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Lumenal adenosine and AMP rapidly increase glucose transport by intact small intestine

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Kimura, Yasuhiro, Jerrold R. Turner, Dwaine A. Braasch, and Randal K. Buddington. Lumenal adenosine and AMP rapidly increase glucose transport by intact small intestine. *Am J Physiol Gastrointest Liver Physiol* 289: G1007–G1014, 2005. First published July 14, 2005; doi:10.1152/ajpgi.00085.2005.—Adenosine modulates the intestinal functions of secretion, motility, and immunity, yet little is known about the regulation of nutrient absorption. Therefore, we measured the carrier-mediated uptake of tracer D-[^{14}C]glucose (2 μM) by everted sleeves of the mouse intestine after a lumenal exposure to adenosine and a disodium salt of AMP. Rates of glucose uptake by intact tissues increased almost twofold after a 7-min exposure to 5 mM adenosine (a physiological dose). The response was slightly more pronounced for AMP and could be induced by forskolin. The response to adenosine was blocked by theophylline and the A_2 receptor antagonist 3,7-dimethyl-1-proparglyxanthine but not by the A_1 receptor antagonist 8-phenyltheophylline. Glucose uptake by control and AMP-stimulated tissues was inhibited by phloridzin, implying that sodium-dependent glucose transporter 1 (SGLT1) is the responsive transporter, but the involvement of glucose transporter 2 (GLUT2) cannot be excluded. Of clinical relevance, AMP accelerated the systemic availability of 3-*O*-methylglucose after an oral administration to mice. Our results indicate that adenosine causes a rapid increase in carrier-mediated glucose uptake that is of clinical relevance and acts via receptors linked to a signaling pathway that involves intracellular cAMP production.

sodium-dependent glucose transporter 1; adaptation; regulation; nongenomic

ADAPTIVE REGULATION of nutrient transporters allows animals to modulate absorptive functions of the small intestine to match changes in the amounts and composition of dietary inputs that occur during the life of individuals (daily, seasonal, and during development). The ability to adaptively regulate nutrient absorption is particularly well developed in omnivores with natural diets that fluctuate in macronutrient composition (5). Exemplary are the regulated expression of the sodium-dependent glucose transporter (SGLT1) when mice are switched between diets high and low in carbohydrates (12) and the initial expression of the gene coding for the fructose transporter GLUT5 when rats are weaned (9).

Historically, transport physiologists interested in the adaptive regulation of nutrient uptake by the brush border membrane (BBM) of enterocytes have focused on how diet, development, and disease states trigger selective gene expression, leading to changes in the abundances of specific transporters. These genomic responses are known to occur over hours to days, during the life history of individuals, and during the evolution of species. Despite the importance of genomic mechanisms of adaptation, the responses are not fast enough for animals to modulate the absorptive functions of the BBM during the processing of individual meals. Moreover, the energetic costs associated with the synthesis of additional transporters for the processing of individual meals would be high.

It has been established that carrier-mediated glucose uptake can be modulated in <60 min, a time scale that is consistent with the processing of individual meals (7, 18, 25). The ability to rapidly regulate nutrient transporters provides an adaptive mechanism that allows absorptive functions to be matched to existing lumenal nutrient concentrations and thereby complements the slower genomic responses to diet composition. Moreover, based on the rapid and pronounced regulation of ion secretion (4, 43), the magnitude of rapid adaptation for the nutrient transporters could exceed what is possible by genomic responses alone. Determining the role of nongenomic regulation in adaptive modulation of transport functions is dependent on measuring changes in absorption that occur in <10 min to avoid the participation of genomic mechanisms. Unfortunately, most studies of rapid regulation have used longer periods of time (e.g., 30 and 60 min), and this may be sufficiently long for the production of new proteins.

A key component of a rapid response is the ability of enterocytes to recognize and respond to changes in lumenal nutrients and to host signaling molecules (38, 44, 51). Corresponding with this, carrier-mediated glucose uptake is higher 30 min after activation of a "glucose sensor" (11). Conversely, enterocyte absorptive functions are lower after an exposure to inflammatory cytokines (14, 29), although the responses are not universal (17). The regulatory influence of adenosine on the intestine is of basic interest and clinical importance. Adenosine is a conditionally essential nutrient for the intestine (34) but also functions as an ubiquitous host signaling molecule that regulates the intestinal functions of ion secretion, motility, and immunity (6, 13, 15, 23, 28, 39, 42, 47, 48). Of particular importance is the participation of adenosine in mediating inflammatory responses at sites of tissue injury.

Despite the multiple roles of adenosine in regulating intestinal health and functions, the influence of adenosine on intestinal absorptive functions is uncertain. Therefore, the role of adenosine as a lumenal signaling molecule that regulates carrier-mediated glucose transport by the small intestine was established using three complementary experimental ap-

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proaches. The first two involved measuring rates of glucose transport by the intact mouse small intestine after chronic (7 days) and acute (from 2 to 10 min) exposure to adenosine and a disodium salt of AMP that is stable at room temperature. The third evaluated the clinical relevance of responses to adenosine using mice to measure in vivo absorption of an oral dose of 3-*O*-methylglucose (3-OMG) in the presence and absence of AMP.

MATERIALS AND METHODS

Animals and Their Care

All aspects of the research using animals were approved by the Mississippi State University Institutional Animal Care and Use Committee and were performed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. Adult mice (6–20 wk of age) of both sexes and of the Swiss-Webster and BALB/c strains were obtained from a commercial supplier (Taconic; Germantown, NY) or were produced by a breeding colony maintained at Mississippi State University. The mice were housed as 5 mice/cage in a controlled environment (12:12-h light-dark cycle, 22 ± 2°C, and 50% relative humidity) and were allowed free access to tap water and a rodent chow (Lab Diet 5001, PMI Feed; St. Louis, MO).

Measurement of Intact Tissue Glucose Transport

The everted sleeve method (24) was used as a sensitive functional assay to detect changes in nutrient transport in response to adenosine and AMP (mol wt = 391.2). Glucose was selected as the model nutrient because it is possible to measure the carrier-mediated component of uptake, because there are selective inhibitors for the apical membrane glucose transporters SGLT1 and GLUT2, and because apical membrane glucose uptake can be rapidly modulated by altering the densities and proportions of SGLT1 and GLUT2 (26).

Mice were killed by CO_2 asphyxiation for harvest of the small intestine. In light of the declining proximal-to-distal gradient for glucose uptake, the majority of studies measured the responses of the proximal small intestine (beginning 5 cm distal to the pyloric sphincter). For studies searching for regional differences in responses, additional tissues were prepared from the distal small intestine (extending 5 cm proximal to the ileocolonic junction). From each region, four to eight sleeves of 1-cm length were secured by silk ligatures onto stainless steel rods that approximated sleeve diameter (1.5, 2, and 3 mm). The sleeves were kept in cold (2–4°C), aerated (95% O_2-5% CO_2) Ringer solution during and after the mounting preparation. Measurements of absorption began 45 min after collection of the small intestine by exposing the sleeves to 37°C mammalian Ringer solution with and without adenosine and AMP and using different conditions [length of exposure, concentrations of adenosine and AMP, presence or absence of inhibitors for SGLT1 (phloridzin) and GLUT2 (phloretin), and adenosine receptor antagonists]. After the exposure, tissues were suspended for 2 min in an uptake solution consisting of Ringer solution that was aerated and stirred (1,200 rpm) and contained tracer concentration (2 μM) of D-[^{14}C]glucose with and without 50 mM unlabeled D-glucose. The tracer L-[^{3}H]glucose was added to the uptake solution for correction of D-glucose in the extracellular fluid and was passively absorbed. After a 2-min incubation, tissues were rinsed for 20 s in cold Ringer solution, carefully blotted to remove adherent fluid, removed from the rods, and placed in tared vials. After the wet mass was recorded, the tissues were dissolved (Solvable, Perkin-Elmer; Boston, MA), scintillant solution was added (Ultima Gold, Perkin-Elmer), and the associated radioactivity was quantified by liquid scintillation counting. Rates of glucose uptake (in nmol or pmol/min) were calculated and normalized to wet tissue mass and, due to the use of L-glucose, represent the carrier-mediated component of absorption.

Chronic Exposure of Mice to AMP

Mice of the BALB/c and Swiss-Webster strains were individually housed and continued to be fed the rodent chow. Each day for 7 days, the mice were provided in the morning (0800–0900 h) a supplement consisting of canned sweet potato (vehicle, 500 mg/mouse) with and without 100 mg AMP (0.26 mmol). After the 7-day period of supplementation, rates of glucose transport by intact tissues from the proximal and distal small intestine were measured with 50 mM glucose in the incubation solution.

Acute Responses of Intact Mouse Tissues

Tissues from the proximal small intestine of BALB/c mice fed the commercial diet were used to study the accumulation of glucose at tracer concentration (2 μM) after exposure to 5 mM adenosine and AMP for varying periods (0–10 min). This was accomplished by exposing the tissues to 37°C aerated Ringer solution and switching to Ringers solution with adenosine or AMP such that the combined length of exposure was 10 min for all tissues. Preliminary studies showed that tissue uptake was stable up to 10 min of exposure, but, after 15 min, rates of uptake began to decline. Phloridzin and phloretin were added to the uptake solution (0.5 mM) to determine whether the response to AMP involved SGLT1 and GLUT2, respectively.

Subsequent studies measured glucose uptake after tissues were exposed for 7 min to different concentrations of adenosine and AMP (0–50 mM). Osmolarity of the solutions was maintained constant by isosmotic replacement of the NaCl fraction in the Ringer solution.

Responses to Adenosine Receptor Antagonists

The possible involvement of an adenosine receptor in mediating the responses was examined using intact tissues from the proximal small intestine that were exposed for 7 min to Ringer solution with adenosine (5 mM) in the presence or absence of theophylline (100 μM), which is a nonselective antagonist of adenosine receptors, before the uptake of tracer D-glucose (2 μM) was measured. Subsequent experiments sought to identify the role of specific adenosine receptors in regulating the uptake of tracer D-glucose by exposing tissues to Ringer solution with adenosine (5 mM) in the presence and absence of antagonists for the A_1 receptor (8-phenyltheophylline; 0.1 μM) and A_2 receptors [3,7-dimethyl-1-propargylxanthine (DMPX); 0.1 μM].

Activation of adenosine receptors expressed on plasma membranes can alter adenylyl cyclase (AC) activity and, hence, intracellular levels of cAMP. Therefore, to determine whether the responses to adenosine were mediated by a signaling pathway that increases intracellular cAMP, rates of glucose uptake were compared among tissues from the proximal small intestine that were exposed for 7 min to Ringer solution with and without 5 mM adenosine and in the presence and absence of forskolin (10 μM), which was used to increase cellular cAMP levels.

Absorption of Orally Administered 3-OMG

To understand whether the responses of intact tissues are of clinical relevance, young adult female BALB/c mice were orally dosed with 3-OMG, a nonmetabolizable glucose analog, which is a substrate for SGLT1 and other glucose transporters. Before the 3-OMG tolerance test, mice were trained over a 3-day period to rapidly consume (in 2–3 min) a vehicle (small, moistened balls prepared with 0.2 g wheat flour, 0.1 g powdered cheese, and 0.1 g water). It was decided to use a vehicle for the 3-OMG challenge instead of oral gavage to avoid stressing the mice, which may cause the release of glucocorticoids and thereby decrease the magnitude of nongenomic responses (18, 37). On the day of the study, 3-OMG (100 μmol of both unlabeled and tracer ^{3}H labeled) was added to the dough balls with and without 26 μmol (10 mg) AMP. After consuming the vehicle with 3-OMG, mice were killed at 10, 15, 30, and 60 min (n = 9–10 for each time and treatment) by CO_2 asphyxiation, and blood samples were immediately

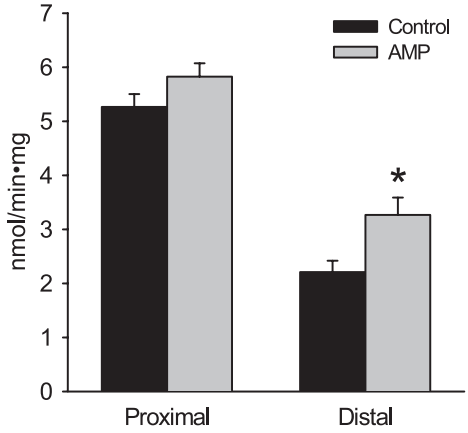

Fig. 1. Rates of glucose transport (*y*-axis) measured at 50 mM by intact tissues from the proximal and distal small intestine of mice fed 100 mg AMP/day for 1 wk. Bars are means ± SE; n = 17–19 mice/group. A significant difference was detected between the mice fed the vehicle with and without AMP (*$P < 0.05$).

withdrawn from the inferior vena cava. The liver was collected, the small intestine was removed (from the pyloric sphincter to the ileocolonic junction), and the contents were flushed out using cold Ringer solution. The samples of whole blood, the entire small intestine, and a portion of the liver (0.5–1 g) were oxidized (model 307, Packard Bioscience; Meriden, CT). The 3-[^{3}H]OMG was recovered in Permafluor E (Perkin-Elmer), and radioactivity was quantified by liquid scintillation counting. Samples of the dough ball were analyzed to determine radioactivity per nanomole of 3-OMG, and this was used to quantify 3-OMG in the samples of blood (nmol/ml) and tissue (nmol/g).

Chemicals

Perkin-Elmer supplied the D-[^{14}C(U)]-glucose and L-[1-^{3}H(N)]-glucose, and the 3-*O*-([^{3}H]methyl)-D-glucose was purchased from Sigma Chemical (St. Louis, MO). The stable form of AMP was generously provided by Otsuka Pharmaceutical. Adenosine and all other chemicals were purchased from Acros Organics (Fairlawn, NJ) and Sigma Chemical and were of the highest purity available.

Statistical Analysis

Rates of nutrient uptake are reported as means ± SE. Data for the chronic exposure study were analyzed by two-way ANOVA to search for the main effects of treatment, strain, gender, and region, with specific differences identified using an unpaired Student's *t*-test. Data for the uptake responses of tissues from BALB/c mice after acute exposures were analyzed by one-way ANOVA to search for differences among incubation times, concentrations of adenosine and AMP, and the treatments with adenosine in the presence and absence of pharmacological agents (phloridzin, phloretin, theophylline, 8-phenyltheophylline, DMPX, and forskolin). When a significant main effect was detected, specific differences were identified by Duncan's test. An unpaired Student's *t*-test was used to determine whether the vehicle with AMP increased in vivo accumulation of 3-OMG in the blood, small intestine, and liver after oral administration. For all analyses, a value of $P < 0.05$ was considered as significant. All statistical analyses were performed using the Statistical Analysis System (version 8.2, SAS Institute; Cary, NC).

RESULTS

Glucose Uptake by Intact Tissues

Chronic exposure to AMP. Strain and gender effects were not detected for the response to chronic dietary exposure to AMP. Therefore, values for both sexes of Swiss-Webster and BALB/c strains of mice were pooled for statistical analysis.

The daily supplement of 256 μmol AMP for 1 wk did not result in differences in body mass (control: 34.9 ± 1.8 g; AMP: 34.9 ± 1.4 g) and intestinal length (control: 47.1 ± 1.0 cm; AMP: 46.9 ± 1.4 cm) and mass (control: 2.3 ± 0.1 g; AMP: 2.3 ± 0.1 g). Rates of glucose uptake measured at 50 mM declined from the proximal to distal small intestine for control mice and those receiving the AMP supplement, with rates of uptake in the proximal small intestine higher compared with the distal region (Fig. 1). However, mice receiving the AMP supplement had higher rates of glucose uptake in the distal small intestine compared with control mice ($P < 0.05$), with a nonsignificant treatment difference for the proximal intestine ($P = 0.11$).

Acute exposure to adenosine and AMP. Exposure of the proximal small intestine to 5 mM AMP for 10 min increased ($P < 0.05$) rates of glucose uptake measured at 50 mM by 30% (Fig. 2) but did not stimulate increased uptake by tissues from the distal small intestine ($P = 0.35$). Exposure of the proximal small intestine to 5 mM AMP increased the uptake of tracer D-glucose (2 μM) by 1.7-fold ($P < 0.05$). The enhanced response when measured at a tracer concentration of D-glucose is explained by the lack of competition between the tracer and the 50 mM unlabeled D-glucose for access to the transporters. As a consequence, accumulation of tracer alone provides a more sensitive indicator of changes in transporter site density and was used for further evaluations of responses. Replacing AMP with adenosine caused a similar increase in tracer glucose uptake.

Rates of tracer glucose uptake by control tissues from the proximal small intestine were reduced by 26% and 92% when phloretin and phloridzin were included in the uptake solution (Fig. 3). After the exposure to AMP, phloretin and phloridzin decreased glucose uptake by 45% and 97%, respectively.

The maximum stimulation of tracer glucose uptake by the proximal small intestine was measured after 7 min of exposure to 5 mM AMP (Fig. 4). Glucose uptake was significantly higher than control tissues after 4 min of exposure ($P < 0.05$),

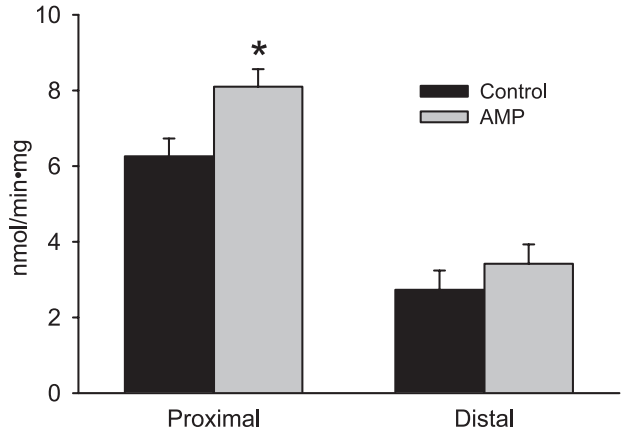

Fig. 2. Rates of glucose transport (*y*-axis) by intact tissues from the proximal and distal small intestine that were exposed to a preincubation solution with 5 mM AMP disodium for 10 min before glucose transport was measured at 50 mM. Bars are means ± SE; n = 7 mice/group. A significant difference was detected between tissues preincubated in Ringer solution with and without AMP (*$P < 0.05$).

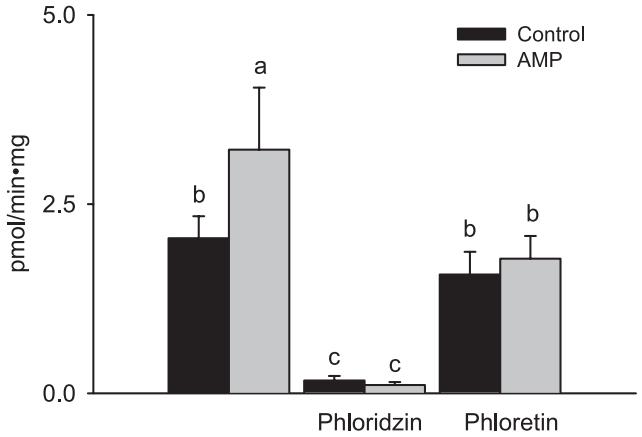

Fig. 3. Rates of tracer glucose transport (*y*-axis) by intact tissues from the proximal small intestine that were exposed to a preincubation solution with and without (control) 5 mM AMP disodium for 10 min before glucose transport was measured at 2 μM in the presence and absence of phloridzin and phloretin to inhibit sodium-dependent glucose transporter (SGLT)1 and glucose transporter (GLUT)2, respectively. Bars are means ± SE; $n = 4–8$ mice/group. Bars with different letters are significantly different ($^{a,b,c}P < 0.05$).

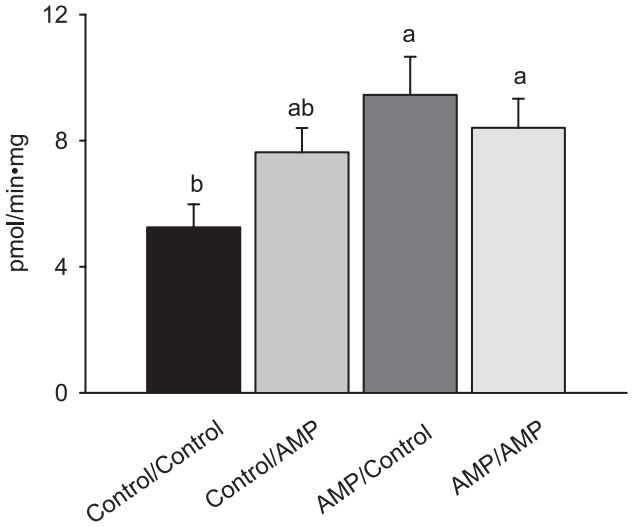

Fig. 5. Rates of glucose transport (*y*-axis) measured at 2 μM by intact tissues from the proximal small intestine that were exposed for 7 min to different combinations of preincubation and incubation solutions (with or without 5 mM AMP disodium). Control/control, preincubated and incubated in the absence of AMP (unstimulated control); control/AMP, preincubated without and incubated with AMP; AMP/control, preincubated with but incubated without AMP; AMP/AMP, preincubated and incubated with AMP. Bars are means ± SE; $n = 10–12$ mice/group. Bars with different letters are significantly different ($^{a,b}P < 0.05$).

with an intermediate, but insignificant ($P = 0.065$), increase after just 2 min. In light of these findings, further experiments used 7-min exposure periods.

Because of the rapid increase, it is possible that the absence of AMP in the uptake solution might result in a partial recovery (decrease) in glucose uptake. Therefore, rates of glucose uptake were compared among tissues that were exposed to *1*) Ringer solution without AMP for 7 min before a 2-min incubation to measure glucose uptake in the absence of AMP (control/control), *2*) Ringer solution without AMP but incubated in the presence of 5 mM AMP (control/AMP), *3*) Ringer solution with 5 mM AMP but incubated in the absence of AMP (AMP/control), and *4*) Ringer solution and incubation solutions with 5 mM AMP (AMP/AMP). The comparison of control/control and AMP/control revealed that the 7-min exposure to AMP increased glucose uptake almost twofold (Fig. 5). This is comparable to our previous comparisons of control and AMP-exposed tissues. Including AMP in both the exposure and incubation solutions (AMP/AMP) did not induce a further increase of glucose uptake. This suggests that the 2-min uptake period without AMP (AMP/control) was not long enough for uptake to return to the basal state. Importantly, restricting the period of AMP exposure just to the 2-min incubation period (control/AMP) resulted in a 45% increase in glucose uptake compared with the control/control treatment ($P = 0.058$). This indicates that upregulation is initiated, but not completed, with only 2 min of exposure to AMP. Therefore, the upregulation of glucose transporters in intact tissues is faster than the downregulation.

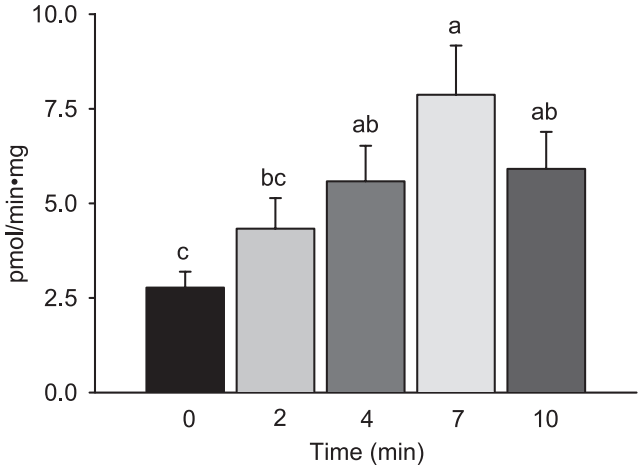

Fig. 4. Rates of glucose transport (*y*-axis) measured at 2 μM by intact tissues from the proximal small intestine that were preincubated for different times in 5 mM AMP disodium. Bars are means ± SE; $n = 8$ mice/group. Bars with different letters are significantly different ($^{a,b,c}P < 0.05$).

A dose-response relationship was detected up to a concentration of 1 mM AMP. Higher concentrations of AMP (5–50 mM) did not elicit further increases in tracer glucose uptake, nor did the higher concentrations cause glucose uptake to decline (Fig. 6).

Influence of adenosine receptor antagonists. Including theophylline (100 μM) in the exposure solution attenuated the increase of glucose transport in response to the presence of 5 mM adenosine (Fig. 7). Theophylline alone did not affect glucose uptake as measured rates did not differ between tissues incubated in adenosine-free Ringer solution with and without theophylline (data not shown).

The increase in glucose uptake after the exposure to adenosine was not prevented when the A_1 receptor antagonist 8-phenyltheophylline (0.1 μM) was included with adenosine (Fig. 8). In contrast, including the A_2 receptor antagonist DMPX (0.1 μM) abolished the response to adenosine. Exposure to the receptor antagonists alone did not trigger a change in glucose uptake (data not shown)

Stimulation of intracellular cAMP production by adding forskolin to the Ringer solution resulted in rates of glucose uptake that were comparable to when tissues were exposed to adenosine and AMP (Fig. 9). Exposing tissues to a combina-

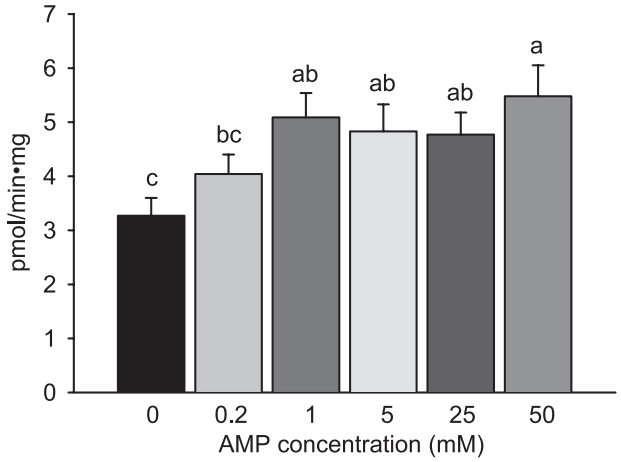

Fig. 6. Rates of glucose transport (*y*-axis) measured at 2 μM after intact tissues from the proximal small intestine were preincubated in various concentrations of AMP disodium for 7 min. Bars are means ± SE; $n = 19$ mice/group. Bars with different letters are significantly different ($^{a,b,c}P < 0.05$).

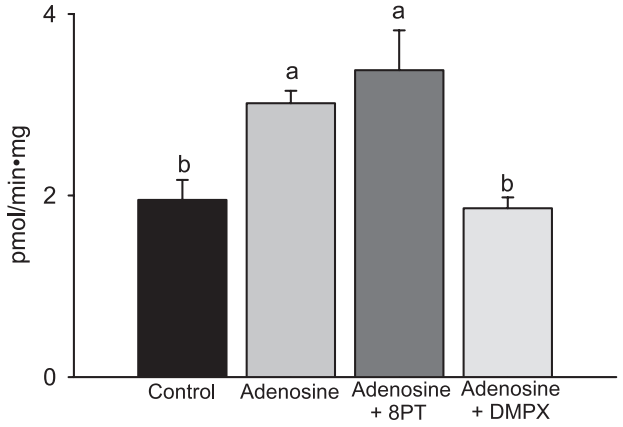

Fig. 8. Rates of glucose transport (*y*-axis) measured at 2 μM by intact tissues from the proximal small intestine after preincubation for 7 min in Ringer solution with or without 5 mM adenosine in the presence and absence of the A_1 receptor antagonist 8-phenyltheophylline (8PT; 0.1 μM) or the A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX; 0.1 μM). Bars are means ± SE; $n = 4$ mice/group. Bars with different letters are significantly different ($^{a,b}P < 0.05$).

tion of forskolin and adenosine did not cause a further increase in glucose uptake.

Absorption of Oral Glucose With and Without AMP

The concentration of 3-OMG in the blood of mice fed 3-OMG alone increased to a maximum at 30 min with similar concentrations measured at 60 min (Fig. 10). Mice that consumed the vehicle with the combination of 3-OMG and AMP had higher concentrations of 3-OMG in whole blood collected at 10 min compared with mice receiving the vehicle with 3-OMG alone, with the difference significant after 15 min. Differences were not detected at later times. There was a corresponding higher concentration of 3-OMG after 15 min in the liver of mice fed the vehicle with AMP ($P = 0.06$) but not at other times (data not shown). Similarly, accumulation of 3-OMG in the entire small intestine tended to be higher at 15 min ($P = 0.09$) for mice that consumed the vehicle with AMP.

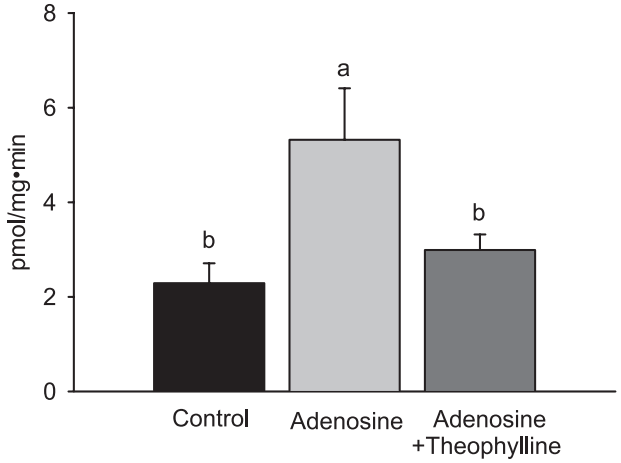

Fig. 7. Rates of glucose transport (*y*-axis) measured at 2 μM by intact tissues from the proximal small intestine after a 7-min preincubation in Ringer solution with 5 mM adenosine in the presence and absence of 100 μM theophylline. Bars are means ± SE; $n = 10$ mice/group. Bars with different letters are significantly different ($^{a,b}P < 0.05$).

DISCUSSION

There is scientific and clinical interest in the identity of the signals that trigger intestinal adaptation. Adenosine is not unique in modulating glucose uptake, as a diversity of host-derived regulatory signals are known to rapidly alter intestinal absorption of nutrients and the secretion of ions, and several of these have been considered for therapeutic purposes. Notable examples include glucagon-like peptide 2 (1), epidermal growth factor (7), insulin (41), glucagon (10), vasoactive intestinal peptide (32), and prostaglandin E_2 (36). Additional signaling molecules that influence nutrient uptake include epinephrine and adrenergic agonists (21) and polyamines (45). Obviously, the regulation of enterocyte functions is complex and in intact organisms involves the integration of multiple

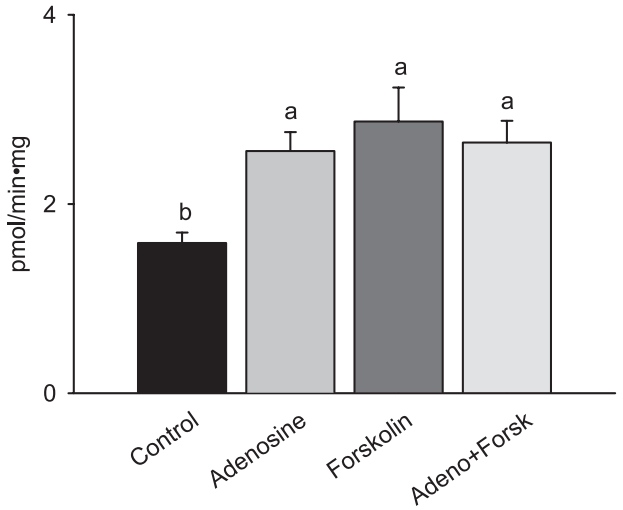

Fig. 9. Rates of glucose transport (*y*-axis) measured at 2 μM by intact tissues from the proximal small intestine after preincubation for 7 min in normal Ringer solution (control) and Ringer solution with 5 mM adenosine, 10 μM forskolin, and a combination of adenosine and forskolin (Adeno+Forsk). Bars are means ± SE; $n = 9$–10 mice/group. Bars with different letters are significantly different ($^{a,b}P < 0.05$).

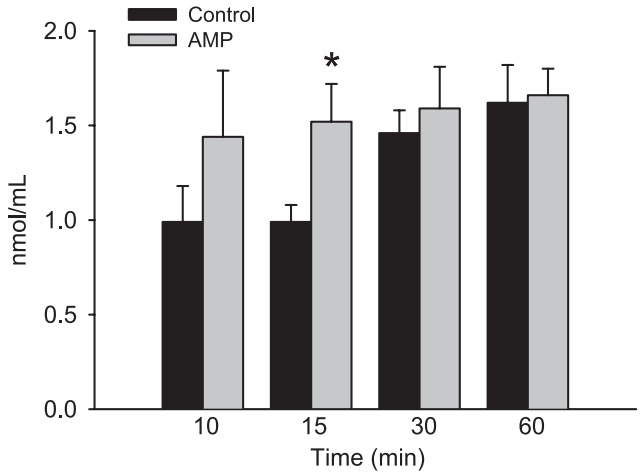

Fig. 10. Concentrations of 3-*O*-methylglucose (3-OMG; *y*-axis) in whole blood of mice fed a vehicle with 100 μmol 3-OMG and with or without 26 μmol AMP disodium. Bars are means ± SE; $n = 9–10$mice/group. A signficant difference between mice fed the vehicle with and without AMP was found ($*P < 0.05$).

regulatory molecules and signal transduction pathways. Unlike many of the other regulatory molecules that modulate nutrient absorption, adenosine is a naturally occurring component of the diet, and this might accelerate the development of therapeutic applications for treatment of malabsorption.

The rapid increase in glucose uptake after the exposure to adenosine and the more rapid accumulation of 3-OMG in small intestine tissue, blood, and the liver of mice that received AMP is also pertinent to the controversy surrounding the higher absorptive capacities measured in vivo compared with those calculated from in vitro rates (30). The present results, and the reports cited above, indicate that dietary and host signaling molecules elicit higher rates of uptake during the processing of meals. However, in vitro studies historically have used unstimulated tissues, which would be transporting glucose at basal, not maximum, rates. In contrast, the infusion of high glucose concentrations into the intestine for in vivo measurements would trigger a nongenomic increase in uptake, resulting in higher transport capacities.

Adenosine, Adenosine Receptors, and Signaling Pathways

Adenosine is somewhat unusual among the regulatory signals that are known to modulate enterocyte functions in that it can originate from two sources: the diet and endogenous production. Although data are not available for dietary intake of adenosine, an estimate can be provided based on information from several sources. Intracellular concentrations of ATP range from 2–10 mM, with lower amounts of ADP and AMP (33). Additional sources of dietary adenosine include cellular DNA, RNA, and NAD, which are hydrolyzed lumenally by pancreatic enzymes, with adenosine released at the BBM by 5′-ectonucleotidase activity (16). Another consideration is that the daily intake of purines by United States adults is 600–1,000 mg/day (35). Because the amount of adenine in a cell is greatly exceeded by adenosine, adenine intake underestimates the daily intake of adenosine. Collectively, these findings suggest 5 mM is a conservative estimate for the concentration of adenosine that would be present in the lumen of the intestine of individuals eating the Western human diet. The second source is endogenous production of adenosine and associated nucleotides (AMP, ADP, and ATP), which are secreted to regulate a diversity of intestinal characteristics and functions. This includes secretion at sites of tissue injury to modulate inflammatory responses (pro- or anti-inflammatory) and the secretion by lumenal neutrophils in response to pathogens (39). Host production of adenosine can be substantial, particularly when secreted adenine nucleotides are included (8). Collectively, these findings indicate that a lumenal concentration of 1 mM adenosine, which elicited the maximum increase in glucose uptake by intact tissues, is physiologically relevant.

The intestinal mucosa expresses four types of adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3, yet little is known about enterocyte expression of adenosine receptors and the potential roles in regulating nutrient absorption and probably other enterocyte functions. The ability to block the response of the everted sleeves to adenosine with theophylline indicates that the BBM of the small intestine includes receptors for adenosine. The subsequent use of two receptor-specific antagonists suggests taht one or more of the adenosine A_2 receptors, but not the A_1 receptor, is responsible for the responses of the BBM of the mouse small intestine, but we do not yet know the identity of the specific type of A_2 receptor.

Adenosine receptors are coupled to G protein-mediated signaling pathways (13), with the specific coupling of G proteins and signaling pathways varying among the four receptor types and among tissues. A_1 and A_3 receptors are usually coupled with G_i proteins that inhibit AC, whereas the A_{2A} and A_{2B} receptors are coupled with G_s proteins that stimulate AC. Activating AC using forskolin caused an 80% increase in glucose uptake by intact tissues (present study), which is consistent with forskolin-induced increases in the densities of SGLT1 transporters in the BBM (49) and higher rates of carrier-mediated uptake of glucose, bile acids, and amino acids (31). The response to forskolin in conjunction with the lack of a response to adenosine in the presence of an antagonist of A_2 receptors implicates the involvement of at least one of the A_2 receptors.

Although the downstream signaling events after cAMP were not determined, the roles of PKA and PKC in regulating glucose uptake have been established using oocytes (50), COS-7 cells (46), perfused segments of the intestine (9, 26), and isolated intact tissues (2). However, the responses to PKA and PKC are not consistent among nutrient transporters and cell types. For example, activating PKA increases glucose uptake by small intestine tissue (2) and oocytes expressing SGLT1 (50), whereas activation of PKC causes a decrease. In contrast, activation of PKC elicits an increase in glucose absorption via GLUT2 (26). The contrasting responses indicate that regulation of nutrient transport systems by adenosine is complex and involves multiple signaling pathways.

Mechanisms for the Increase in Glucose Transport

The majority of studies reporting rapid responses for SGLT1 and other nutrient transporters have used exposure periods of 30–60 min. This is sufficiently long to allow for at least partial genomic responses. The higher rates of glucose transport measured in <5 min after an exposure to adenosine is too rapid to be explained by genomic mechanisms, which would require

the synthesis and insertion of functional transporters into the BBM. Hence, nongenomic mechanisms must play a central role in the rapid adaptation of glucose uptake.

In unstimulated intestinal tissue, glucose uptake is by a "basal" density of SGLT1 that is maintained in the BBM (18), with the majority of BBM glucose transporters (SGLT1 and GLUT2) associated with an intracellular fraction. The rapid increase in tracer glucose uptake is consistent with the trafficking of glucose transporters from an intracellular site to the BBM (27).

Moreover, the virtual elimination of BBM tracer glucose uptake when control and adenosine-stimulated tissues were exposed to phloridzin suggests that SGLT1 is responsible for the majority of basal glucose uptake and the increase in response to adenosine. Corresponding with this, exposing the intact rat intestine to polyamines (45) induces rapid and simultaneous increases in glucose uptake (apparent by 5 min) and the abundance of SGLT1 in the BBM (measured at 15 min). It is possible there was a simultaneous upregulation of GLUT2 in conjunction with SGLT1, as previously reported (18, 26). Because GLUT2 is a lower-affinity transporter, it is difficult to detect changes in rates of uptake based on the accumulation of tracer concentrations of labeled glucose via GLUT2. Still, including phloretin in the incubation solution decreased the tissue accumulation of tracer D-glucose by control tissues and those exposed to AMP.

An alternative mechanism is that transporter site density in the BBM may not change in response to adenosine. Instead, the activity of transporters in the BBM could be regulated. Although there is less direct evidence for the activation/deactivation of BBM transporters, the "in situ" regulation of transporters in the BBM by phosphorylation remains a distinct possibility (21). SGLT1 includes several potential sites for phosphorylation by PKA and PKC (20), and there is evidence SGLT1 activity may be regulated by interactions with other intracellular proteins (e.g., heat shock proteins) (19).

A New Paradigm of Transporter Regulation

Nongenomic regulation has not been adequately recognized as a mechanism for the adaption of nutrient transport. Specifically, the 70% or more increase in carrier-mediated glucose uptake by the intact mouse small intestine after a 7-min exposure to adenosine or AMP is comparable in magnitude to the slower genomic response that occurs over several days when mice are switched between diets with 0% and 55% sugar (24). Hence, adaptative regulation of nutrient transport includes nongenomic responses that rapidly (<5 min) modulate the densities or activities of transporters in the BBM, whereas genomic responses establish the total cellular abundance of transporters that are available for rapid regulation.

Perspectives

Of particular importance is learning how adenosine can be prosecretory and proabsorptive. This will require a better understanding of the types, proportions, and distributions of adenosine receptors expressed by enterocytes and the associated signaling pathways. These efforts will be complicated by the differential targeting of the receptor types to the apical and basolateral membrane domains of polarized cells (3, 22, 40, 48), the trafficking of receptors to the apical membrane after exposure to adenosine (48), and how the proportions of the different receptors in the membrane domains are responsive to health status (28, 42).

There is also a need to understand whether the response to adenosine is unique to SGLT1 or if it is shared by transporters for other nutrients, such as amino acids and fatty acids. Learning which transporters are responsive to adenosine, and other signaling molecules, will assist in identifying nutrients that are most likely to become limiting during disease states as well as the development of therapeutic strategies to reduce the incidence and severity of malabsorption.

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