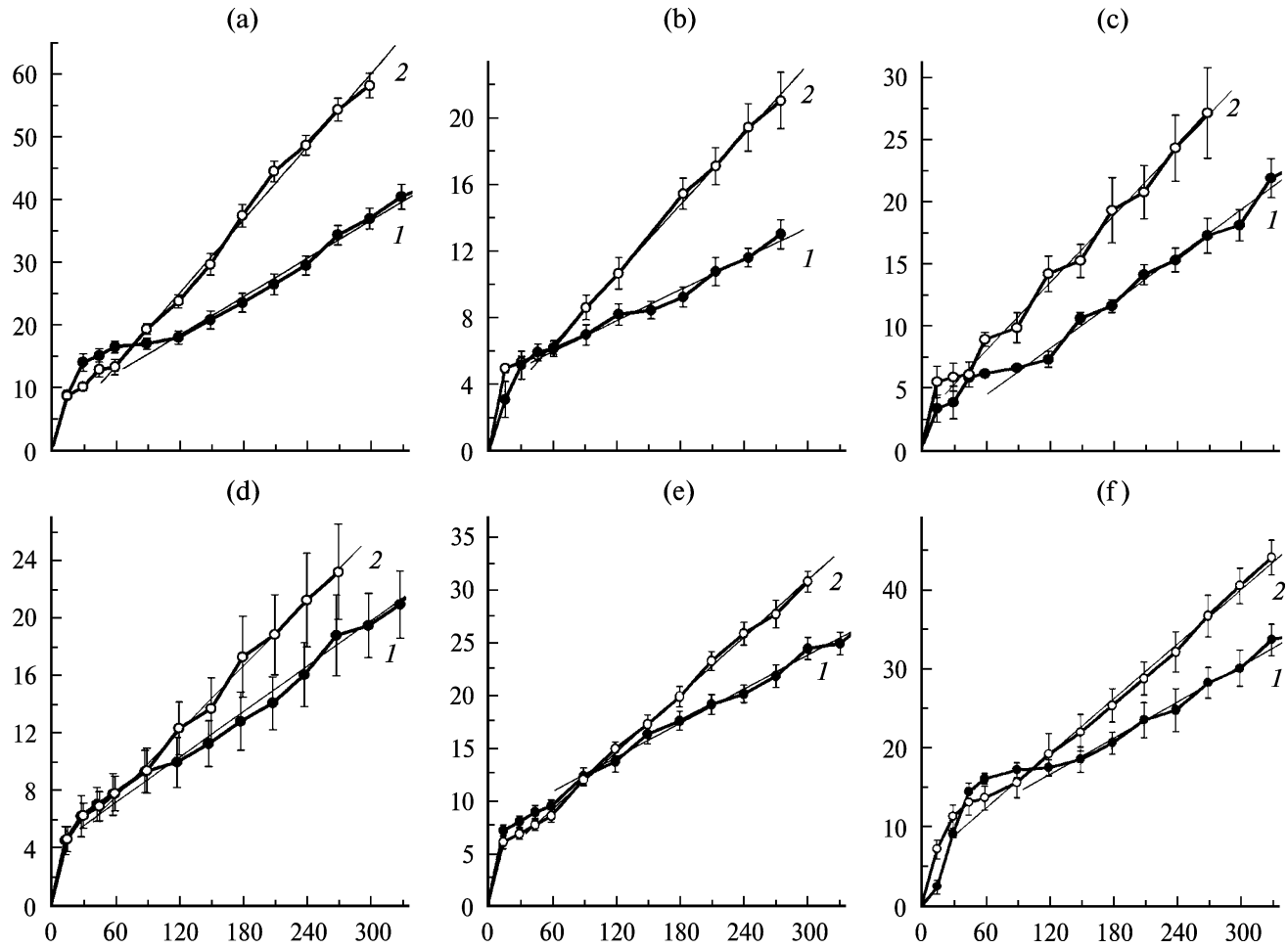

Fig. 2. Temporal dynamics of free uptake of concentrated glucose solution (200 g/L) by rats from different strains in the daytime (*1*) and in the evening (*2*). *Ordinate*: mean volume (M ± m) of glucose solution (mL) drunk by rats; *abscissa*: time from the onset of the experiment (min). (a), (b), (c), (d), (e), (f) Rat strains Sprague Dawley, Wistar Kyoto, Long Evans, SHR, Wistar and HT (with high CNS excitability thresholds), respectively. *Thin straight lines*—linear regression lines, reflecting a mean uptake rate of glucose solution within the interval of 60–330 min since the onset of the experiment.

— ***Long Evans rats.*** Of 6 individuals (body weight 250.8 ± 8.5 g) involved in experiments, only 4 drank glucose solution (200 g/L). Interestingly, in the daytime the stationary uptake regime was established only 90–120 min after the onset of the experiment (Fig. 2c, line *1*), when the mean uptake rate was 62.0 ± 2.4 μL/min. However, in the evening time, as soon as 15 min after the onset of the experiment, the rate of free uptake of glucose solution became practically constant and exceeded that in the daytime experiment nearly 1.5 times: 90.2 ± 3.4 μL/min (Fig. 2c, line *2*).

Thus, in Long Evans rats, the glucose absorptive capacity of the small intestine was relatively high, but in our experiments more than one third of them did not drink concentrated glucose solution whatsoever.

— ***SHR rats.*** The experiment was carried out on 6 individuals, weighting 221.0 ± 12.3 g. In the daytime, the mean uptake rate of glucose solution was 57.2 ± 2.3 μL/min (Fig. 2d, line *1*) while in the evening time it was 75.5 ± 2.4 μL/min (Fig. 2d, line *2*).

— ***Wistar rats.*** 12 individuals (body weight 289.9 ± 3.9 g) were used in experiments. The obtained results are presented in Fig. 2e. In the daytime experiment carried out within the interval of 90–360 min beginning from its onset (line *1*), the mean rate of free uptake of glucose solution was 54.1 ± 1.7 μL/min. In the evening (line *2*), this

rate within the interval of 60–300 min was nearly twice as high (90.9 ± 1.8 μL/min).

— Rats with high excitability threshold (HT) of the central nervous system. Tentative experiments were carried out on 10 individuals (body weight 366.1 ± 9.4 g) but 5 of them, despite pre-fasting for 20 h, did not drink glucose solution at all. In the other 5 animals of this strain used in the major experiment, the dynamics of glucose solution uptake was characterized by the fact that in the daytime (Fig. 2f, line *1*) the steady state (rate constancy) of this process was achieved as late as 150 min since the onset of the experiment. Within the interval of 150–300 min, the mean rate of glucose solution uptake was quite high: 76.3 ± 3.1 μL/min.

In the experiment carried out 4 days thereafter in the evening time, already 8 of 10 animals drank glucose solution after pre-fasting. At the same time, 90 min after the onset of the experiment, a relatively constant mean rate of glucose solution uptake was established in the range of 119.3 ± 2.4 μL/min (Fig. 2f, line *2*).

— Rats with low excitability threshold (LT) of the central nervous system. The experiment was carried out on 10 individuals (body weight 371.8 ± 20.7 g). The dynamics of glucose solution uptake in these animals did not differ significantly from that in HT rats. A relative rate constancy of this process was observed as soon as 30 min after its onset. In the daytime, the mean rate of glucose solution uptake was 49.7 ± 2.6 and in the evening 69.2 ± 1.8 μL/min.

DISCUSSION

The method we used in our experiments allowed us to objectively and quantitatively evaluate the glucose absorptive capacity of the small intestine in different strains of laboratory rats that are widely used now in experimental studies.

With regard to the absorptive capacity level of the small intestine, our estimates are consistent with data obtained previously in analogous experiments by A.M. Ugolev and his associates [9] on free uptake of concentrated (400 g/L) glucose solution in adult Wistar rats.

In all animals that we studied herein, there was observed a similar temporal dynamics of uptake of concentrated (200 g/L) glucose solution: 60–120 min after the onset of the experiment, the mean uptake rate for this solution reached a plateau and remained nearly constant for the next 4–5 h. At the same time, the regression slope reflects a mean rate of free uptake of glucose solution that we consider as a criterion of the glucose absorptive capacity of the small intestine.

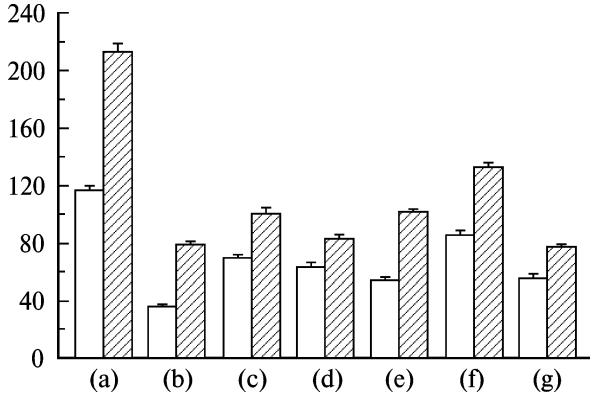

Fig. 3. Glucose absorption rates in the small intestine of rats from different strains. *Vertical*—glucose absorption rate (μmol/min). (a), (b), (c), (d), (e), (f), (g) Rat strains Sprague Dawley, Wistar Kyoto, Long Evans, SHR, Wistar, HT (with high CNS excitability threshold) and LT (with low CNS excitability threshold), respectively. *Light columns*—daytime; *hatched columns*—evening time.

Figure 3 shows a comparison of glucose uptake (absorption) rates (μmol/min) in seven rat strains calculated from the volume-normalized uptake rates (μL/min) of solution with a glucose concentration of 200 g/L. As can been seen, in experiments carried out in the daytime (most convenient for these purposes) a maximal rate of glucose solution uptake (hence of its absorption in the small intestine) was observed in Sprague Dawley rats (116.7 ± 3.1 μmol/min) being minimal in Wistar Kyoto rats (35.6 ± 1.1 μmol/min). Rats of the latter strain are characterized by a lesser proneness to uptake concentrated glucose solution (even 4 out of 12 animals pre-fasted for 20 h drank the solution in none of the experiments).

Relatively high glucose absorption rates in the daytime experiment were found in HT rats (with high CNS excitability thresholds) as well as in Long Evans and SHR strains: 84.8 ± 3.4, 68.9 ± 2.7 and 63.6 ± 2.6 μmol/min, respectively.

Approximately the same, though appreciably

lower, uptake rates of glucose solution in the daytime were detected in Wistar and LT (with low CNS excitability thresholds) rats: 54.1 ± 1.9 and 55.2 ± 2.9 μmol/min, respectively.

It is important to point out that in experiments carried out in the evening time (*hatched columns* in Fig. 3), i.e. at the beginning of the active digestive phase, in all rat strains the uptake rates of glucose solution (and hence the glucose absorption rate in the small intestine) were much higher than in the daytime. Such an increase was maximal in Wistar Kyoto rats (2.2 times). The absorptive capacity of the small intestine also increased considerably in the evening time in Sprague Dawley (1.8 times) and Wistar (1.7 times) rats. In other strains, differences between daytime and evening experiments were less pronounced: by 56, 45, 39 and 32% in HT, Long Evans, LT and SHR rats, respectively.

This finding is consistent with the presence of circadian rhythmicity in absorbing glucose in the small intestine. At the same time, as shown by some authors [13, 14], changes in glucose absorption over the day correlate with changes in expression of the SGLT1 transporter which carries out active glucose transfer across the apical membrane of enterocytes. As to the GLUT2 transporter involved in glucose transfer across the basolateral membrane by means of facilitated diffusion, such a correlation was not observed [13].

The fact that the absorptive capacity of the small intestine in Wistar Kyoto, Sprague Dawley and Wistar rats is considerably increased in the evening vs. daytime indicates that such an increase may owe mainly to the enhancement of the mechanism of active glucose transport. Apparently, this is also true, albeit to a different extent, for other rat strains studied herein.

This circumstance should definitely be taken into account when planning evening experiments to evaluate the absorptive capacity of the small intestine, because daytime experiments, more preferable in terms of their organization, on condition of appropriate extrapolation can be quite adequate for evaluating the absorptive capacity of the small intestine in animals that are in conditions close to natural.

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