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Running title: RELM-β increases glucose absorption

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ABSTRACT

OBJECTIVE: An increased expression of RELM-β (Resistin-Like Molecule-β), a gut-derived hormone, is observed in animal models of insulin resistance/obesity and intestinal inflammation. Intestinal sugar absorption is modulated by dietary environment and hormones/cytokines. The aim of this study was to investigate the effect of RELM-β on intestinal glucose absorption**.** RESEARCH DESIGN AND METHODS: Oral glucose tolerance test (OGTT) was performed in mice and rats in the presence and the absence of RELM-β. The RELM-β action on glucose transport in rat jejunal sacs, everted rings and mucosal strips was explored as well as downstream kinases modulating SGLT-1 and GLUT2 glucose transporters. RESULTS: OGTT carried out in rodents showed that oral administration of RELM-β increased glycemia. Studies in rat jejunal tissue indicated that mucosal RELM-β promoted absorption of glucose from the gut lumen. RELM-β had no effect on paracellular mannitol transport suggesting a transporter-mediated transcellular mechanism. In studies with jejunal mucosa mounted in Ussing chamber, luminal RELM-β inhibited SGLT-1 activity in line with a diminished SGLT-1 abundance in brush border membranes (BBMs). Further, the potentiating effect of RELM-β on jejunal glucose uptake was associated with an increased abundance of GLUT2 at BBMs. The effects of RELM-β were associated with an increased amount of PKC βII in BBMs and an increased phosphorylation of AMPK. CONCLUSIONS: The regulation of SGLT-1 and GLUT2 by RELM-β expands the role of gut hormones in short-term AMPK/PKC mediated control of energy balance.

The family of proteins called « RELMs » for resistin-like molecules has been reported to be involved with insulin resistance, diabetes and inflammatory processes. Resistin was initially identified as an adipokine which inhibits insulin action and adipocyte differentiation (1). RELM-β is a protein homologous to resistin which is localized mainly within the digestive tract (2,3). RELM-β is highly expressed in goblet cells of murine colon and is secreted in response to bacterial colonisation. It plays an important role in host defense and innate immunity (4,5). We have also shown that RELM-β may have a direct effect on intestinal goblet cell secretion (6) and others have shown that RELM-β can also act as a hormone. An acute perfusion of RELM-β in rat induced a hepatic insulin resistance (7).

Recently, a concomitant increase of serum concentration and intestinal expression of RELM-β has been reported in insulin-resistant models such as obese db/db mice and high-fat-fed mice (8). Interestingly, the intestinal expression of RELM-β in mice is controlled by fasting and also by various nutritive elements such as glucose and saturated free fatty-acids (9). Glucose reduces the enterocyte expression of RELM-β while insulin and TNFα can upregulate its expression (9). This suggests that intestinal RELM-β may not only be associated with inflammation but can also be a regulator of energy homeostasis.

Glucose, the main source of energy in humans, comes from the digestion of carbohydrates and is absorbed in the small intestine. Intestinal sugar absorption constantly adapts to the dietary environment (10). One risk factor for developing non-insulin-dependent diabetes (NIDDM) is the excessive consumption of diets containing high levels of carbohydrates. An important defect in NIDDM is the increased ability of intestine to absorb monosaccharides by intestinal sugar transporters (11). Intestinal glucose is actively transported by the Na^+/glucose cotransporter-1 (SGLT-1) and passively by glucose transporter2 (GLUT2), (10). Moreover, it is also becoming increasingly evident that the gut is not just site of nutrient absorption but is also an active endocrine organ (12,13). A paracrine

regulation of hexose absorption by intestinal hormones such as glucose-dependent insulinotropic polypeptide (GIP) and proglucagon-derived peptides GLP-1 and GLP-2 has been shown (10). Indeed, certain gastro-intestinal peptides secreted at the luminal side of intestine such as leptin, angiotensin II and EGF have a mucosal effect on hexose transport (14-17). Even though glucose is the main regulator of its own absorption, the modulatory effect of gut-derived-molecules on intestinal sugar absorption plays a critical role in the adaptation to dietary environment. However, to our knowledge, nothing is known concerning the effect of RELM-β on intestinal absorption of glucose. A local action of RELM-β express in the jejunum is likely even though the highest expression of RELM-β is found in colonic goblet cells (2). There is also evidence that RELM-β expression can be upregulated in rat (18) and mice (19) goblet cells of proximal intestine. Considering that RELM-β expression is regulated by nutrients/insulin and inflammatory cytokines, it seems important to explore whether RELM-β can regulate glucose jejunal absorption.

In the present study, we show that exogenous RELM-β acted as a luminal effector in enhancing glycemia during OGTT carried out in mice and rats. The use of rat jejunal strips mounted in Ussing chamber indicated that RELM-β can directly inhibit SGLT-1 activity induced by glucose. The activation of AMPK has been shown to down-regulate SGLT-1 transport and upregulate glucose uptake by GLUT2 (20). The *in vitro* and *in situ* experiments performed with rat jejunal segments indicate that RELM-β increases transepithelial glucose transport by switching the active transport into passive entry. The mechanism involved activation of AMPK and PKC kinases and an insertion of GLUT2 transporters in jejunal brush border membranes. These data suggest that RELM-β, a gut-derived hormone, can directly modulate intestinal glucose transport.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats weighing 220-240 g and male C57BL/6J mice of 20-25 g weight were from Centre Elevage Janvier, Le Genest-St-Isle, France. The animals had free access to tap water and standard food and were treated in accordance with European Community guidelines concerning care and use of laboratory animals.

Glucose tolerance test. Gavages of conscious rats or mice with 1 g of glucose per kg of body weight were performed after a 16 h fast using a D-glucose solution without (control) or with RELM-β The total bolus volume for mice and rats was 0.25 ml and 1 ml respectively and the amount of glucose in each bolus was adjusted for the animal weight. The recombinant murine RELM-β (18 kDa, PeproTech, Neuilly-sur-Seine, France) used was highly purified by high-performance liquid chromatography and was endotoxin-free. RELM-β, at a final concentration of 0.1 and 1 nmol/L, results in dose range of 0.01-0.1 ug per Kg of body weight. RELM-β is a stable molecule as high amounts of the homodimer form have been detected in mice and human stools (21). Before starting the oral glucose tolerance test (OGTT), blood samples were taken from the tail and fasting blood glucose levels (mg/dl) were determined (ACCU-CHEK; Roche Diagnostic, Meylan, France). The bleeds were further taken at 15, 30, 60 and 120 min after oral glucose administration. These experiments were performed at least with 6-10 individual animals.

Tissue preparation and short-circuit measurement. Rats were fasted for 16 h and were killed by pentobarbital overdose. The proximal jejunum was dissected out and four adjacent samples were mounted in Ussing chambers as described previously (22). The tissues were bathed with 4 ml of KRB solution (pH 7.4) with 10 mmol/L glucose at 37°C. In the solution bathing the mucosal side of the tissue, glucose was replaced by mannitol. Both solutions were gassed with 95% O_2/5% CO_2. Electrogenic ion transport was monitored continuously

as short-circuit current (Isc) by using an automated voltage clamp apparatus (DVC 1000; WPI, Aston, England) linked through a MacLab 8 to a MacIntosh computer. KRB alone (vehicle) or containing RELM-β (0.001-1 nmol/L) was added in the mucosal bath 2 min before a glucose challenge. Carbachol (100 mmol/L) was added at the end of each experiment as a control. Further, similar tests were performed with RELM-β incubated overnight at 4°C with a rabbit polyclonal antibody raised against RELM-β. Results were expressed as the intensity of the Isc ($\mu A/cm^2$) or as the percentage of the peak Isc obtained after glucose challenge (measured after 3 min) over basal Isc (measured just before the addition of glucose).

Transmural transport of hexoses. The experiments were performed using jejunal sacs from adult Wistar rats. Animals were fasted for 16 h and were killed by pentobarbital overdose. The proximal jejunum was dissected out and rinsed in cold saline solution. Jejunal loops (4 cm long) were prepared and 0.5 ml of KRB solution without (control) or with 1 nmol/L RELM-β was inserted inside the jejunal lumen. The jejunal loops were incubated for 15 min in oxygenized KRB at 37°C and conditions were maintained during hexose transport assay. The corresponding jejunal loops were filled with 1 ml of KRB solution without (control) or with 1 nmol/L RELM-β and containing 0.02 μCi/ml of the isotopic tracer D-[1-^{14}C] glucose (49.5 mCi/mmol) and glucose to obtain a final concentration of 10, 30 and 100 mmol/L. Similarly, we studied the paracellular transport with 30 mmol/L mannitol and the isotopic tracer D-[1-^{14}C] mannitol (59 mCi/mmol) at 0.2 μCi/ml. All the jejunal loops were incubated in 10 ml of KRB solution during the indicated time. The radioactivity was measured in the collected samples of serosal KRB solution and used to calculate glucose or mannitol transport as pmoles per mg of jejunal protein. Five independent experiments were performed and significance is expressed as * $p<0.05$,** $p<0.01$.

Western blot analysis. Rats were anesthetized by pentobarbital and laparotomized for *in situ* experiments. Three jejunal segments (5 cm long) were tied and filled with 3 ml of KRB without (control) or with 1 nmol/L RELM-β. After 3 min of *in situ* incubation, 3 ml of 60 mmol/L glucose solution were injected in the lumen so as to obtain a final concentration of 30 mmol/L. After a further 3 min, these loops were removed and opened along the mesenteric border and the mucosa was scraped off on ice with a glass blade. Total cell protein extracts and BBMs were prepared from the scrapings as previously described (23) and enrichment was estimated by determination of alkaline phosphatase activity (20-fold increase of activity in BBMs). Solubilised proteins were resolved by electrophoresis on 10 % SDS-PAGE gels and transferred onto nitrocellulose membranes for immunoblot analysis. The following rabbit antibodies were used at a 1:1000 dilution; SGLT-1 (AB 1352; Chemicon International, Temecula, CA); GLUT2 , PKC βII (sc-9117 , sc-210, Santa Cruz Biotechnology, Tebu-Bio, France); phospho-AMPK-α (Thr172) , AMPK-α (23A3), phospho-PKC (pan (2531, 2603, 190D10; Cell Signalling Technology, Ozyme, France). The intensity of the specific immunoreactive bands was quantified using NIH Image (Scion, Frederick, MD).

Glucose uptake experiments. Uptake experiments were performed using rat intestinal everted rings as previously described (24). Briefly, groups of 8 intestinal rings were incubated at 37°C for 15 min in oxygenized KRB buffer in the absence (control) and the presence of RELM-β (1 nmol/L) and cytochalasin B as indicated. Then the rings were incubated for 2 min in an uptake solution corresponding to a KRB buffer containing 30 mmol/L glucose and 0.1 μCi/ml of the isotopic tracer D-[1-^{14}C] glucose. After adding the uptake solution, the rings were washed in ice-cold KRB solution and radioactivity incorporated in the tissue was quantified by liquid scintillation. Data were not corrected for extracellular substrate since RELM-β was found not to affect paracellular diffusion as

shown in Fig. 3A. Total protein from homogenized tissue with a Dounce homogenizer was determined with BCA reagent from Pierce (Thermo Scientific, Brebières, France). Results are expressed as µmol glucose/g tissue protein.

Chemicals. Recombinant murine RELM-β was purchased from PeproTech (Neuilly-sur-Seine, France). Antibody raised against RELM-β was a gift from Dr. Blagoy Blagoev (University of Southern Denmark). D-[1-14C] Mannitol, GE Healthcare Amersham Biosciences, (les Ulis, France) and D-[1-14C] glucose, Perkin Elmer, (Boston USA), compound C from Merck Sharp & Dohme-Chibret (Paris, France). All other chemical reagents were purchased from Sigma (St. Louis, MO).

Statistical analysis. All results were expressed as means ± SE. One-way ANOVA with Turkey-Kramer multiple comparisons posthoc-test was performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA). The level of significance was set at $p<0.05$.

RESULTS

Effect of RELM-β on glucose tolerance tests. Oral administration of RELM-β increased glycemia during oral glucose tolerance test carried out in mice (Fig. 1A) and rats (Fig. 1B) as compared with control groups. The area under the curve was significantly ($p<0.05$) increased in mice when RELM-β was used at 1 nmol/L and the two doses used (0.1 and 1 nmol/L) were effective in rats (insets, Fig. 1A and B). A similar 15% increase in blood glucose as compared to control was observed in both mice and rats. These data show that luminal administration of exogenous RELM-β is active in vivo as previously described (6).

Mucosal RELM-β inhibits Na^+-dependent glucose transport. The route of glucose entry can involve active Na^+-dependent glucose transporter (SGLT-1) or diffusive transporter GLUT2. We used the Ussing chamber to characterize the effect of mucosal RELM-β on rat

intestinal active glucose transport (SGLT-1). Rat jejunal mucosa mounted in the chamber was allowed to reach a steady state (usually 40 min). Addition of 10 mmol/L glucose in the mucosal bath induced a rise in Isc (maximum after 3 min) representing an increase in SGLT-1 activity. Addition of RELM-β to the mucosal side 2 min before glucose challenge induced a marked and dose-dependent inhibition of glucose-induced Isc. As shown in Fig. 2A, addition of RELM-β to the mucosal side inhibited glucose transport by jejunal mucosa in a concentration-dependent manner. Maximal inhibition was achieved with 0.1 nmol/L RELM-β. The concentration that produced a half-maximal inhibition (IC_{50}) of glucose transport was 3 pmol/L. Further, an overnight incubation of 0.1 nmol/L RELM-β with an antibody raised against RELM-β countered the inhibition of glucose-induced Isc by RELM-β as shown in Fig. 2B. We next investigated if the observed inhibition by RELM-β was associated with an altered abundance of SGLT-1 in jejunal brush border membranes (BBMs). A typical immunoblot of SGLT-1 protein in BBMs after glucose challenge in the presence or the absence of RELM-β is shown in Fig. 2C. The mean densitometry of three separate blots shows that glucose increased the amount of SGLT-1 protein in BBMs by 1.5-fold as compared to control. This increase is reduced by half in the presence of RELM-β.

RELM-β modulates jejunal glucose transport. We isolated rat jejunum and performed *in vitro* studies of transmural glucose transport to directly evaluate the effect of RELM-β on intestinal transport. As shown in Fig. 3A, luminal glucose in jejunal loops significantly increased net mucosal to serosal glucose flux. A dose-dependent effect on glucose transport was observed with increasing glucose concentrations 10, 30 and 100 mmol/L. Further, RELM-β, at 1 nmol/L, significantly enhanced the jejunal transport of 10, 30 and 100 mmol/L glucose but not that of 30 mmol/L mannitol. Thus, increased jejunal glucose transport induced by RELM-β is unlikely to have been caused by changes in paracellular

permeability. This is in line with histological studies of the jejunum tissues used in these experiments which did not show any visible mucosal deterioration (data not shown). Further, experiments were performed to identify if the effect of RELM-β implicated potential downstream kinases, PKC and AMPK, which are known as key effectors of intestinal glucose transport (6, 20, 23, 25). The AMPK inhibitor, compound C, and the PKC inhibitor, chelerythrine, inhibited the 30 mmol/L glucose-induced jejunal glucose transport as shown respectively in Fig. 3B and 3C. The insets represent the area under the curve of the 30 min glucose transport kinetics. RELM-β significantly increased glucose transport by approximately 2-fold and this effect was blunted by compound C and chelerythrine as shown respectively in Fig. 3B and 3C. These results indicate a likely involvement of PKC and AMPK in the luminal effect of RELM-β on glucose uptake.

RELM-β stimulates phosphorylation of AMPK and translocation of PKC βII. The above results prompted us to assess by *in situ* experiments the cellular effects of RELM-β on AMPK and PKC. Jejunal segments were injected with a KRB solution alone or containing 30 mmol/L glucose in the absence and the presence of 1 nmol/L RELM-β. The mucosal scrapings of jejunum were examined by Western blot analysis. The results indicated that glucose as well as RELM-β stimulated AMPK phosphorylation over control values as shown in Fig. 4A. The corresponding mean densitometric analysis indicates that RELM-β induced a 3-fold increase in AMPK phosphorylation. A further increase in the phosphorylation of AMPK occurred when glucose and RELM-β were added together. Similarly, RELM-β induced a 3-fold increase in PKC phosphorylation in line with our data (6) in the mouse colon (data not shown). This prompted us to study the translocation of cytosolic PKC βII of enterocytes to BBMs, a mechanism which is associated with intestinal glucose transport (20, 23). We performed Western blot analysis to determine the expression

of PKC βII in BBMs obtained from intestinal segments which were incubated with glucose (30 mmol/L) or RELM-β (1 nmol/L) as described above. As shown in Fig. 4B, RELM-β or glucose induced respectively 1.5 or 2-fold increase of PKC βII at the BBMs as compared to control values. A further increase (2.7-fold compared to the control) of PKC βII at the BBMs was observed when RELM-β and glucose were added together. These results suggest that RELM-β stimulates the phosphorylation of AMPK and PKC as well as an increased shift of PKC βII to the BBMs of rat jejunal tissue.

RELM-β increases GLUT2 activity and its expression at BBMs.

We explored whether RELM-β could enhance the glucose-induced GLUT2 translocation to the apical membrane of enterocytes. To this end, we measured glucose uptake in rat everted jejunal rings in the presence of RELM-β and without or with cytochalasin B, a competitive inhibitor of GLUT2 (26). As shown in Fig. 5A, cytochalasin B inhibited glucose uptake by 50 % in agreement with the implication of GLUT2 in glucose uptake (27, 28). RELM-β enhanced glucose uptake by 2-fold and this effect was also strongly inhibited by cytochalasin B. Further, we performed Western blot analysis to determine the expression of GLUT2 in BBMs obtained from intestinal segments that had been incubated with RELM-β under the same conditions as above. We observed that glucose or RELM-β alone induced respectively a 4- or 3-fold increase in the amount of GLUT2 found in the brush-border fraction respectively (Fig. 5B). When glucose and RELM-β were added together, a further increase in the amount of GLUT2 was observed (5-fold as compared to control). This suggests that RELM-β enhanced glucose uptake is due to an increased insertion of GLUT2 into the BBMs.

DISCUSSION

In the present study, we show that RELM-β, a resistin-like molecule, can directly increase jejunal absorption of glucose in the rat. Several lines of evidence suggest that trans-epithelial transport of glucose in the small intestine can be mediated by an active absorption through Na^+/glucose cotransporter (SGLT-1) as well as by a diffusive component GLUT2 at the apical membrane (27). We found that RELM-β inhibited the activity of SGLT-1 while enhancing the presence of GLUT2 at the brush border membrane of enterocytes. Morever, the increased jejunal glucose transport induced by RELM-β was inhibited by cytochalasin B in agreement with a functional role of GLUT2. The underlying molecular mechanism involves the activation of PKC βII and AMPK kinases as described for such reciprocal regulation of glucose transporters (20).

The acute treatment of rat jejunum with RELM-β had no significant effect on passive mannitol movement suggesting that RELM-β increases glucose transport by the use of transporters. We found that mucosal RELM-β inhibited the activity and the translocation of cytosolic SGLT-1 to cell membranes. This effect was blunted by an antibody raised against RELM-β. Other peptides such as leptin, angiotensin II and CCK-8 have also been shown to inhibit SGLT-1 activity (14, 16, 28). We show that in contrast to CKK-8 which seems to have no effect on GLUT2 (28), RELM-β can induce GLUT2 translocation. Indeed, in response to RELM-β the amount of GLUT2 was increased in the BBMs. Similarly, a rapid insertion of GLUT2 to apical membrane in response to another gut-peptide, GLP-2, has been reported (29, 30). Taken together, these findings suggest the involvement of GLUT2 in RELM-β stimulated glucose uptake.

The mechanisms responsible for RELM-β effect may involve the activation of PKC (6) and AMPK (25) which have been shown to regulate jejunal glucose transporters (20). This is

sustained by the report that luminal EGF increases jejunal glucose transport in rabbit through PKC activity (31). We found that chelerythrine, an inhibitor of PKC, blocked RELM-β stimulation of glucose uptake. The effect of RELM-β is accompanied by an increase in the amount of PKC βII at the BBMs. The activation of PKC has been shown to inhibit SGLT-1 mediated transport of hexoses (14, 32). In oocytes expressing rat and rabbit SGLT-1, the activation of PKC decreases the maximum rate of transport for both isoforms. This change is accompanied by proportional change in the number of SGLT-1 molecules at the plasma membrane, indicating that PKC regulates endocytosis of the vesicles containing the transporter (33). Further, SGLT-1 contains a consensus site of PKC phosphorylation and thus PKC phosphorylation of the transporter could control its activity (34). These data are in line with our results showing that RELM-β inhibits SGLT-1 activity and its translocation to BBMs in rat small intestine.

The activation of AMPK has been shown to downregulate SGLT-1-dependent glucose transport but also to enhance GLUT2 translocation to the apical membrane of the jejunum (20). This effect of AMPK leads to an increased glucose uptake in jejunum (20) as well as in muscle (35). Interestingly, several hormones have been shown to regulate AMPK in a strictly tissue-specific manner (36). We demonstrate that the compound C blocked RELM-β stimulation of glucose transport in rat intestine suggesting the involvement of AMPK. Humans and rodents express two isoforms of the catalytic subunit (α1, α2) which form the heterotrimeric complex AMPK (α, β and γ) known to serve as a regulator of energy balance (37). We showed that RELM-β can increase the phosphorylation of the conserved threonine residue (Thr-172) of α1 in the jejunal mucosa which is crucial for the AMPK activity. As evoked above, activated PKC decreases the number of SGLT-1 transporters but can also activate the translocation of GLUT2 to BBMs as described elsewhere (23). Thus luminal

RELM-β can directly enhance glucose transport by mustering GLUT2 at BBMs through PKC and AMPK activation.

This inverse regulation of SGLT-1 and GLUT2 by luminal RELM-β may be important when enterocytes require energy as shown in stress-induced pathology (38). The energy sensor molecule AMPK as well as PKC can increase the GLUT2 energy-independent pathway to override that of SGLT-1 which requires energy (20, 32). In agreement with this concept, the SGLT-1-mediated absorption of nutrients such as galactose and glucose is decreased during either systemic (39) or intestinal inflammation (40). Further, to meet the increased metabolic demand of inflamed tissue, it has been shown that pro-inflammatory cytokines (IL-8, IL-6 and IL-1) can increase jejunal absorption of glucose without changes in BBM SGLT-1 content (41). An increased expression of RELM-β has been described in the intestine during jejunal inflammation (19, 42 43). The expression of RELM-β is increased by several pro-inflammatory cytokines and by LPS (9, 21) and they may act together to modulate intestinal glucose absorption. Thus, the enhanced glucose absorption by RELM-β in response to inflammatory stimulus may contribute to the associated energy demand.

Obese and insulin-resistant rodent models which are characterized by a low grade inflammation are also associated with an increased expression of the gut-derived RELM-β (8). Expression of RELM-β has been shown in mice (18) and rat (19) proximal intestine and the peptide may act locally in a paracrine manner or as a circulating hormone linking the gut to the liver. When given by gavage, it is possible that RELM-β could be partly absorbed by the small intestine to reach the blood as demonstrated for leptin (44). In favour of a hormonal effect, the infusion of intestinal RELM-β in mice has been shown to promote a marked increase in the rate of hepatic glucose production (7). This was associated with a rapidly induced hepatic but not peripheral insulin resistance. We observed that acute RELM-β administration in the intestinal lumen of rodents resulted in an increased glycemia in OGTT.

This could result from the observed increased intestinal glucose absorption and an acute hepatic insulin resistance (7). This is in line with a local intestinal and distant action of this gut peptide. As to whether RELM-β may also counteract the described insulin inhibition of intestinal sugar absorption remains to be established (45). There may be different RELM-β thresholds in insulin target tissues as described for resistin (46). A better understanding of RELM-β action on different tissues could emerge if putative RELM-β receptors were identified. In this context, the effect of RELM-β may even involve rapid neuronal activation since RELM family members RELM-α (47) and RELM-β (4) can bind to neurons. Other than short-regulation, RELM-β can have a chronic effect as shown in diet-induced metabolic disorders (25). Transgenic mice over-expressing circulating RELM-β exhibited significant hyperglycemia, hyperlipedemia when fed on high fat diet. In conclusion, our study shows that RELM-β increases intestinal glucose transport. Further studies may reveal if this process in conjunction with an adverse nutritional and inflammatory status, can participate in the onset of diabetes.

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FIGURE LEGENDS

FIG. 1: Oral glucose tolerance tests (OGTT) in mice and rats. OGTT (1g/kg) were performed in overnight-fasted mice (A) or rats (B) with a 15% D-glucose solution without (○, dotted lines) or with RELM-β (0.1 nmol/L, ● and 1 nmol/L, □). Glucose concentration was determined in blood samples from the tail and are expressed as mg/dl over the time points (minutes). Each point is the mean ± SE; n= 6-10. *p <0.05 versus control. Area under the curve (inset) is expressed in arbitrary units (AU). In absence (0, clear bar) and in presence of 0.1 nmol/L (black) and 1 nmol/L (hatch) RELM-β.

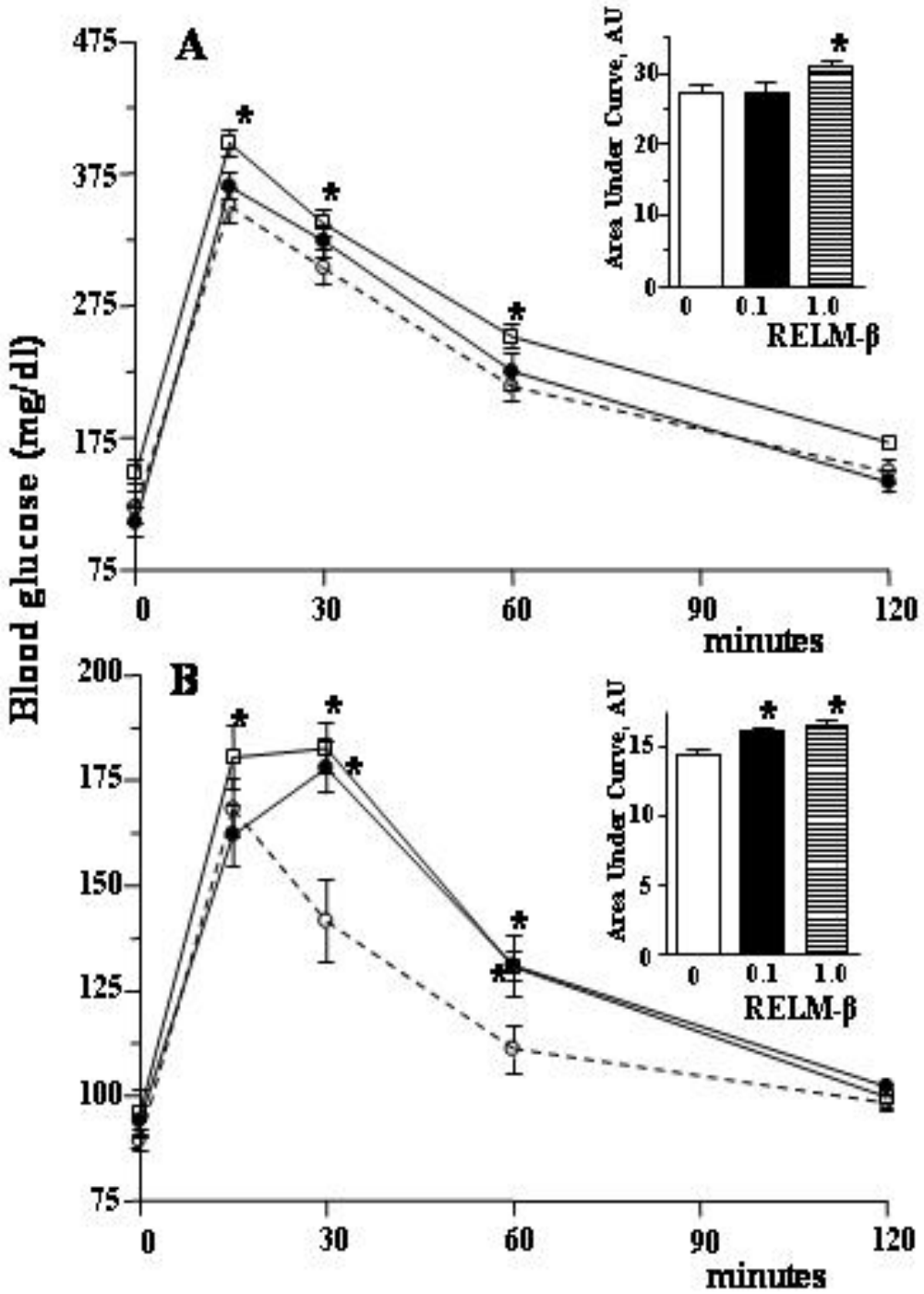
FIG. 2: Effect of luminal RELM-β on glucose-induced Isc. Rat jejunal mucosa was mounted in Ussing chamber and the increase in Isc was studied at steady state. Electrogenic (Na^+) transport was followed as an index of the active glucose transport by cotransporter SGLT-1. RELM-β was added in the mucosal bath 2 min before challenging the tissues with 10 mmol/L glucose. Values for Isc were standardized to control values and expressed as percentage of controls. A dose-dependent inhibition is shown in (A). The effect of 0.1 nmol/L RELM-β after an overnight incubation with an antibody against RELM-β is shown in (B). Brush border membranes (BBMs) were prepared from rat jejunum loops incubated *in situ* with and without luminal RELM-β during 6 min. A representative SGLT-1 immunoblot of solubilized BBMs is shown in (C). Densitometric analysis of immunoreactive bands was performed using NIH Image analysis program. The densitometry represents the amount of SGLT-1 relative to β-actin and is representative of at least three separate experiments. Each point of the Isc study represents the mean ± SE of four to eight noncumulative values from five separate experiments. Significant differences from control (*$p < 0.05$ and **$p < 0.01$).

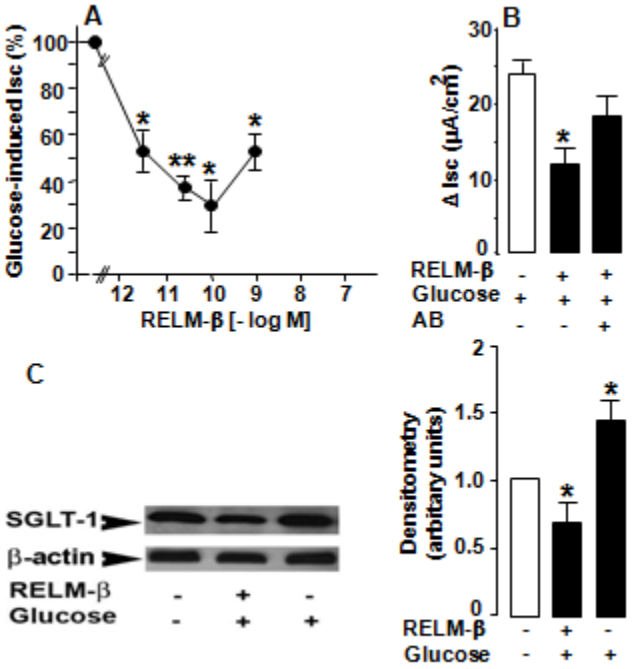
FIG. 3: Effect of luminal RELM-β on transmural transport of hexoses in rat jejunal loops. Transmural transport of glucose or mannitol was performed in jejunal sacs from adult Wistar rats. In (A), intestinal sacs were incubated at 37°C during 15 min with 1 nmol/L RELM-β (■) or vehicle (□) in oxygenized KRB buffer with glucose at 10, 30 and 100 mmol/L and the isotopic tracer D-[1-^{14}C] glucose. The radioactivity measured in the collected samples was used to calculate glucose transport as pmoles per mg of jejunal protein per min. Results from a similar experiment using mannitol (30 mmol/L) are shown in (A). The kinetics of glucose (30 mmol/L) transmural transport is shown in the absence (○, dotted line) and in the presence of 1 nmol/L RELM-β (■) in B and C. In figure B, the AMPK inhibitor (compound C or CC) was incubated without (◆) or with RELM-β (▲). Similarly in (C), the PKC inhibitor (chelerythrine chloride or CCL) is incubated without (◆) and with RELM-β (▲). The insets show the corresponding area

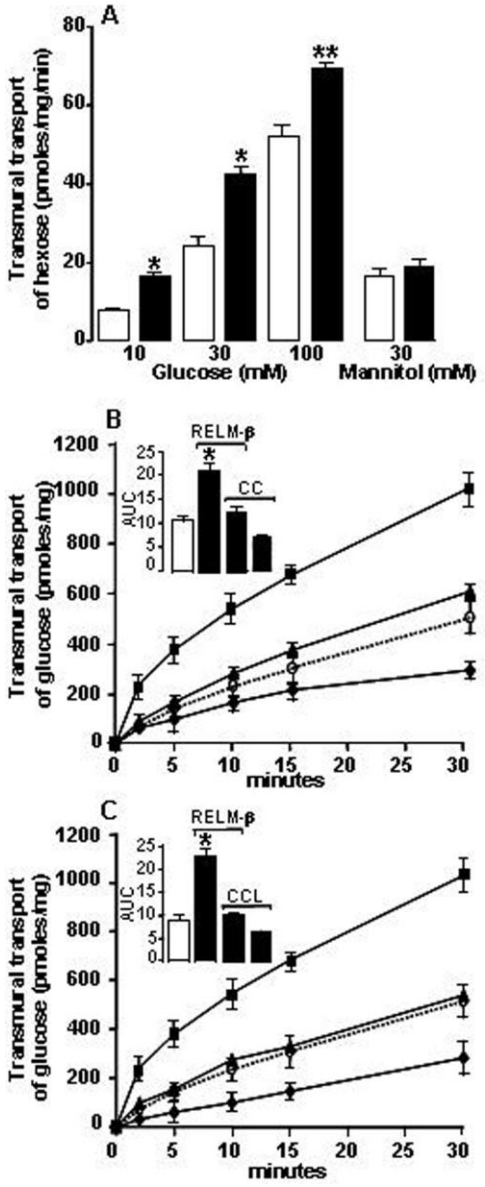
under the curve. The data are representative of the mean ± SE of at least 4 individual experiments (*p <0.05 and **p <0.01 versus control).

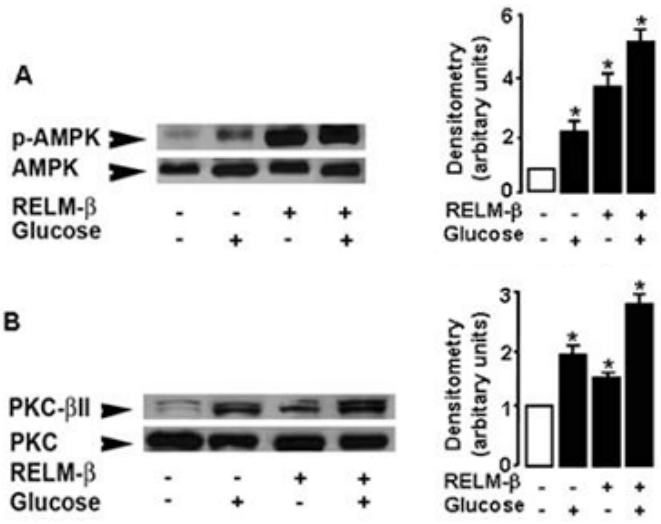
FIG. 4: RELM-β induces phosphorylation of AMPK and translocation of PKC βII. Rats were anesthetized and their jejunal loops were used for *in situ* experiments. The loops were filled with KRB-buffer with or without 1 nmol/L RELM-β. After 3 min, glucose (30 mmol/L) was added in this mucosal bath. After a further 3 min, loops were excised and kept on ice before scraping off the mucosa. Total protein extraction and BBMs preparation were performed immediately as described in Methods. Representative immunoblots for phospho-AMPK (A) in mucosal extracts and PKC βII (B) in BBMs are shown. The densitometry in A represents the amount of phosphorylated kinases relative to total AMPK. The amount of PKC βII in BBMs in B is relative to total PKC. The data are representative of three separate experiments. *Significantly different from control (p <0.05).

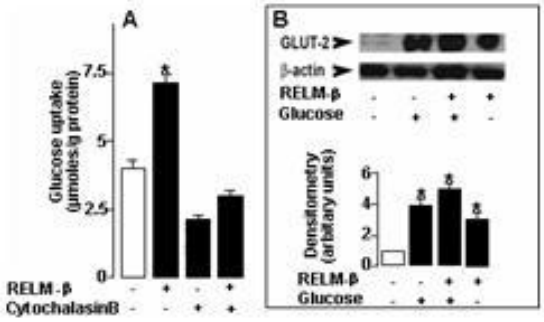
FIG. 5: Effect of RELM-β on glucose uptake and GLUT2 trafficking in BBMs. In (A), rat jejunal everted rings were incubated in oxygenized KRB-buffer without (control) or with 1 nmol/L RELM-β. The presence of cytochalasin B is indicated. Then, the rings were incubated for 2 min in a KRB buffer containing 30 mmol/L glucose and 0.1 µCi/ml of the isotopic tracer D-[1-^{14}C] glucose). The radioactivity incorporated in the tissue was determined by liquid scintillation. The amount of glucose incorporated is expressed as µmol per g of tissue protein. In panel (B), rat jejunal loops were treated 6 min with a mucosal bath containing 30 mmol/L glucose with or without 1 nmol/L RELM-β and BBMs were prepared as described in Methods. A representative Western blot analysis of the corresponding protein lysates with GLUT2 antibody is shown. Densitometric analysis of immunoblots indicates an increase of GLUT2 in the presence of glucose or RELM-β. The data are expressed relative to β-actin and is representative of at least three separate experiments, *Significantly different from control (p <0.05).


A
B
Blood glucose (mg/dl)
minutes
Area Under Curve, AU
RELM-β
0
0.1
1.0
475
375
275
175
75
200
150
125
100
30
60
90
120
10
20
30
5
15


A
Glucose-induced Isc (%)
100
80
60
40
20
0
12
11
10
9
8
7
RELM-β [- log M]
B
Δ Isc (μA/cm²)
30
20
10
0
RELM-β - + +
Glucose + + +
AB - - +
C
SGLT-1
β-actin
RELM-β - + -
Glucose - + +
Densitometry (arbitary units)
2
1.5
1.0
0.5
0
RELM-β - + -
Glucose - + +


A
Transmural transport of hexose (pmoles/mg/min)
80
60
40
20
0
*
*
**
10
30
100
30
Glucose (mM)
Mannitol (mM)
B
Transmural transport of glucose (pmoles/mg)
1200
1000
800
600
400
200
0
RELM-β
*
CC
AUC
25
20
15
10
5
0
0
5
10
15
20
25
30
minutes
C
Transmural transport of glucose (pmoles/mg)
1200
1000
800
600
400
200
0
RELM-β
*
CCL
AUC
25
20
15
10
5
0
0
5
10
15
20
25
30
minutes


A
p-AMPK
AMPK
RELM-β
Glucose
Densitometry (arbitary units)
RELM-β
Glucose
B
PKC-βII
PKC
RELM-β
Glucose
Densitometry (arbitary units)
RELM-β
Glucose


A
Glucose uptake (μmoles/g protein)
7.5
5
2.5
0
&
RELM-β
CytochalasinB
B
GLUT-2
β-actin
RELM-β
Glucose
Densitometry (arbitrary units)
6
4
2
0
&
RELM-β
Glucose