Original Research Article

Daily expression of sodium-dependent glucose cotransporter-1 protein in jejunum during rat ontogeny

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ABSTRACT

It is widely known that intestinal capacities such as the enzymatic hydrolysis of carbohydrates, lipids and proteins, and the subsequent absorption of the hydrolyzed products, are evolutionary matched to dietary loads and feeding behaviors. In this study, we demonstrate that the protein expression of apically located sodium-dependent glucose cotransporter-1 (SGLT-1) throughout rat ontogeny is daily adjusted to afford glucose uptake when the load of this metabolically essential monosaccharide in the intestinal lumen is maximum. The jejunal expression of SGLT-1 protein in 14 one-day-old suckling pups was found to increase at dark and early light phase ($P < 0.05$), when they have a better access to mother milk. In weaning 21-d-old and juvenile 28-d-old rats, the cotransporter expression was high throughout the entire day ($P < 0.05$). Finally, adult 90-d-old rats showed a well-developed circadian rhythm for SGLT-1 protein ($P < 0.05$), whose expression increased at late light and dark phase when the highest intestinal glucose load was achieved. To our knowledge, these results are the first reporting the daily profile of SGLT-1 expression during rat early developmental stage and may contribute to understand the biological significance of a well-established molecular capacity to deal with the crucial increase of glucose load in the diet during the weaning process.

1. Introduction

The energy intake necessary for animal growth and development are provided by food processing in the gastrointestinal tract. Since these processes are dynamic and the gastrointestinal tract has phenotypic plasticity, intestinal functional capacities should be adjusted to adequately meet increasing energy demands (Karasov and Martinez del Rio, 2007; Iossa et al., 1999). In mammalian species, the development of the small intestine during the early stages of life is a fast and challenging process, going from liquid diet during suckling to solid diet after weaning (Ferraris et al., 2000). In rats, this switch involves a weaning stress of pups and the intestinal physiology must be therefore adjusted to match these changes and provide a continuous flux of nutrients, thus increasing the digestive system efficiency to match the needs of growing rats (Ferraris et al., 2000; Pácha, 2000; Henning, 1986).

Glucose is the most important nutrient acting as an energy source for highly energy-demanding cells. In mammals, intestinal D-glucose uptake is largely carried out by the sodium-dependent glucose cotransporter-1 (SGLT-1) located in the apical membrane of the enterocytes that line intestinal villi (Poulsen et al., 2015; Wright et al., 2004). The SGLT-1 cotransporter protein has a predicted molecular weight (MW) of 73 kDa (Wright et al., 2004; Lee et al., 1994). Expression of this protein in Sf9 cells using a baculovirus expression system showed the existence of a deglycosylated SGLT-1 protein of 55 kDa MW (Smith et al., 1992). However, the SGLT-1 protein presents several N-glycosylation sites, which results

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in the presence of several MW immune-reactive bands in Western blot assays (Hirayama and Wright, 1992; Smith et al., 1992). In addition, there is growing evidence that the SGLT-1 cotransporter consists of functional polymeric arrangements (i.e. homotretameric forms) at the enterocyte apical membrane (Stevens et al., 1990).

To our knowledge, only one study has reported the SGLT-1 protein expression during the ontogeny development of rats, in which it was found that the expression of this protein was higher in weanling rats compared with suckling and adult ones (Khan et al., 2000).

Intestinal transporters expression and absorptive functions have long been known to exhibit daily rhythms in animals (Hussain and Pan, 2015) and particularly in adult rats (Corpe and Burant, 1996; Castelló et al., 1995; Stevenson and Fierstein, 1976). This daily rhythm is matched to nutrient availability and cued to the feeding rhythm rather than to the light cycle, unlike the endogenous circadian rhythms generated by the suprachiasmatic nucleus (Damiola et al., 2008). A daily rhythm of intestinal glucose absorption has been well established, with a peak of glucose transporter activity that matches the nocturnal feeding habits of these rodents (Fisher and Gardner, 1976; Stevenson et al., 1975). Furthermore, several studies have determined that circadian glucose uptake is associated to the expression of mRNA and protein of the intestinal SGLT-1 in adult rats (Stearns et al., 2009; Balakrishnan et al., 2008; Pan et al., 2004; Tavakkolizadeh et al., 2001). However, no information has yet been reported on the daily expression rhythm of the SGLT-1 cotransporter protein for suckling, weaning and weanling rats, even though day to day rhythmic changes may be of importance for this animal's physiology. A daily rhythm of the SGLT-1 cotransporter protein would secure an optimal absorption of the nutritional substrates during rat ontogeny development and prevent a waste of energy and membrane space (Diamond, 1991).

Hence, our goal was to characterize the daily expression profile of the jejunal SGLT-1 protein during rat ontogeny, focusing on this protein expression during the first stage of postnatal development (first month of life), when the switch from a liquid diet provided by the mother to a self-independently gathered solid diet triggers pivotal adjustments of the digestive and absorptive functions (Pácha, 2000; Henning, 1986). We hypothesize that rats will exhibit different daily profiles for intestinal SGLT-1 protein expression throughout their ontogeny development, and we predict that suckling rats will exhibit a maximal diurnal cotransporter protein expression when mothers rest and they thus have more access to the mother's milk. At the weanling stage, the SGLT-1 expression would be high along the entire day to maximize glucose absorption to afford the highly growing rate at this ontogeny stage, and finally the adult pattern of SGLT-1 expression would rise at night matching the typical rat nocturnal feeding habits.

2. Materials and methods

2.1. Animals

The experimental protocol was approved by the Institutional Care and Use Committee for Experimental Animals, from the Universidad Nacional San Luis, Argentina (N° B-31/06). Adult Sprague−Dawley rats (10 males, 20 females) were purchased from BIO-FUCAL S.A. (Del Viso, Buenos Aires, Argentina) and housed in the animal facilities at Universidad Nacional de San Luis. Rats were acclimated during 2 weeks to a 12 h light-dark photoperiod (light period from 06:00 to 18:00), with a room temperature (RT) of $22 \pm 2\ ^\circ\mathrm{C}$ and a relative humidity of $(55 \pm 5)\%$. Rats had *ad libitum* access to water and standard rodent chow (A.C.A., División de Nutrición Animal, Argentina. Composed by maximun of 23%

protein, 5% lipids, 6% crude fiber, 10% minerals, 12% humidity and 44% carbohydrates. Metabolizable energy 2,900 to 3,100 kcal/kg) throughout the entire study.

Two female and 1 male were mated in the same cage for one week, and all animals were then housed individually. Pregnant rats were regularly watched to record the pup births (recorded as d 1). On the second day after birth, the littermates were adjusted to 10 pups by mother in order to uniform the pups demand on the mother's milk. At 21 d of age, pups were artificially weaned to simulate the natural process (Redman and Sweney, 1976) and maintained at the same room and feeding conditions mentioned above during entire experimental period.

2.2. Experimental design

Twenty four 14-d-old male pups and 20 male rats of 21-, 28- and 90-d-old from different litters were weighed and euthanized by decapitation at zeitgeber time (ZT): ZT3 (09:00), ZT9 (15:00), ZT15 (21:00) and ZT21 (03:00); specifically, 6 rats each time for 14-d-old and 5 rats each time for rest ages. Light in animal room was on at 06:00 (ZT0) and off at 18:00 (Fig. 1).

Immediately after euthanization, the entire small intestine was removed by a fast laparotomy and placed in ice-cold mammals Ringer's saline solution (pH 7.4). After cleaning to remove the connective and adipose adjacent tissues, it was rinsed inside with ice-cold Ringer saline solution, and weight and length were recorded. Finally, it was divided into 3 equivalent segments and only the middle segment, representative of the jejunum, was kept for the subsequent experiments. All tissues were immediately snap-frozen in liquid nitrogen and maintained at $-120\ ^\circ\mathrm{C}$ in an ultrafreezer (Revco - Ultima II).

2.3. Sample preparation

Jejunal tissues were thawed at $4\ ^\circ\mathrm{C}$, cut into small pieces and mechanically homogenized in 5 mL/g wet tissue of ice-cold Tris-EDTA hypotonic lysis buffer (pH 7.4), with 1 mmol/L of phenyl-methylsulfonyl fluoride (PMSF) and 1% proteases inhibitor cocktail (Sigma). Homogenates were then centrifuged at $2,500 \times g$ at $4\ ^\circ\mathrm{C}$ for 20 min to separate the cellular debris. Supernatant was recovered and quickly separated into 20 μL aliquots, which were immediately snap-frozen in liquid nitrogen and stored at $-120\ ^\circ\mathrm{C}$. The protein concentration of the samples was assessed by the bicinchoninic acid (BCA) assay with a commercial kit (Pierce, BCA Protein Assay Kit) according to the manufacturer's instructions.
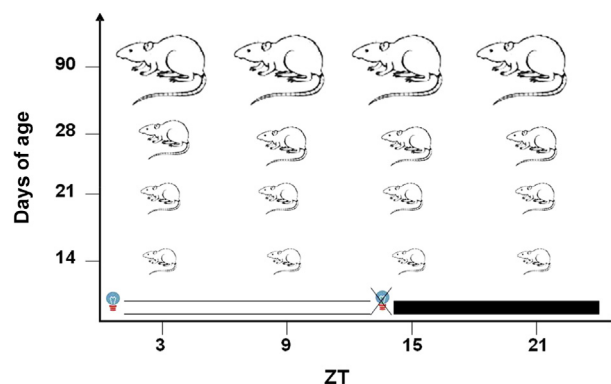

Fig. 1. The scheme represents the ages and zeitgeber time (ZT) of the day for jejunal sampling.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot assay

After thawing the homogenates aliquots at 4 °C, 50 μg of total proteins were boiled for 5 min in Laemmli sample buffer. Proteins were resolved by electrophoresis in 8% polyacrylamide gel with SDS and subsequently electroblotted onto a polyvinylidene fluoride (PVDF) membrane (Sigma) for 2 h at 4 °C. The PVDF membrane was cut into 2 pieces at 45 kDa MW standard. The upper membrane was used for SGLT-1 immune-detection, and the lower piece for α-actin immune-detection. Alpha-actin protein was used as gel loading control and for optical densitometry (OD) normalization in the densitometric analysis of the bands.

For the immunodetection of the SGLT-1 protein, the PVDF membranes were hydrated in absolute methanol for 30 s, washed 5 min in Tween-Tris buffered saline solution (TTBS, 0.1% Tween 20 in Tris Buffer solution, pH 7.5) and blocked for 1 h at room temperature with 5% (wt/vol) of non-fat dry milk diluted in TTBS. Then, the membranes were rinsed once with TTBS and incubated overnight at 4 °C with a rabbit anti-rat SGLT-1 primary antibody (Chemicon International, AB1352), diluted 1:2,000 in TTBS with 1% (wt/vol) non-fat dry milk and 0.02% (vol/vol) Timerosal (pH 7.5). Afterward, the membranes were washed with TTBS 5 times for 5 min to remove the residual unbound primary antibody and, incubated 2 h at room temperature with a goat anti-rabbit immunoglobulin G (IgG) secondary antibody conjugated to horseradish peroxidase (HRP) enzyme (Chemicon International, AP307P), diluted 1:10,000 in the same solution as primary antibody. Finally, the membranes were rinsed with TTBS 5 times for 5 min to remove residual secondary antibody, incubated with a chemiluminescent substrate (Pierce, Super Signal WestPico), and immediately exposed to a X-ray film (Kodak, BioMax Light) into a dark room.

The immunodetection procedure for α-actin protein was the same as that for the SGLT-1 protein, with the following difference: the membrane was incubated for 2 h at room temperature with a rabbit anti- α-actin primary antibody (Sigma, A2066) diluted 1:1,000.

All steps of the immunodetection procedure were carried out with gentle agitation. The immunodetection of each sample was performed in 2 independent experiments in order to verify the experimental reproducibility. The OD analysis of the bands was performed with the free-access ImageJ software (NIH software). The OD values of SGLT-1 bands were relativized by the α-actin OD values for each sample and this ratio was used for the statistical analyses.

2.5. Inhibition assay

A representative sample from each experimental age was resolved by SDS-PAGE and immunoblotted as described above, except that the primary anti-SGLT-1 antibody, diluted 1:3,000, was previously incubated for 2 h at room temperature with a 30-fold molar excess of its control immunogenic peptide (Chemicon International, SGLT-1 control peptide AG661) to check the primary antibody specificity. In parallel to this inhibition assay, a control assay was carried out without the immunogenic peptide under the same experimental conditions.

2.6. Statistical analyses

Data are presented as the mean value ± SEM. One-way analysis of variance (ANOVA) was applied to test for statistic differences and Fisher (LSD) post-hoc test was used for intergroup comparison. Values were considered as statistically significant at $P < 0.05$. The numerical values of SGLT-1:α-actin OD ratio were logarithmically transformed to accomplish with the data normality assumption, which was confirmed by the Shapiro–Wilk test. Sodium-dependent glucose cotransporter-1 protein expression at each experimental ZT was calculated by the sum of all MW bands immunodetected at each experimental time, and the total daily SGLT-1 protein expression at each age was calculated as the sum of all immunodetected bands at all experimental time for each age.

3. Results

3.1. Body and small intestine mass

The growth rate of live body mass was slower than that of small intestine during early developmental stages (from birth to 28-d-old). Subsequently, the body mass increased until the individuals became adult rats, and the small intestine mass showed a progressive growth rate among the different ages studied (Fig. 2). The different growth rate is clearly shown by the ratio of small intestine mass to body mass of the same individual, with the highest ratio being obtained for 21- and 28-d-old rats, an intermediate ratio for 14-d-old pups and the lowest for adult rats (Fig. 2).

3.2. Ontogeny and circadian rhythms of sodium-dependent glucose cotransporter-1 protein expression

Western blot assays of jejunal homogenates showed 4 different immune-reactive bands for SGLT-1, 3 of them with relative MW of 55, 60 and 72 kDa corresponding to nude, partially and whole glycosylated protein variants, respectively, and one 225 kDa band corresponding to the tetrameric arrangement of apical SGLT-1 cotransporter (Khan et al., 2000; Stevens et al., 1990). Jejunal samples from suckling 14-d-old pups showed the 4 different immune-reactive bands with relative MW of 55, 60, 72 and 225 kDa. The 3 lower MW bands were present at every experimental time, but the 225 kDa band was only present at ZT3 (Fig. 3A). Weaning 21-d-old rats showed 3 immune-reactive bands, with relative MW of 55, 72 and 225 kDa, at every experimental time studied (Fig. 3B). Juvenile 28-d-old rats exhibited 3 immune-reactive bands at ZT9, ZT15 and ZT21 with relative MW of 55, 72 and 225 kDa, but they showed only the 72 kDa band at ZT3 (Fig. 3C). Finally, adult 90-d-old rats showed 4 immune-reactive bands at ZT9, ZT15 and ZT21 with relative MW of 55, 60, 72 and 225 kDa, but only 72 kDa band could be detected at ZT3 in adult rats (Fig. 3D).
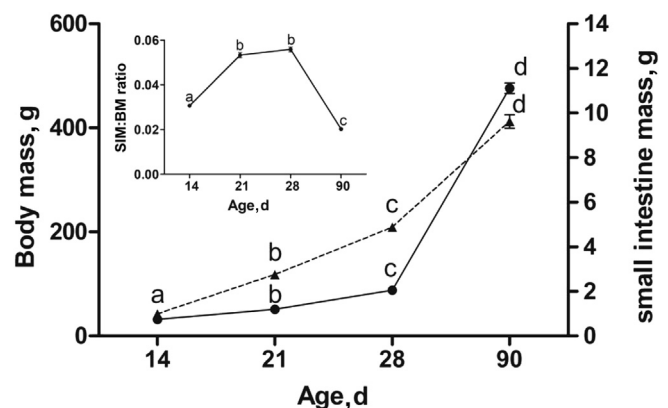

Fig. 2. Body mass (filled circle, continuous line) and small intestine mass (filled triangle, dotted line) through rat ontogeny. The insert graph denotes the ratio of small intestine mass to body mass (SIM:BM ratio) vs. age. The values represent mean values ± SEM. Data with different letters differ at $P < 0.05$, and $n = 20$ individuals for each age.
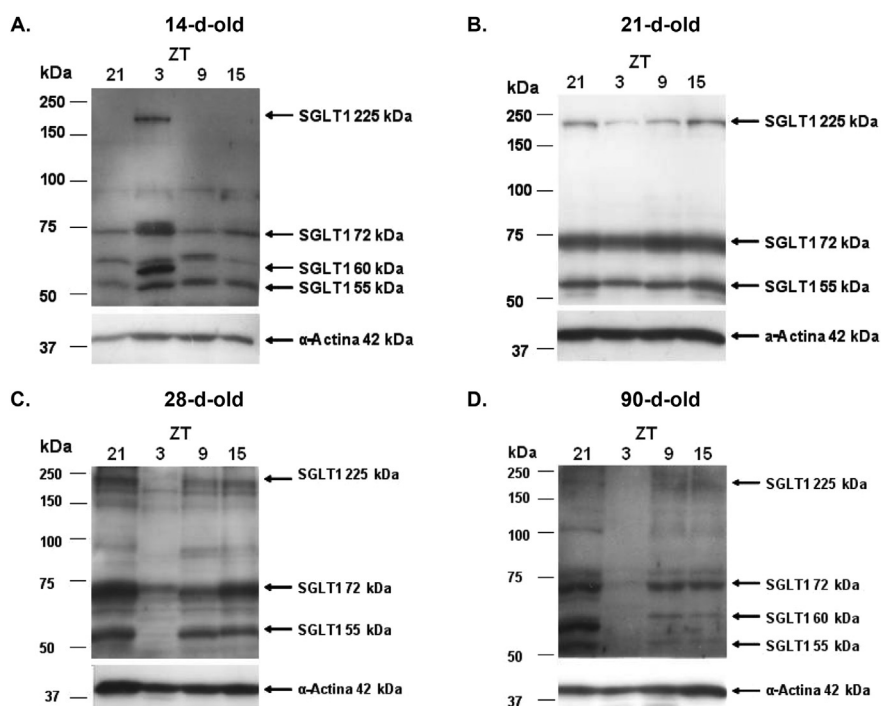
Fig. 3. Western blot images are representative of 2 independent assays illustrating the molecular SGLT-1 protein expression in jejunum at the different experimental zeitgeber time (ZT) in 14- (A), 21- (B), 28- (C) and 90-d-old (D) rats. Fifty microgram of total homogenated proteins were loaded by line in an 8% acryl-bisacrylamide denaturizing gel. Molecular weight standards (kDa) are indicated in the left side of each immunoblot. The 42 kDa α-actin protein was employed as a loading control.

3.3. Inhibition assay of anti-sodium-dependent glucose cotransporter-1 antibody

To evaluate the specificity of SGLT-1 immune-reactive bands detected by Western blot assays, we carried out the blockade of the anti-SGLT-1 primary antibody with its antigenic peptide. The primary antibody incubation with a 30-fold molar excess of their specific antigenic peptide yielded an almost complete inhibition of all immune-reactive bands at every age studied. This result supports the anti-SGLT-1 primary antibody specificity for the immune-detected bands and indicates the SGLT-1 protein identity of the Western blot assays.

3.4. Circadian expression of sodium-dependent glucose cotransporter-1 throughout different developmental stages

Circadian expression of the SGLT-1 cotransporter protein was calculated by adding up all immune-detected bands by Western blot at each experimental time, in order to avoid any under estimation of the cellular expression capacity for such protein.

Circadian changes on protein expression of SGLT-1 cotransporter only were found in adult rats, showing a significant decrease of SGLT-1 expression at ZT3 ($P < 0.05$; Fig. 4). Cotransporter levels at ZT9 decreased in suckling 14-d-old pups ($P < 0.05$; Fig. 4), but weaning 21-d-old and juvenile 28-d-old rats exhibited comparatively high and almost constant daily levels of SGLT-1 expression (Fig. 4).

3.5. Total daily expression of sodium-dependent glucose cotransporter-1 protein at the different stages of rat ontogeny

In order to evaluate the total SGLT-1 protein expression throughout the whole day, we summed the values of all immune-detected bands at the 4 experimental time by each age.
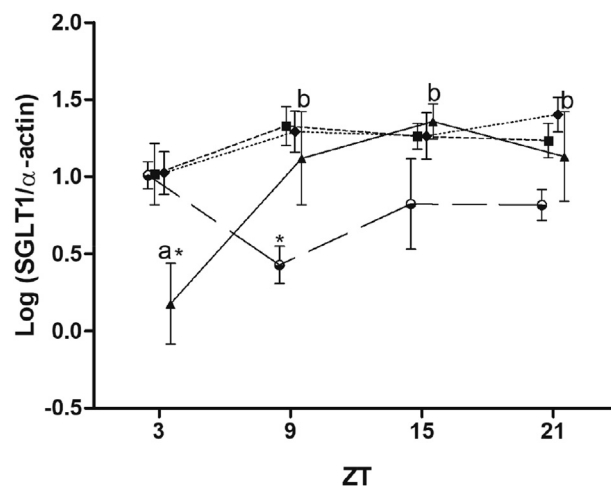

Fig. 4. Daily expression of SGLT-1 protein by 14-(○, long dashed line), 21-(■, short dashed line), 28- (♦, dotted line) and 90-d-old (▲, solid line) rats at each experimental zeitgeber time (ZT). Data represent mean values ± SEM of the logarithmic-transformed of SGLT-1:α-actin ratio, and the value of SGLT-1 is the sum of all immune-detected bands at each ZT. Significant differences ($P < 0.05$) among ZT at the same age are indicated by different letters and among ZT at different ages by *, $n = 4$ to 5 by ZT at each age.

There was a significant increase in the whole daily SGLT-1 protein expression levels in 21- and 28-d-old rats compared with suckling 14-d-old rats ($P < 0.05$), but the daily cotransporter expression exhibited a moderate decrease when the rats became adults and consequently no significant differences were observed between these 3 premature ages and the 90-d-old adults rats (Fig. 5).
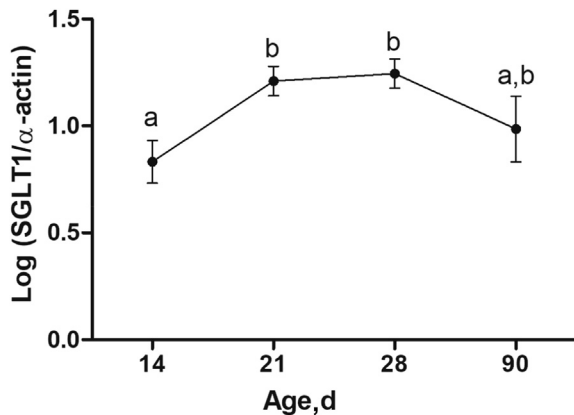
Fig. 5. Total daily expression of SGLT-1 protein at each experimental age. Data represent mean values ± SEM of the logarithmic-transformed of SGLT-1:α-actin ratio, and the value of SGLT-1 is the sum of all immune-detected bands at the 4 experimental zeitgeber time (ZT). Significant differences ($P < 0.05$) among ages are indicated by different letters, $n = 19$ to 20 by age.

4. Discussion

Although it has been well established that nutrient transport changes with age in mammalian species, little is known about daily variations and ontogeny at molecular level in rats. The main objective of this study was to examine the daily profile of the expression of the jejunal SGLT-1 during rat ontogeny, focusing on the period of diet switch around of first month of life.

In agreement with previously reported research (O'Connor and Diamond, 1999; Toloza and Diamond, 1992), we found a significant increase of the small intestine growth rate respect to the body mass growth rate for 21- and 28-d-old rats, and a lower but still significant growth rate for 14-d-old rats. This pattern is reasonable because at these early ontogeny stages the small intestine is a key organ in the acquisition of energy to sustain the active growth rate of the whole organism (Hulbert and Else, 2000; Cant et al., 1996). Hence, the understanding of the molecular machinery that affords this high growing rate is quite relevant in developmental physiology.

The lower SGLT-1 protein expression in 14-d-old rats in the afternoon is tightly associated to the feeding behavior reported for suckling rats. The increased SGLT-1 protein expression during night and early morning allows to suckling rats have larger amounts of cotransporter protein when the access to mother milk is available. During this developmental stage, pups feed exclusively on maternal milk by short time periods because, since their psychomotor development is not mature enough to follow the mother into the nest, they suck the mother's milk when it is resting during the last night phase and early morning (Redman and Sweney, 1976). However, during the weaning stage the liquid and poor-carbohydrates diet ingested by the suckling rats turn into a solid and rich-carbohydrates one (O'Connor and Diamond, 1999; Redman and Sweney, 1976). Thus, weaning rats need a highly developed biochemical machinery to hydrolyze the complex polysaccharides present in the solid diet (Henning, 1979, 1986; Stevenson and Fierstein, 1976), and they need an adequate transporter machinery to carry out the active uptake of the released monosaccharides, including glucose, in order to satisfy the highly energetic growth demands (Ferraris, 2001; Pácha, 2000). Hence, the steady state of intestinal SGLT-1 cotransporter protein expression throughout the entire day during the weaning stage suggests that the molecular capacity to uptake glucose in rat jejunum is huge at this ontogeny stage, providing the necessary molecular support

for the high nutritional requirements needed for the growth rate of several organs, including the small intestine.

To date, there are few studies reporting circadian rhythms of the biochemical capacity to digest and transport carbohydrates during rat ontogeny. However, the lack of circadian rhythms at the post-natal stage in rat ontogeny is not only restricted to SGLT-1 cotransporter protein expression. The presence of circadian rhythms has been reported for the biochemical activity of intestinal disaccharidases (i.e. lactase, maltase, isomaltase and sucrase) in 30-d-old rats, when the weaning has ended, but not in 16- and 19-d-old rats (Kojima et al., 1998; Saito et al., 1978).

A plausible explanation for the lack of circadian rhythms of SGLT-1 protein expression in early developmental stage during rat ontogeny can lie in the fact that the genes that constitute the peripheral circadian clock of several organs have not yet been completely established during the first month of life (Polidarová et al., 2014; Damiola et al., 2008; Stratmann and Schibler, 2006). To date, we have not found any study about the peripheral circadian clock genes expression in the small intestine tissue during rat ontogeny. The relevance of these master peripheral clock genes lies in the fact that they codify transcriptional-regulator molecules (i.e. transcription factor) whose main function is to switch on or off cellular genes expression (Hussain and Pan, 2015; Konturek et al., 2011; Damiola et al., 2008; Stratmann and Schibler, 2006).

Iwashina et al. (2011) have reported that the circadian rhythms of the mRNA expression of the hexose transports genes, e.g., SGLT-1, GLUT5 and GLUT2, in adult mice are driven by the food supply schedule, peaking at the late light-phase, and that this daily expression profile is tightly regulated by the peripheral core-feedback clock genes (Hussain and Pan, 2015; Iwashina et al., 2011).

Only adult 90-d-old rats revealed a well-established circadian rhythm expression for the cotransporter SGLT-1 protein. Similar results are already reported by other studies (Stearns et al., 2009; Balakrishnan et al., 2008). The physiological meaning of this daily profile for the molecular expression of the cotransporter protein is relevant, because it is closely associated with the diurnal profile exhibited by adult rats for intestinal glucose absorption rate assessed *in vivo* (Fisher and Gardner, 1976) and cotransporter levels measured *in vitro* in duodenum (Pan et al., 2002) and jejunum (Balakrishnan et al., 2008; Houghton et al., 2008), which showed that the intestinal active glucose absorption rate was 50% to 60% higher at night time respect to day time, matching the enhanced levels of the SGLT-1 protein expression with the typical nocturnal feeding behavior inherent to the adult rats. Accordingly, the results obtained in adult individuals corroborate one of our hypotheses that the circadian SGLT-1 protein expression levels in adult rats match their typical nocturnal feeding habits. Therefore, the intestinal machinery is ready when the maximal glucose bulk is in the intestine to maximize the absorption of this metabolically essential molecule.

The short-term changes of the cotransporter protein levels throughout the day displayed by adult rats require the presence of one or more quick-action molecular mechanism that allow enterocytes to carry out SGLT-1 protein synthesis, degradation, vesicular recruitment, or a combination of them. There are studies exploring this issue that propose several molecular processes such as transcriptional SGLT-1 gene regulation (Balakrishnan et al., 2008; Rhoads et al., 1998), post-transcriptional or post-translational regulation exerted by the vagal innervations (Houghton et al., 2006), or intracellular SGLT-1 protein recruitment into early endosomes (Khoursandi et al., 2004; Kipp et al., 2003). The results of these studies are controversial, and a conclusive answer has not been yet achieved related to how the luminal nutrients can influence the circadian SGLT-1 expression. Some *in vivo* studies sustain that the diurnal rhythms of the intestinal peptides transporter 1,

and the SGLT-1 cotransporter, expression in adult rats is regulated by the feeding conditions (Pan et al., 2004; Corpe and Burant, 1996). However, in an *in situ* study, Stearns et al. (2009), using a Thiry-Vella loop in the jejunal segment from adults, to exclude the luminal nutrients, demonstrated that the circadian cotransporter protein expression is independent of the local luminal factors placed into the jejunum, suggesting that the entrained mechanism involved in the circadian SGLT-1 expression should be systemic rather than local (Stearns et al., 2009).

Our results agree with the ontogeny profiles during rat growth for D-glucose absorption rate measured *in situ* by a perfusion technique (Khan et al., 2000) and the cotransporter uptake activity measured *in vitro* by everted sleeves assay (Toloza and Diamond, 1992). In such profiles, the jejunal D-glucose absorption rate was the lowest during the suckling period, peaked at the weaning stage and slightly decreased when the rats reached adulthood, matching the profile reported here for the total daily SGLT-1 protein expression.

5. Conclusion

In summary, one of the most relevant aspects of this study lies in the fact that, to date, our results are the first reporting the daily profile of SGLT-1 protein expression during rat early ontogeny. This may contribute to understand the biological significance of a well-established intestinal molecular capacity to deal with the crucial increase of glucose load in the diet composition during the weaning process. In this context, we have demonstrated that the daily induction of SGLT-1 anticipates the onset of feeding bulk. A further contribution of this study lies in the demonstration of the molecular support provided by the developmental profile of SGLT-1 protein expression to the physiological cotransporter activity measured *in vivo* and *in vitro* during rat ontogeny.

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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