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Psychological stress impairs \( \text{Na}^+ \)-dependent glucose absorption and increases GLUT2 expression in the rat jejunal brush-border membrane

Gaëlle Boudry, Christopher I. Cheeseman, and Mary H. Perdue

1Intestinal Disease Research Program, Department of Pathology and Molecular Medicine, Faculty of Health Science, McMaster University, Hamilton, Ontario, Canada; and 2Membrane Protein Research Group, Department of Physiology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

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Boudry G, Cheeseman CI, Perdue MH. Psychological stress impairs \( \text{Na}^+ \)-dependent glucose absorption and increases GLUT2 expression in the rat jejunal brush-border membrane. Am J Physiol Regul Integr Comp Physiol 292: R862–R867, 2007. First published October 19, 2006; doi:10.1152/ajpregu.00655.2006.—Chronic psychological stress impairs many functions of the gastrointestinal tract. However, the effect of stress on nutrient absorption is poorly documented. This study was designed to investigate glucose transporters in rats submitted to different periods of water-avoidance stress (WAS). Rats were subjected to WAS (1 h/day) for 1, 5, or 10 consecutive days. Four hours after the last WAS session, rats were killed and segments of jejunum were mounted in Ussing chambers to study electrophysiological properties of the jejunum and \( \text{Na}^+ \)-dependent glucose absorption kinetics. Mucosa was obtained to prepare brush-border membrane vesicles (BBMV) used to measure \([14\text{C}]\)fructose uptake as well as sodium-glucose transporter 1 (SGLT-1) and GLUT2 expression by Western blot analysis. Exposure of animals to WAS induced a decrease in \( \text{Na}^+ \)-dependent glucose absorption \( V_{\text{max}} \) after 1, 5, and 10 days without any change in SGLT-1 expression. Potential difference across the jejunum was decreased for all stressed groups. Furthermore, we observed an increase in phloretin-sensitive uptake of \([14\text{C}]\)fructose by BBMV after 1, 5, or 10 days of WAS, which was not present in control animals. This suggested the abnormal appearance of GLUT2 in the brush border, which was confirmed by Western blot analysis. We concluded that psychological stress induces major changes in glucose transport with a decrease in \( \text{Na}^+ \)-dependent glucose absorption and an increase in GLUT2 expression at the brush-border membrane level.

STRESS, EITHER PHYSICAL OR psychological, is a normal component of life. All life forms have developed mechanisms to cope with stressors, in order to maintain homeostasis and assure survival. Although stress is not inherently bad, major stressful events may have long-term consequences (17). Stress-induced pathology can develop in conditions where defense mechanisms are challenged over a long period of time or adaptation fails, i.e., when one is exposed to chronic stress (15). Hyper- or hypofunction of the stress system activity involves a number of human health problems of enormous impact on the society, such as metabolic syndrome, diabetes, inflammatory bowel disease, irritable bowel syndrome, etc. (5). One of the main characteristics of exposure to chronic psychological stress is a drop in body weight in adults or a decrease in body weight gain in growing individuals (16). These changes in body weight are assumed to be related to changes in food intake and/or a shift in metabolism of nutrients from anabolism to catabolism to prioritize nutrients used for coping with stress rather than for physiological processes like growth (7). However, disturbances of the gastrointestinal system may also result in body weight disorders. Impact of stress on gastrointestinal function has been widely recognized, both at a clinical level and in animal models. So far, the effect of stress on gastrointestinal function has been shown to involve changes in motility (30) and mucosal function (26), with appearance of early signs of inflammation (22, 27). Despite the importance of intestinal nutrient absorption for health, the impact of stress on nutrient absorption has been poorly documented. Among nutrients, glucose plays a key role in energy metabolism, and its mechanisms of absorption at the intestinal level are well described. Glucose is actively transported through SGLT-1, but a facilitated pathway is also involved through the rapid, glucose-dependent activation and recruitment of the facilitative glucose transporter GLUT2 to the brush-border membrane. The regulation involves a protein kinase C-dependent pathway activated by glucose transport through SGLT-1 and also involves mitogen-activated protein kinase signaling pathways (13). A recent report indicated that environmentally stressed rats exhibited decreased apical GLUT2-facilitated glucose absorption in fed conditions (25). However, the environmental stress (construction work in the building) as used in this study was poorly defined; animals were assigned to the stressed groups retrospectively and the stress was most likely a mild stress, since no behavioral disturbances were observed in the animals.

Our objective was to study the effect of psychological stress on intestinal glucose transporters in a model of stress [water-avoidance stress (WAS)] already validated and known to induce disturbances of intestinal physiology. This model of psychological stress induces minimal physical stress and seems to be an appropriate model of psychological stress, since it is widely used by psychiatrists as a model of depression. We previously showed in our group that WAS in rats induces enlargement of the adrenal glands with hypertrophy of the steroid-producing zona fasciculata of the cortex, and increased serum corticosterone values (27), two characteristics of a chronic stress state. Moreover this model has been shown to trigger intestinal inflammation (27), alter motility, and alter visceral sensitivity (3).

MATERIALS AND METHODS

Animals. We used Brown Norway background rats obtained from our breeding colony at McMaster University [original breeders from

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Dr. Y. Kitamura, Osaka, Japan (19)]. These rats have been shown to be sensitive to stress and to stop gaining weight when exposed to chronic psychological stress (27). Body weight (250–350 g)- and age (14–20 wk)-matched rats were maintained under pathogen-free conditions on a 12:12-h dark–light cycle. They were provided with food and water ad libitum, except during the last 4 h before death when they had no access to food. Food intake was measured daily and expressed in grams per day per 100 grams body weight. All procedures were approved by the Animal Care Committee at McMaster University.

Stress protocol. To acclimatize the rats to manipulation by humans, all rats (stressed and controls) were handled daily for 1 wk. The stressed rats were then submitted to WAS for 1 h each day for 1 (WAS 1 day), 5 (WAS 5 days), or 10 (WAS 10 days) consecutive days. The procedure was performed between 8:00 and 10:00 AM to minimize the effect of circadian rhythm. Rats were weighed and then placed on a platform (8 × 6 cm) in a plastic container (56 × 50 cm) with water (25°C) to 1 cm below the platform. Control rats were still handled and weighed daily but remained in their home cages.

Four hours after the last WAS session, rats were killed by decapitation and 5-cm long intestinal segments from the proximal jejunum (starting 5 cm distal to the Treitz ligament) were taken for studies in Ussing chambers. In another group of rats, 15-cm-long intestinal segments (also starting 5 cm distal to the Treitz ligament) were immediately flushed with ice-cold PBS. Mucosal scrapings were immediately snap-frozen in liquid nitrogen for subsequent preparation of brush-border membrane vesicles (BBMV).

Transport and kinetic studies in Ussing chambers. Jejunal segments were stripped of their seromuscular layers and mounted in Ussing chambers (World Precision Instruments, Narco Scientific, Mississauga, ON, Canada) with an exposed area of 0.6 cm². They were bathed on each side with 8 ml of oxygenated Krebs buffer containing (in mM) 115 NaCl, 1.25 CaCl₂, 1.2 MgCl₂, 2 KH₂PO₄, 25 NaHCO₃, 10 mannitol, and 10 fructose (pH 7.4). Temperature was maintained at 37°C with water-jacketed reservoirs. Transmural potential difference (PD) was measured using calomel electrodes connected to the chambers with Krebs-Agar (2%) bridges. The tissues were continuously short-circuited with an automatic voltage clamp (model EVC-4000; World Precision Instruments) and Ag-AgCl electrodes connected to the chambers via Krebs-Agar bridges. Every 30 s, the short-circuit current (Isc) was transiently shut off and the open-circuit PD across the mucosa was used to allow the calculation of tissue conductance (G) using Ohm’s law.

After a 20-min equilibration period, increasing amounts of 3-O-methylglucose (3-OMG, Sigma) were added to both mucosal and serosal buffers every 5 min, resulting in final concentrations of 2, 4, 8, 16, 32, and 64 mM. Maximal ΔIsc at each concentration was recorded. The Kₘ and Vₘₐₓ for Na⁺-dependent glucose absorption were then calculated using GraphPad Prism (San Diego, CA).

Preparation of BBMV. BBMVs were prepared from mucosal scrapings using a standard procedure (4). Briefly, the mucosa of two rats from the same group were pooled and placed in 65 ml of ice-cold mannitol/Tris buffer (300 mM mannitol, 5 mM EGTA, 12 mM Tris-HCl, pH 7.4, and 0.1 mM PMSF). The tissue was homogenized in a Polytron homogenizer for 2 min before addition of MgCl₂ to a final concentration of 12 mM. After being stirred on ice for 15 min, the solution was centrifuged at 1600 g for 15 min to remove debris. The supernatant was further centrifuged at 20,000 g for 30 min. The pellet was homogenized in half-strength mannitol/Tris buffer (in mM: 150 mannitol, 2.5 EGTA, 6 Tris-HCl, pH 7.4, and 0.05 PMSF) with a glass homogenizer before further addition of MgCl₂ (final concentration 12 mM). After being stirred on ice, the centrifugations were repeated as before. The supernatant of the last centrifugation was discarded, and the vesicle preparation was diluted in Tris buffer (300 mM mannitol, 5 mM Tris-HCl, pH 7.4) to a protein concentration of 6 mg/ml (Bio-Rad protein assay).

Fructose uptake measurement. Fructose uptake measurements were carried out with the rapid filtration procedure (2). A quantity of 20 μl of the uptake buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 7 μCi[^14C]fructose; Amersham Biosciences, Piscataway, NJ) and nonlabeled fructose (to reach the desired concentration) was first pipetted into the bottom of a polystyrene tube (10 × 15 mm). Then 10 μl of BBMV suspension, previously incubated or not for 1 h with 100 μM of phloretin (Sigma) was spotted onto the side of the tube in two separate drops right above the uptake buffer with a Microman pipette. After 10 s of warming up at room temperature, uptake incubation was initiated by a foot-switch-activated vibromixer, and the process was terminated after 3 s by the addition of 1.125 ml of ice-cold wash solution (in mM: 125 NaCl, 2 Tris-HCl, 1 phloridzin, 0.25 phloretin). Uptake mixture (1,000 μl) was then rapidly pipetted onto 0.45-μm cellulose acetate filters (presoaked with the wash solution) mounted in a Mannifold filtration unit, which was connected to a vacuum source. The filters were immediately washed four times with 5 ml of ice-cold washing solution. The uptake was measured in triplicate for each concentration. The remaining solution in the incubation tubes was collected and at the end of the experiment was pooled and counted for the average initial radioactivity in the uptake media. After 30 min of extraction in 5 ml of Ecolume scintillant, filters were counted with a liquid scintillation analyzer with automatic quench correction. The nonspecific binding of[^14C]fructose to filters was also measured and subtracted. The Kₘ and Vₘₐₓ for fructose uptake were calculated using GraphPad Prism (San Diego, CA).

Western blot analysis. BBMV (15 μg protein) were solubilized in Laemmli sample buffer and run on a SDS-polyacrylamide gel (10% gel) using a Mini-Protein II cell (Bio-Rad). Molecular markers were also run on the gel. The proteins were blotted onto nitrocellulose membrane (Bio-Rad) by electrotransfer for 75 min at 4°C using the Mini-Trans-Blot Cell (Bio-Rad). Blocking of the membrane was carried out in 5% nonfat dry milk in PBST (0.05% Tween 20PBS, pH 7.4) for 1 h and then incubated with 1:1,000 rabbit polyclonal antibody to rat GLUT2 [Chemicon, Temecula, CA]; antigenic sequence was equivalent to AA402–442 in 3% nonfat dry milk in PBST overnight at 4°C. The membrane was washed three times in 3% nonfat dry milk/PBST for 15 min. The nitrocellulose membrane was then incubated with a secondary antibody, anti-rabbit IgG coupled to horseradish peroxidase (Amersham Biosciences) diluted 1:2,000 in 3% nonfat dry milk/PBST for 1 h. Three subsequent washes followed as described above. Finally, the membrane was treated with the enhanced chemiluminescence detection solution (Amersham Biosciences) before autoradiography for 30 s by using Kodak XAR-5 film with an intensifying screen. Autoradiographic images were scanned with an HP Scanjet 4300C, and the data were analyzed using Scion Image software. The optical density for each peak was calculated by using a standard curve generated with Kodak density strips. In all cases, the peaks were determined to be on the linear portion of the standard curve. Western blot analysis for GLUT2 frequently produced two bands apparently representing differing levels of glycosylation. There was no consistent alteration in one band vs. the other for a given condition, so both bands were quantified together for each measurement of GLUT2 abundance.

Statistical analysis. Results are expressed as means ± SE. One-way ANOVA was performed using the General Linear Model (GLM) of SAS (SAS Institute, Cary) for body weight gain, food intake, Na⁺-dependent kinetics, and fructose uptake kinetics. Testing for the effect of stress treatment was by the Snedecor F-test. Differences between groups were then tested by the Bonferroni test when appropriate. For the fructose uptake measurement, a two-way ANOVA was performed using GLM, to test the stress treatment and the fructose dose effects. Differences between doses and groups were tested by a Bonferroni test when appropriate. For the Western blot analysis, we
could not study all the conditions on the same gel. Therefore, data were analyzed by a Student’s t-test to compare the control animals vs. WAS 1 day, WAS 5 days, or WAS 10 days. For all analyses, a P value < 0.05 was considered significant.

RESULTS

Body weight gain and food intake. During the week preceding the onset of WAS procedures, all rats gained weight consistently (in g/day: control, 1.9 ± 0.2; WAS 1 day, 2.3 ± 0.4; WAS 5 days, 1.7 ± 0.4; and WAS 10 days, 2.6 ± 0.3). They stopped gaining weight as soon as the WAS sessions started (in g/day: WAS 5 days, −0.8 ± 0.4 and WAS 10 days, 0.2 ± 0.3, P < 0.05 compared with the period before the onset of stress). Their food intake was slightly reduced by 10%, although the reduction was significant only in the WAS 10 days group (Table 1).

Electrophysiological properties of the jejunum. One session of WAS (WAS 1 day) did not alter Isc (Table 2). However, after 5 and 10 consecutive days of WAS (WAS 5 days and WAS 10 days), jejunal Isc was significantly decreased compared with controls (Table 2). In contrast, after one session of WAS, rat jejunum exhibited a higher G than the jejunum from control animals but not after 5 and 10 days of WAS (Table 2). This resulted in a decrease of the PD across the mucosa for all groups (Table 2).

Glucose-evoked changes in Isc and SGLT-1 expression. We used the rise in Isc after 3-OMG addition into the mucosal side of the chambers as an index of Na\(^+\)-dependent glucose absorption. 3-OMG is a nonmetabolizable analog of glucose. Measurement of the rise in Isc after addition of increasing doses of 3-OMG allowed us to build dose-response curves and calculate Na\(^+\)-dependent glucose V\(_{\text{max}}\) and K\(_{\text{m}}\). V\(_{\text{max}}\) was reduced by half after 1, 5, and 10 days of WAS (Table 3). Moreover, K\(_{\text{m}}\) was decreased after 1 and 5 days of stress (Table 3).

The abundance of SGLT-1 in BBMV was measured semiquantitatively by Western blot analysis. There was no significant difference in SGLT-1 abundance after 1, 5, and 10 days of WAS compared with control (Fig. 1).

GLUT2 activity and expression at the brush-border membrane level. We evaluated GLUT2 activity in the brush-border membrane by the measurement of fructose uptake by BBMV in presence or absence of the specific GLUT2 inhibitor phloretin. GLUT2 can facilitate the entry of glucose and fructose equally well and is phloretin sensitive (13). In contrast, the only other known route for fructose entry is mediated by GLUT5, which is believed to be selective for this hexose and is phloretin insensitive. Therefore, phloretin-sensitive fructose uptake into BBM vesicles should specifically measure uptake mediated by GLUT2.

In control animals, fructose uptake was phloretin insensitive, indicating that it was mainly mediated by GLUT5 in these animals (Fig. 2). However, after 1, 5, or 10 days of WAS, BBMV exhibited a significant increase in fructose uptake compared with control animals. Incubation of the BBMV with phloretin reversed this increased uptake to the control animal values (Fig. 2), suggesting the presence of GLUT2 (phloretin-sensitive uptake) in addition to GLUT5 (phloretin-insensitive intake). Fructose uptake kinetics obtained from these curves confirmed that V\(_{\text{max}}\) was significantly enhanced in the stressed groups compared with control animals (Table 4). Moreover, addition of phloretin to the BBMV reduced V\(_{\text{max}}\) to a level similar to control levels (Table 4). K\(_{\text{m}}\) was not altered by stress except in the WAS 5 days group, which exhibited a significant increase in K\(_{\text{m}}\), that was inhibited by phloretin (Table 4).

This phloretin-sensitive fructose uptake in the BBMV of the stressed animals suggests the presence of GLUT2 in the BBMV that was confirmed by Western blot analysis. Expression of GLUT2 in BBMV of rats after 1, 5, or 10 days of WAS was significantly increased compared with control (Fig. 3).

DISCUSSION

Using a model of chronic psychological stress, we demonstrated in our study that WAS induced a decrease in Na\(^+\)-dependent glucose absorption together with an increased expression and activity of GLUT2 at the brush-border membrane level in animals that had no access to food for several hours, i.e., at a moment when GLUT2 should not be expressed (13).

We used different techniques to study glucose transporters: Ussing chambers and BBMV. Indeed, GLUT2 trafficking to the brush-border membrane and activity are under the control of PKC-II, which is rapidly lost after excision of the tissue (10). GLUT2 activity is therefore impossible to detect in Ussing chambers. This is the reason why we investigated GLUT2 activity in BBMV prepared from intestine flushed with ice-cold solutions and kept on ice during the entire isolation.
process, previously shown to prevent the loss of GLUT2 (10). On the other hand, we chose to use Ussing chambers for Na\(^+\)-dependent glucose absorption study since it also gives data on the electrophysiological properties of the tissue (\(I_{\text{sc}}, G_{\text{R}}, \text{ and PD}\)). Na\(^+\)-dependent glucose absorption was measured by the rise in \(I_{\text{sc}}\) in Ussing chambers after the addition of different concentrations of 3-OMG. This change in \(I_{\text{sc}}\) reflects both the activity of Na\(^+\)-K\(^+\)-ATPase pumping the sodium across the basolateral membrane and the resulting activity of SGLT-1 mediating the coupled entry of glucose and sodium at the apical side. The decrease of \(V_{\text{max}}\) observed after 1, 5, or 10 days of WAS could be due to either a decrease in SGLT-1 activity and/or a decrease in Na\(^+\)-K\(^+\)-ATPase one. No difference in SGLT-1 abundance was noticed in the BBMV, suggesting that the difference observed in Na\(^+\)-dependent glucose absorption could be accounted for by a decrease in the activity of the Na\(^+\)-K\(^+\)-ATPase. This is in agreement with the decreased PD across the mucosa observed in all stressed animals.

The influence of stress on nutrient absorption has, to date, been poorly documented. A report in 1980 investigated glucose absorption in the intestine of rats submitted to a restraint stress for 14 h (31). This acute restraint stress produced a slight increase, although not significant, in glucose absorption measured by an isolated perfused intestine technique. However, the effects were relatively small and no indication of the transporters involved was provided. Very recently, Shepherd et al. (25) described the effect of environmental stress on glucose absorption in Wistar rats. Environmentally stressed animals (by construction activity during expansion of the department) exhibited a 42\% decrease in the rate of phloretin-sensitive glucose absorption, paralleled by a decrease in GLUT2 expression at the brush-border membrane level; the SGLT-1 component of glucose absorption was not altered. This seems contradictory to our results since we observed an increase in GLUT2 expression and alteration of SGLT-1 kinetics. The discrepancies between Shepherd’s study and ours might be accounted for by the difference in the stress model. Indeed, it is well recognized that modeling stress, especially chronic stress, is rather difficult and can give rise to different results. For example, no significant changes of GLUT2 were observed in rat jejunum after a restraint stress of 2 h (32). The stress duration can therefore represent a key variable in the expression of GLUT2.

### Table 4. Effect of WAS on fructose uptake by brush border membrane vesicles kinetics

<table>
<thead>
<tr>
<th>WAS Duration</th>
<th>(V_{\text{max}}), pmol/mg protein (\cdot s^{-1})</th>
<th>(K_m), mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2363 ± 586*</td>
<td>1096 ± 231*</td>
</tr>
<tr>
<td>WAS 1 day</td>
<td>5894 ± 1466bc</td>
<td>1929 ± 441bc</td>
</tr>
<tr>
<td>WAS 5 days</td>
<td>10724 ± 1526ab</td>
<td>4537 ± 1726ab</td>
</tr>
<tr>
<td>WAS 10 days</td>
<td>4108 ± 1572d</td>
<td>2754 ± 1394d</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from control animals (\(P < 0.05\)). **Significantly different from WAS 1 day (\(P < 0.05\)). Values are means ± SE, \(n = 4–5\) blots/group. GLUT2 expression was measured in BBMV obtained from control rats or rats submitted to 1, 5, or 10 consecutive days of WAS.
changes in water absorption were observed in the study by Shepherd et al., whereas this is a common feature in the WAS model (23, 27). Moreover, the environmental stress in Shepherd et al.’s study seemed to be a mild stress since no changes in feeding behavior were noticed and a 2-day period without stress seemed to be enough to restore normal glucose absorption. On the other hand, in the WAS model, we observed a slight but significant decrease in food intake in accordance to what had already been reported by our group (22). We did not study the time necessary to restore normal glucose absorption, but the studies performed on barrier epithelial function in our laboratory showed that a minimum of 3 days was necessary to recover normal barrier function (22), suggesting that WAS is a more intense stressor compared with environmental stress. Another explanation for the discrepancies between Shepherd et al.’s study and ours could be the difference in animal strains used since reactivity and coping with stress can be very different between strains. Whatever the explanations for the discrepancies are, their data and ours demonstrate that psychological stress can impair glucose absorption, although the mechanism may differ.

The term “stress” in the literature is used regardless of the type of stressor (external, either physical or psychological, or internal, such as inflammation or hemorrhage). Despite the wide range of stressors, the mechanisms underlying the stress response are often similar although with various intensities involving activation of the sympathetic system and a neuroendocrine response (HPA axis) (24). This leads to a general catabolic state (7). The model of 10-day WAS we used in this study was previously shown to induce enlargement of the adrenal glands with hypertrophy of the steroid-producing zona fasciculata of the cortex, and increased serum corticosterone values (27), suggesting a catabolic state. We did not measure endocrine response to stress in our study since it had already been well documented by previous studies in our laboratory (27), but we carefully monitored daily food intake and body weight before and during stress periods. The stressed animals all stopped gaining weight and slightly reduced their food intake as previously described (22, 27). However, stress magnitude per se was not really measured by the endocrine response and could account for some of the variability observed in the results. The enhanced glucocorticoid levels and catabolic state are characteristic of other stress conditions, such as major surgery, heat stress, or sepsis (18). In studies of heat-stressed birds, absorption of methionine was decreased compared with control animals, with a decrease in the energy-dependent uptake; energy-independent uptake was increased but not enough to compensate for the decrease in energy-dependent uptake (6). This is analogous to our findings of an increased expression of the energy-independent transport of glucose, GLUT2, and alteration of the energy-dependent one. Similarly, [14C]glucose uptake was decreased in BBMV prepared from rats submitted to surgical manipulation compared with controls (20). Nutrient absorption is also decreased during inflammation, either systemic (1) or intestinal (28–29). Taken together, these results suggest that decreased nutrient absorption could be a common feature of stress responses in general.

In addition to the mechanisms involved at the animal level, we are now testing some hypotheses on the intracellular mechanisms implicated at the enterocyte level. The first one is the involvement of the AMP-activated kinase (AMPK). AMPK is a key sensor of energy within the cell and is activated by any stress that causes a rise in the cellular AMP-to-ATP ratio. Its activation switches on catabolic pathways that generate ATP while switching off anabolic pathways and any other nonessential processes that consume ATP (9). Its induction under chronic psychological stress has not yet been demonstrated, but the induction of catabolism induced by stress is likely to upregulate this kinase. A recent study reported that AMPK activation reduced mRNA expression and total cellular levels of SGLT-1 in murine small intestine, paralleled by an appearance of GLUT2 in the brush-border membrane (32). If AMPK is actually upregulated in our model of WAS, this intracellular mechanism could explain the changes in transporters expression and activity we observed.

Another possible mechanism could involve muscarinic receptors and their downstream effectors. Indeed, the rapid activation and recruitment of GLUT2 to the brush-border membrane correlates with the activation of PKC-βII by glucose transport through SGLT-1 (10) and also involves mitogen-activated protein (MAP) kinase signaling pathways (11). It is clearly demonstrated now that psychological stress effects in the intestine is partly mediated by acetylcholine through muscarinic receptors at the enterocytes level (8, 14, 21, 23), which in turn activates the phosphorylation of several intracellular mediators, such as PKC (8) and MAP kinase (12). Therefore, the activation of muscarinic receptors, by using the same downstream signals as the one involved in GLUT2 trafficking, could promote the insertion of GLUT2 in the brush-border membrane as a side effect. However, those two hypotheses need further investigation.

In conclusion, we demonstrated that psychological stress alters glucose transporters in rats. The mechanisms involved, either at the neuroendocrine or at the intracellular levels, are yet to be elucidated. It is difficult with our results to draw a firm conclusion on glucose absorption capacity of these rats at the whole intestinal level. However, in terms of energy savings under stressful conditions, this increase in facilitated passive transport of both glucose and fructose and the decrease in the active transport should be beneficial to the animal.

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Present address of G. Boudry: Unité Mixte de Recherche Systèmes d’Elevage, Nutrition Animale et Humaine, Institut National de la Recherche Agronomique/Agrocampus, St-Gilles, France.

REFERENCES