Acute increase, stimulated by prostaglandin E$_2$, in glucose absorption via the sodium dependent glucose transporter-1 in rat intestine

B Scholtka, F Stümpel, K Jungermann

Abstract

Background/Aims—Acute stimulation by cAMP of the sodium dependent glucose cotransporter SGLT1 has previously been shown. As prostaglandin E$_2$ (PGE$_2$) increases intracellular cAMP concentrations via its receptor subtypes EP2R and EP4R, it was investigated whether PGE$_2$ could enhance intestinal glucose absorption.

Methods—The action of PGE$_2$ on carbohydrate absorption in the ex situ perfused rat small intestine and on 3-0-[$^14$C]methylglucose uptake in isolated villus tip enterocytes was determined. Expression of mRNA for the PGE$_2$ receptor subtypes 1–4 was assayed in enterocytes by reverse transcriptase polymerase chain reaction (RT-PCR).

Results—In the perfused small intestine, PGE$_2$ acutely increased absorption of glucose and galactose, but not fructose (which is not a substrate for SGLT1); in isolated enterocytes it stimulated 3-0-[$^14$C]methylglucose uptake. The 3-0-[$^14$C]methylglucose uptake could be inhibited by the cAMP antagonist Rp-cAMPS and the specific inhibitor of SGLT1, phlorizin. High levels of EP2R mRNA and EP4R mRNA were detected in villus tip enterocytes.

Conclusion—PGE$_2$ acutely increased glucose and galactose absorption by the small intestine via the SGLT1, with cAMP serving as the second messenger. PGE$_2$ acted directly on the enterocytes, as the stimulation was still observed in isolated enterocytes and RT-PCR detected mRNA for the cAMP-increasing PGE$_2$ receptors EP2R and EP4R.

Keywords: glucose absorption; sodium dependent glucose transporter (SGLT1); prostaglandin; intestine; rat

Carbohydrate absorption in small intestine involves a number of distinct transporters. On the luminal (apical) side, glucose and galactose are transported into the enterocytes via the sodium dependent glucose cotransporter-1 (SGLT1)$^1$ and fructose via the glucose transporter-5 (GLUT5).$^2$ On the serosal (basolateral) side, all three carbohydrates are released into the circulation via GLUT2.$^2$ Acute stimulation by the intracellular messenger cAMP of intestinal glucose absorption via the SGLT1 has been shown. cAMP enhanced sugar accumulation in chicken epithelial cells$^7$ and augmented glucose uptake into brush border membrane vesicles prepared from rat enterocytes.$^9$ Pancreatic glucagon, a hormone that elevates cAMP levels, acutely increased galactose uptake into rat enterocytes.$^1$ In addition, in the isolated perfused small intestine and in suspensions of mature enterocytes of the rat, enteroglucagon-37 (oxyntomodulin) cAMP-dependently increased glucose absorption via the SGLT1.$^8$

Of the five naturally occurring prostanoids, prostaglandin (PG) D$_2$, E$_2$, F$_2$ and thromboxane A$_2$, only PGE$_2$, PGD$_2$ and PGF$_2$ are known to increase intracellular cAMP concentrations.$^1$ For PGE$_2$, four receptor subtypes are known, named EP1R, EP2R, EP3R, and EP4R. EP1R is coupled to a phospholipase C stimulating G protein and increases intracellular calcium concentrations, EP3R is coupled to an inhibitory G protein and decreases intracellular cAMP concentrations, and EP2R and EP4R are linked to a stimulatory G protein and thus increase intracellular cAMP concentrations.$^7$ The synthesis of PGE$_2$ has been shown in epithelial and subepithelial layers of the rat intestine.$^7$ In mice and rats mRNA for the PGE$_2$ receptor subtypes EP2R and EP4R has been detected in the intestinal mucosal layer.$^{10}$ PGE$_2$ was previously shown to stimulate adenylate cyclase in rat intestinal epithelial cells via a receptor mediated mechanism.$^{11}$ and to enhance bicarbonate secretion in guinea pig intestine.$^{12}$ The mRNA expression of G$_i$ linked EP2R and EP4R,$^{10}$ the observed activation of adenylate cyclase by PGE$_{12}$ and the reported stimulation of 3-O-methylglucose uptake by dbcAMP in enterocytes suggested that PGE$_2$ should enhance intestinal glucose absorption. The possible regulation of glucose absorption by PGE$_2$ has so far not been studied in the isolated perfused small intestine, only in isolated mice enterocytes, where, surprisingly, PGE$_2$ had no effect on α-methylglucose uptake.$^{13}$

Thus it was the aim of this study to investigate the possible stimulation by PGE$_2$ via cAMP of intestinal carbohydrate absorption. It

Abbreviations used in this paper: dbcAMP, dibutyryl adenosine 3',5'-cyclic monophosphate; EP4R, prostaglandin E$_2$ (subtype 4) receptor; GLUT3, glucose transporter-5; PG, prostaglandin; Rp-cAMPS, R-stereoisomer of 3',5'-cyclic adenosine monophosphothioate; RT-PCR, reverse transcriptase polymerase chain reaction; SGLT1, sodium dependent glucose cotransporter-1.
could be shown that PGE₂ acutely enhanced intestinal glucose absorption via the SGLT1 in perfused small bowel and enterocytes isolated from the rat. In addition, mRNA for the Gₛ-linked PGE₂ receptor subtypes EP₂R and EP₄R was detected in the villus tip enterocytes.

**Materials and methods**

**MATERIALS**

All chemicals were of reagent grade and from commercial sources. Enzymes were purchased from Boehringer (Mannheim, Germany), PGE₂, dextran, and bovine serum albumin from Appli Chem (Darmstadt, Germany), dibutylryl adenosine 3',5'-cyclic monophosphate (dbcAMP) from Sigma (Deisenhofen, Germany), and the R-stereoisomer of adenosine 3',5'-cyclic mononphosphothioate (R⁻dbcAMPs) from Calbiochem-Novabiochem GmbH (Bad Soden, Germany).

**ANIMALS**

Male Wistar rats were supplied by Harlan-Winkelmann (Borchen, Germany). They were kept on a 12 hour day/night rhythm with free access to water and food (standard diet of Ssniff, Soest, Germany). Preparation of the organ perfusion and isolation of enterocytes were started at 9 am. Treatment of the animals was in accordance with the German Law on Protection of Animals.

**PERFUSION OF ISOLATED RAT INTESTINE**

The preparation was performed as described previously. In brief, rats (300–350 g) were anaesthetised by intraperitoneal injection of pentobarbital (60 mg/kg) followed by a midline laparotomy. Then a vascular non-recirculating perfusion of the intestine was started by cannulating the superior mesenteric artery and the coeliac trunk with the outflow in the portal vein. Intestinal contents were washed out with a warmed saline solution through a catheter placed into the caecum and transferred to an organ bath filled with warmed saline solution. Experiments were started at the given time points. Metabolites in the vascular inflow and outflow were measured using standard enzymic techniques. Luminal flow and thus transit were due to physiological intestinal peristalsis. The time interval from application of the carbohydrate bolus to appearance of the carbohydrate in the small intestinal effluent was taken as the transit time. As the luminal outflow occurred through a catheter placed into the caecum, luminal substrates could be absorbed only from the small intestine. Therefore the isolated perfused intestinal preparation used corresponded functionally to perfused small intestine.

**ISOLATION OF VILLUS TIP ENTEROCYTES**

A low temperature method was used to isolate villus tip enterocytes as SGLT1 is expressed mainly in mature enterocytes located on the villus tips. In brief, everted small pieces of the proximal one third of the small intestine were first stirred on ice for 5 minutes in oxygenated Hanks balanced salt solution containing 0.5 mmol/l dithiothreitol, and then subjected to a mechanical wash out in oxygenated calcium chelate buffer (27 mmol/l trisodium citrate, 5 mmol/l Na₃HPO₄, 96 mmol/l NaCl, 8 mmol/l KH₂PO₄, 1.5 mmol/l KCl, 20 mmol/l ß-sorbitol, 20 mmol/l sucrose, 2 mmol/l glutamine, 1.5 mmol/l dithiothreitol, 1 mg/ml hyaluronidase) for another 20 minutes. The separated enterocytes in the supernatant were collected by centrifugation (1000 g for six minutes at 4°C) and washed twice in incubation medium (80 mmol/l NaCl, 100 mmol/l mannitol, 20 mmol/l Tris, 3 mmol/l K₂HPO₄, 1 mmol/l MgCl₂, 2 mmol/l glucose, and 1 mg/ml bovine serum albumin) as described by Kimmich. Viability, assessed by the trypan blue exclusion method, was greater than 90%. Cells were suspended in incubation medium to a final concentration of 2 × 10⁶/ml. Light microscopy and alkaline phosphatase activity were used to verify the purity of the villus tip enterocyte fraction, as alkaline phosphatase activity is highest in the upper villus zone and almost absent in the crypts.

**CARBOHYDRATE UPTAKE EXPERIMENTS WITH ISOLATED ENTEROCYTES**

A rapid filtration technique was used to determine glucose uptake into isolated enterocytes. A 200 µl sample of the stock suspension of cells was added to several glass flasks each containing 100 µl incubation medium, and incubated in a thermostatically controlled water bath (37°C). After an incubation period of 10 minutes with or without effector substances, the experiments were started by adding 10 µCi 3-O-[¹⁴C]methylglucose (specific radioactivity 320 mCi/mmol; NEN, Bad Homburg, Germany) diluted in 100 µl incubation medium to a final glucose concentration of 50 µM. The experiments were stopped at the given time points by rapid transfer to a Whatman filter...

<table>
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<th>Oligonucleotide sequence (5'→3')</th>
<th>Receptor and primer position (GenBank accession no)</th>
<th>Temperature profile</th>
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<td>1 min 95°C, 1 min 55°C, 2 min 72°C</td>
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r, rat; m, mouse.

(Whatman, Maidstone, Kent, UK; pore size 0.45 µm). Adhering radioactivity was washed from the retained cell pellet with 10 ml ice cold incubation medium. Dried filters were directly transferred to scintillation fluid (LSC Hydrolymax; Baker, Deventer, The Netherlands) and counted for radioactivity.

**Results**

**ACUTE INCREASE IN GLUCOSE ABSORPTION STIMULATED BY PGE2 IN ISOLATED PERFUSED INTESTINE**

The possible stimulatory effect of PGE2 on intestinal carbohydrate absorption was first examined with isolated perfused rat intestine. Basal intestinal glucose absorption from the small bowel without addition of any effector was measured after application of a first glucose bolus into the intestinal lumen. The glucose concentration in the portal vein as a result of intestinal glucose absorption increased from 4.9 (0.1) mmol/l to a peak value of 6.1 (0.1) mmol/l (fig 1). The total amount of glucose absorbed was 388 (48) µmol, as indicated by the corresponding area under the portal glucose concentration curve v time (11.8 (2.9) µmol/ml/min) multiplied by the portal flow (33 ml/min) (fig 1). On infusion of PGE2 (1 µM) into the superior mesenteric artery, the portal glucose concentration after the second glucose bolus was raised to a peak value of 10.2 (0.6) mmol/l. Thus PGE2 stimulated intestinal glucose absorption by 3.2-fold to 1246 (184) µmol (area under the curve = 71.8 (10.6) µmol/ml/min × flow = 33 ml/min). When, as a control, the second glucose bolus was given without arterial infusion of PGE2, the maximum increase in portal glucose concentration as well as the total amount of glucose absorbed were not different from glucose absorption after the first glucose bolus (fig 1). Lactate release by the small intestine during the entire experiment was at a low constant level and was not altered by the infusion of PGE2 (data not shown). The vascular flow of the perfusion system remained essentially constant during the whole experimental period—that is, it was not modified by the infusion of PGE2 (fig 1). The intestinal transit time did not differ significantly between the first glucose bolus without and the second glucose bolus with arterial PGE2 infusion (fig 1).

**SGLT1 AS TARGET OF THE STIMULATORY EFFECT OF PGE2 ON GLUCOSE ABSORPTION**

Glucose and galactose are absorbed by SGLT1, and fructose by GLUT5.12 Glucose and galactose are absorbed by SGLT1 (as a competitive inhibitor of SGLT120 (see below). These results, obtained with the two experimental systems, indicate that PGE2 stimulated intestinal glucose absorption by 3.2-fold to 1246 (184) µmol (area under the curve = 71.8 (10.6) µmol/ml/min × flow = 33 ml/min). When, as a control, the second glucose bolus was given without arterial infusion of PGE2, the maximum increase in portal glucose concentration as well as the total amount of glucose absorbed were not different from glucose absorption after the first glucose bolus (fig 1). Lactate release by the small intestine during the entire experiment was at a low constant level and was not altered by the infusion of PGE2 (data not shown). The vascular flow of the perfusion system remained essentially constant during the whole experimental period—that is, it was not modified by the infusion of PGE2 (fig 1). The intestinal transit time did not differ significantly between the first glucose bolus without and the second glucose bolus with arterial PGE2 infusion (fig 1).
Prostaglandin E₂ and intestinal glucose absorption

35th minute of the experiment, PGE₂ (10 µmol/l) was infused into the superior mesenteric artery. Glucose was absorbed as indicated by the increase in portal glucose concentration. From the 23rd to the 35th minute, PGE₂ (10 µmol/l) was infused into the superior mesenteric artery from the 23rd to the 35th minute. Carbohydrate absorption (µmol) was calculated from the area under the portal glucose concentration v time curve (mmol/1/min) multiplied by the flow (ml/min) (fig 1). The left panel shows the basal carbohydrate absorption after the first luminal carbohydrate bolus, and the right panel the increase in carbohydrate absorption stimulated by PGE₂, after the second carbohydrate bolus. Values are means (SEM) from three or four experiments. *p<0.05 v corresponding basal absorption.

Figure 1 Prostaglandin E₂ (PGE₂) stimulated increase in glucose absorption with constancy of flow rates and transit time in the isolated perfused small bowel of the rat. Rat small intestine was perfused through the coeliac trunk and superior mesenteric artery with Krebs-Henseleit buffer containing 5 mmol/l glucose, 2 mmol/l lactate, 0.2 mmol/l pyruvate, 1 mmol/l glutamine, 3% dextran, and 1% bovine serum albumin, equilibrated with O₂/CO₂ (19:1, v/v). After the intraluminal bolus of 5.5 mmol glucose in the 6th minute, glucose was applied again as a luminal bolus of 5.5 mmol in the 26th minute. As a control, the second glucose bolus was given without infusion of PGE₂. Values are means (SEM) from four experiments. Flow in the superior mesenteric artery (SMA) was measured with a flow meter and in the portal vein (PV) by fractionated sampling. Flow in the coeliac trunk (CT) was the difference between flow in the superior mesenteric artery and that in the portal vein. Transit time was the time interval from carbohydrate bolus application to appearance in the luminal effluent from the cecum.

The action of PGE₂ on glucose transport was evaluated using enterocytes, dibutyryl cAMP (dbcAMP), and with PGE₂, in the presence of the protein kinase A inhibitor, the R₃-stereoisomer of 3',5'-cyclic adenosine monophosphothioate (RpcAMPS). In the isolated villus tip enterocytes, RpcAMPS (10 µM) enhanced 3-O-[¹⁴C]methylglucose uptake to 220% (fig 3A), and RpcAMPS completely blocked the stimulation of 3-O-[¹⁴C]methylglucose transport by PGE₂. Incubation of the enterocytes with RpcAMPS alone did not affect 3-O-[¹⁴C]methylglucose uptake (data not shown). These results clearly indicate that cAMP mediates the increase in glucose absorption by PGE₂.

Expression of Gₛ linked PGE₂ subtype 4 and 2 receptor mRNA in villus tip enterocytes

To verify the direct action of PGE₂ on villus tip enterocytes, the expression of the four PGE₂ receptor subtypes was examined at the mRNA level by RT-PCR. Detection of SGLT₁ mRNA served as a positive control confirming correct isolation of villus tip enterocytes and their RNA as a template for the RT-PCR (fig 4). mRNA for Gₛ linked PGE₂ receptor subtypes 2 and 4 appears to be strongly expressed in the enterocytes (fig 4). mRNA of the Gₛ linked subtype 1 receptor was also detectable, but that of the Gₛ linked subtype 3 receptor was undetectable (fig 4). The PCR products were sequenced; the sequence was identical with the corresponding receptor sequence. To exclude the possibility that EP₃R mRNA was missed because of non-functioning primers or ineffective reverse transcription or PCR, mRNA from isolated primary hepatocytes, which express EP₃R, served as a positive control (data not shown).
Discussion

Basal glucose absorption in isolated perfused rat intestine

The experimental system of the single path vascularly perfused intestine used in this investigation ensured sufficient oxygen supply, as indicated by little lactate release, which remained constant during the experiments (data not shown). Basal intestinal glucose absorption, vascular flow rates, and intestinal transit time (fig 1) were comparable with previous results.\(^6\)\(^{-}\)\(^{24}\) As rats consume about 20 g of food a day containing 75% carbohydrates,\(^3\) the intraluminal glucose bolus applied of 1 g amounted to less than 7% of the daily ingested carbohydrates. Water movements across the intestinal wall into the lumen induced by the intraluminal carbohydrate bolus through osmotic forces and out of the lumen through absorption of the carbohydrates were balanced; there was a constant luminal net movement of water of less than 0.3 ml/min during the entire experiment (data not shown), which is comparable with the results of previous investigations.\(^2\)\(^{-}\)\(^{24}\) The constancy of flow in the coeliac trunk and superior mesenteric artery excluded the possibility of any deterioration of the preparation from tissue oedema or development of microembolisms.\(^9\)\(^{\text{25}}\)

Acute increase in glucose absorption stimulated by PGE\(_2\), via SGLT1 in isolated perfused intestine of the rat

In isolated perfused small intestine, PGE\(_2\) acutely increased glucose and galactose but not fructose absorption (figs 1 and 2). As glucose and galactose are absorbed via SGLT1,\(^1\) and fructose via GLUT5,\(^2\) this indicates that SGLT1 was involved in the stimulation of absorption by PGE\(_2\). These results are supported by the data obtained with isolated enterocytes, in which phlorizin, a specific inhibitor of SGLT1,\(^20\) markedly decreased the PGE\(_2\) stimulated 3-O-\(^{14}\)C)methylglucose uptake (fig 3B). The different responses to PGE\(_2\), of glucose and galactose absorption on the one hand and fructose absorption on the other also ruled out stimulation via the paracellular route, which has been proposed previously.\(^28\)\(^{-}\)\(^{29}\) In addition, no stimulatory effect would be detectable in isolated enterocytes because, in cell suspensions, no paracellular pathways are present. This is in accordance with the previous finding of negligible paracellular intestinal glucose transport in the rat.\(^30\) Also PGE\(_2\), induced changes in intramucosal flow are unlikely to cause, or contribute to, the increase in glucose and galactose absorption. Firstly, no alteration in total vascular flow or flow through the superior mesenteric artery and coeliac trunk (fig 1) could be measured. Secondly, and more importantly, a change in intramucosal blood flow would alter all absorptive processes not only the absorption of glucose and galactose (fig 2). An alteration in intestinal motility may also be involved in the increase in glucose absorption. However, intestinal motility, as measured by the transit time of the glucose load, was not significantly modified by PGE\(_2\), (fig 1). In conclusion, the stimulatory effect of PGE\(_2\), in the isolated perfused intestine must occur via SGLT1.

Figure 3

Involvement of dibutyryl cAMP (dbcAMP) in the prostaglandin E\(_2\) (PGE\(_2\)) stimulated increase in 3-O-\(^{14}\)C)methylglucose uptake in isolated villus tip enterocytes. Villus tip enterocytes were isolated collagenase free in chelate buffer and incubated in single glass flasks placed in a thermostatically controlled water bath (37°C, except for temperature dependence experiment). The experiments were started by the addition of 3-O-\(^{14}\)C)methylglucose. Uptake at 10 minutes in controls without addition of an effect was taken as 100%. Phlorizin (50 µmol/l), dbcAMP (10 µmol/l), PGE\(_2\), (10 µmol/l), PGE\(_2\) plus RpcAMPS (10 µmol/l), or PGE\(_2\) plus phlorizin was added 10 minutes before the start of the experiment. The retained cell pellet was washed and counted for radioactivity. Data are means (SEM) from four to six experiments.

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The rapid onset of the stimulatory effect of PGE, (fig 1) clearly differs from the well known adaptive increase in carbohydrate absorption known to occur during pregnancy, lactation, streptozotocin induced diabetes mellitus, and high carbohydrate diets in rats. In all these, the underlying mechanism was modulation of the number of SGLT1 molecules. In line with this, the luminal carbohydrate content controlled the expression of SGLT1 in several animals and man. The acute increase in glucose and galactose absorption produced by PGE, in the present investigation excludes the possibility of de novo synthesis of the SGLT1. The rapid action of PGE, is in line with a chemical modification or translocation of the SGLT1 from intracellular storage pools to the cell membrane. No evidence is so far available for a chemical modification. Electrophysiological data obtained with SGLT1 transfected oocytes showed cAMP dependent translocation of the SGLT1, which became detectable only after more than 10 minutes. The half time of insulin dependent insertion of GLUT4 into the plasma membrane in skeletal muscle and adipose tissue is about five minutes. PGE, infusion in the present investigation was started only two minutes before application of the carbohydrate bolus; thus the increase in glucose absorption occurred more rapidly than SGLT1 could be expected to be inserted into the plasma membrane, making this mechanism unlikely. However, as oocytes have a larger volume than enterocytes or adipocytes, the longer time lag of insertion observed in oocytes does not definitively rule out SGLT1 trafficking in the smaller enterocytes. The rapid stimulation of glucose absorption by PGE, is in accord with several recent observations. In the jointly perfused intestine and liver of the rat, luminal glutamine and portal insulin acutely stimulated glucose absorption. Electrophysiological data from mice show a CAMP dependent short term increase in the activity of SGLT1, and, in the isolated rat intestine, enteroglucagon-37 rapidly enhanced glucose absorption.

**PGE, STIMULATION OF AN ACUTE INCREASE IN INTESTINAL GLUCOSE ABSORPTION VIA cAMP**

The intracellular signalling that elicits the rapid stimulation of SGLT1 mediated transport is not understood in detail. It probably involves the intracellular messenger cAMP. This is the case for PGE, stimulated intestinal glucose absorption, because, in isolated enterocytes, RpcAMPS, an inhibitor of protein kinase A, completely prevented the stimulation of 3-O-[14C]methylglucose uptake by PGE, (fig 2). This is in accord with data showing a stimulatory effect of CAMP on glucose absorption. Maybe, cAMP stimulates phosphorylation of the translocator via protein kinase A. Consensus sites for phosphorylation have been described. In addition, a regulatory subunit (RS1) of SGLT1 has recently been described, which may be involved in the PGE, dependent stimulatory effect via cAMP.

In the present study mRNA for the G, linked PGE, receptor subtypes EP2R and EP4R was detected in villus tip enterocytes from rat. In mice, expression of EP4R mRNA has previously been observed by in situ hybridisation in the duodenum and ileum and in rats, northern blot analysis showed EP4R receptor gene expression in the intestinal mucosal layer. Apparently, PGE, receptors that increase cAMP are present on enterocytes. Moreover, PGE, synthesis has previously been shown in epithelial and subepithelial layers of rat intestine. Thus PGE, formed in the intestine in response to as yet unknown stimuli, may act directly via its receptors on the enterocytes. This is supported by the direct stimulatory effect of PGE, on 3-O-[14C]methylglucose uptake in isolated villus tip enterocytes (fig 3). In a previous study with mice enterocytes, PGE, did not alter α-methylglucose uptake; this discrepancy with the present finding cannot be readily explained at present.

**CONCLUSION**

PGE, stimulation of glucose absorption has been shown in isolated perfused small intestine and isolated villus tip enterocytes of the rat. The increase in glucose absorption was elicited via G, linked PGE, receptors of subtype 4 and 2, found expressed in villus tip enterocytes, and thus mediated by cAMP; it occurs via the sodium-dependent glucose cotransporter SGLT1.

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