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EVIDENCE FOR GLUT2-MEDIATED GLUCOSE INFLUX IN RAT JEJUNUM AT LOW GLUCOSE CONCENTRATION

by
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AN ABSTRACT OF THE THESIS

Rita Antoine Berkachy for Master of Science
Major: Physiology

Title: Evidence for GLUT2-mediated glucose influx in rat Jejunum at low glucose concentration

Background: Intestinal glucose absorption is an integral component of overall glucose homeostasis. It occurs through active or facilitated mechanisms via the sodium dependent glucose transporter-1 (SGLT1) and sodium independent transporter-2 (GLUT2), depending on intraluminal glucose concentration. The study aims to characterize the relative contribution of SGLT1 and GLUT2 to glucose absorption under various glucose concentrations, *in-vitro* and *in-vivo*, and to investigate the possible control of glucose receptors by the extrinsic gastrointestinal innervation, in particular capsaicin sensitive primary afferents (CSPA).

Methods: Glucose absorption was measured *in-vivo* by intraluminal perfusion of various concentrations of glucose in the jejunum of control rats and rats subjected to systemic CSPA fibers ablation or subdiaphragmatic vagotomy. 1-minute glucose uptake by jejunal strips was also measured *in-vitro*. The effects of adding various concentrations of phlorizin or phloretin, specific antagonists to SGLT1 and GLUT2, respectively, on glucose uptake were also studied *in-vitro* and *in-vivo*. The expression of SGLT1 and GLUT2 mRNA was determined in intestinal mucosal scrapings by real-time PCR.

Results: Systemic desensitization to capsaicin decreased 1-minute glucose uptake (*in-vitro*) of 1 and 20 mM glucose by 55% and 30% *($p<0.001$),* respectively. However, it had no significant effect on the absorption of 50 mM glucose. The effect of selective inhibitors of SGLT1 and GLUT2 was more pronounced at lower concentration of glucose, and less effective in inhibiting glucose transport in rats subjected to CSPA fibers ablation. Systemic desensitization to capsaicin decreased basal absorption of 20 and 50 mM glucose *in-vivo* by 24% *($p<0.01$)* and 40% *($p<0.001$)* respectively. The addition of 1mM phloretin or phloridzin to the perfusate resulted in a decrease of absorption in both sham and CSPA ablated rats. This decrease was not significantly altered by CSPA ablation at 20 mM glucose. Furthermore, subdiaphragmatic vagotomy increased baseline absorption of 5 and 50 mM glucose, with a more potent reduction after the addition of receptors antagonists. Finally, a 47% decrease in GLUT2 and 52% decrease in SGLT1 mRNA expression were detected in capsaicin-treated rats as compared to sham.

Conclusions: At very low concentration of glucose, GLUT2 can play a key role in the influx of glucose to enterocytes. Furthermore, capsaicin sensitive afferents modulate glucose absorption depending on its intraluminal concentration. Our results suggest an involvement of CSPA in the regulation of both SGLT1 and GLUT2 transporters and a role of these fibers in sensing glucose concentrations.

CONTENTS

CHAPTER I

INTRODUCTION

A. Preamble

Diabetes and obesity present some of the biggest public health challenges of the 21st century. One of the reasons contributing to the rampant spread of these two intertwined diseases may be the increasing consumption of sugar-rich meals. Subsequent adaptations or changes in small intestinal structure and function (such as glucose absorption) are often observed in obese and diabetic individuals and animals (Gorboulev et al., 2012).

Intestinal glucose absorption is an integral component of overall glucose homeostasis. It occurs through active and facilitated mechanisms (Gruzdkov et al., 2012). Normal glucose homeostasis is tightly regulated by multiple neural and humoral mechanisms. Extrinsic intestinal innervation through the autonomic afferent (sensory) and efferent (motor-secretory) fibers appears to play a crucial role in glucose absorption and homeostasis (Koeppen and Stanton, 2009).

B. Small Intestine

The small intestine in mammals plays a central role in the digestion and absorption of nutrients, e.g., carbohydrates, proteins and fats. It is about 20 feet (~6 m) long and is composed of three sections: the duodenum, jejunum, and ileum. The chemical digestion of food takes place in the duodenum. The jejunum is the middle segment of the

small intestine; it is important in secretion and absorption, via both active and passive transport mechanisms. Once food reaches the ileum, it contains far fewer nutrients, and passes through to the lower GI tract at a faster speed (Schneeman 2002; Hamilton et al., 2013).

Epithelial cells of the small intestine serve as a barrier against invasion from the environment and play a key role in the absorption of ions (i.e., Na^+), solutes (i.e., glucose), and water. Many transporters (ion channels, facilitated transport carrier proteins, and primary and secondary active transport proteins) work in concert to perform absorption (Bourzac et al., 2013; Hamilton et al., 2013).

C. Glucose Homeostasis

Glucose serves as the most important metabolic substrate and provides energy for normal activity in humans (Yoshikawa et al., 2011). Glucose from dietary carbohydrates is absorbed in the small intestine (Aschenbach et al., 2009; Chichger, 2011). Glucose homeostasis is well regulated in mammals. Maintaining constant blood glucose level is fundamental for the proper operation of physiological processes such as cerebral metabolism; dysregulation thereof triggers many metabolic disorders (Chichger 2011).

Glucose homeostasis is regulated by the collaboration between the central nervous system and the gastrointestinal tract where peripheral signals originating from the gut inform the nervous system to monitor the status of peripheral glucose stores. These gut signals are either hormonal or neuronal (Sellin 2001; Migrenne et al, 2006).

Regulation of blood glucose levels is achieved by modulating the amount of glucose entering and leaving the circulation. The brain, particularly the hypothalamus, has an important role in the regulation of energy and in controlling glucose homeostasis as it directly senses hormones such as insulin, leptin, glucagon-like peptide-1 (GLP-1), pancreatic peptide (PP), peptide YY (PYY) and nutrients such as fatty acids and glucose (Pénicaud et al, 2006; Lam et al 2009).

1. Intestinal Glucose Absorption

Dietary carbohydrates contribute up to 50% of the daily intake of calories. They are hydrolyzed in the small intestine, by pancreatic amylase and brush-border membrane disaccharidases, to monosaccharides, glucose, galactose, and fructose. (Wright 2003; Wei et al., 2011).

Much of the fructose and almost all the galactose are rapidly converted into glucose by the liver. Thus glucose, representing ~80% of the final products of carbohydrate digestion in the alimentary tract, becomes the final common molecule transported to cells. The major site of glucose absorption in the body is the epithelial cells of the small intestine where transport rates are highest in the jejunum and least in the ileum; whereas reabsorption of glucose is carried out by epithelial cells of the proximal tubule of the nephron (Hummel et al., 2011; Hamilton et al., 2013).

The cell membranes are hydrophilic and therefore glucose must traverse the lipid bilayer via facilitative or active transport mechanisms (Chichger 2011). The monosaccharides are absorbed across the intestine via a carrier-mediated process, through

which luminal glucose is transported into the cell by a hexose transporter (Chaudhry et al., 2011). These transporters speed up or facilitate the equilibration of the sugar across the membrane (Stümpel et al., 2001; Wright et al., 2006).

a. Active Glucose Transport

Active transporters are energy-requiring symporters, which move specific nutrient substrates. The energy required for this transport is provided from electrochemical gradient coupling of nutrient transport to specific ions (Hummel et al., 2011).

The transporter responsible for carrying glucose from the intestinal lumen into enterocytes is primarily the sodium-dependent glucose transporter SGLT1 that is expressed on the brush-border membrane of villus cells (Moran et al., 2010; Batchelor et al., 2011). SGLT transporters are large homotetramer proteins with twelve transmembrane segments and a leucine zipper domain on one extracellular loop (Hummel et al., 2011).

b. Facilitated Glucose Transport

The facilitative glucose transporters mediate the movement of glucose down its concentration gradient, thus transport is dependent on the glucose concentrations across the membrane. GLUT proteins contain twelve transmembrane domains, which generate internal and external ligand binding sites, N- and C-terminal cytoplasmic domains and a single glycosylation site on one of the extracellular loops associated with glucose transport (Mueckler et al., 1985; Wilson-O'Brien et al., 2010; Chichger 2011).

GLUT2, the sodium-independent glucose transporter, is an example of a facilitated transport carrier protein, as the function of GLUT2 is dependent solely on the concentration gradient of the sugars across the basolateral membrane of the epithelial cell. Once inside the cell, sugar molecules are transported into the portal venous circulation (figure 1) (Bird et al., 1996).

c. Classical Model of Intestinal Sugar Transport

Glucose absorption in the small intestine relies on the coordinated functions of transporters at the surface membrane of enterocytes. These transporters are expressed in the duodenum and jejunum and at lower levels in the ileum (AU et al., 2002; Bajaj-Elliott et al., 2008; Ait-Omar et al., 2011; Chaturvedi et al., 2013).

On the apical membrane, SGLT1 actively transports glucose from the lumen of the intestine into enterocytes. It co-transports two Na^+ ions into the cell with one glucose molecule and has a high-affinity for glucose and galactose; however, SGLT1 has a low capacity for glucose transport (figure 1) (Sabino et al., 2010). SGLT1 is powered by the downhill Na^+ gradient maintained by the Na K pump found on the basolateral membrane (figure 1).

Glucose transported into the cell through SGLT1, is carried across the basolateral membrane via GLUT2- dependent mechanisms, which provides an exit pathway of glucose from the cytosol into the blood (Moran et al, 2010; Liu et al, 2013). GLUT2 is a high-capacity, low-affinity glucose transporter, which can also transport galactose, glucosamine, mannose and fructose (Uldry et al., 2002; Kellett and Helliwell, 2000; Marks et al., 2003; Chichger 2011). In the classical model of sugar absorption,

GLUT2 was thought to be located only at the basolateral membrane where it transports glucose into blood (figure 1) (Bourzac JF et al, 2013).

The electrochemical gradient for Na^+ is generated and maintained by the primary active Na-K pump (Bourzac et al., 2013; Hamilton et al., 2013). Two Na^+ ions coupled with glucose are transported into the cell; they exit the cell across the basolateral membrane via the Na-K pump, which transports three Na^+ ions out in exchange for two K^+ ions, against their respective electrochemical gradients. This is directly coupled with the expenditure of ATP (Figure 1). K^+ is recycled back across the basolateral membrane via K^+ channels, aiding in the maintenance of the negative membrane potential of the epithelial cell, which contributes to the electrochemical gradient for Na^+ entry across the apical membrane. (Göstemeyer 2010; Yoshikawa et al., 2011; Bourzac et al, 2013; Hamilton et al., 2013).

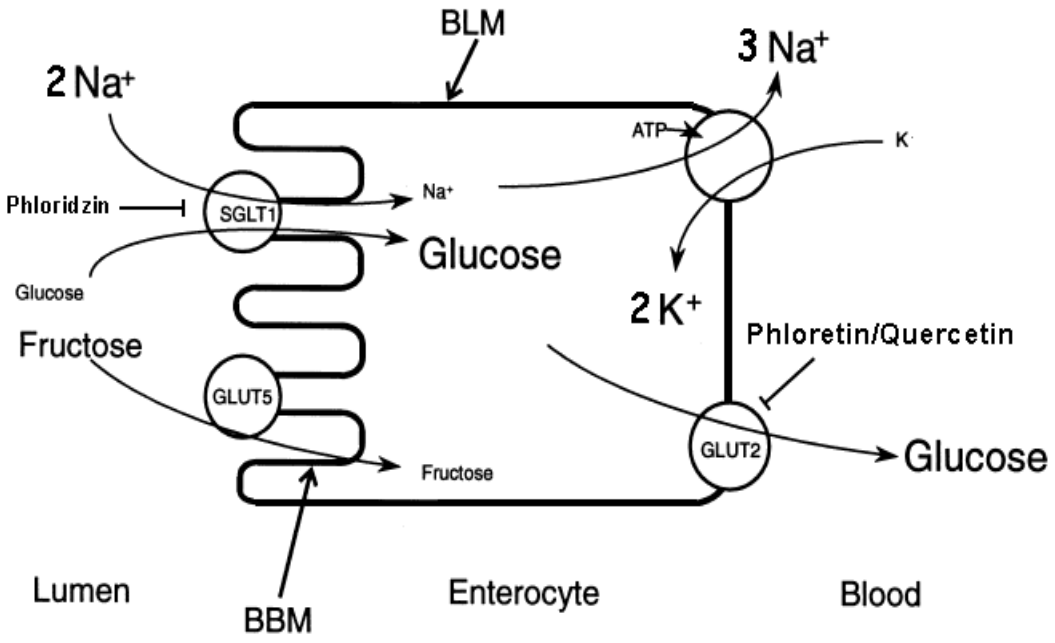


Figure 1: Sugar transport across the Enterocyte of the Small Intestine.

(From The Online Metabolic and Molecular Bases of Inherited Disease, with modifications)

The transepithelial transport of Na^+ contributes to a charge separation across the epithelium, providing a driving force for Cl^- absorption via the paracellular pathway. The absorption of Na^+, Cl^-, and glucose generates a slight osmotic gradient across the epithelium, providing a driving force for water absorption via both transcellular and paracellular pathways (Liu et al., 2011; Bourzac et al, 2013; Hamilton et al, 2013).

d. Glucose Concentration and Transporters Saturation

The intraluminal concentration of glucose after a meal varies from 0.8 to 45 mM (Wright et al., 1994) and the concentration at the brush border membrane may be much higher. It has been recognized for a number of years that the apical uptake phase has two distinct elements: a saturable, phlorizin-sensitive (SGLT1) fraction and a diffusive component, suggesting that more than one transporter may be involved (Alzaid et al., 2013).

The traditional hypothesis describes accurately glucose absorption measured at low luminal concentrations of glucose (< 30 mM) where absorption occurs via SGLT1 through active transport. However, once the glucose concentration in the intestine is beyond the transport saturation of SGLT1 (for example after a carbohydrate rich meal), this theory cannot explain the marked increase in glucose absorption at luminal concentrations of glucose (> 30 mM) even when SGLT1 is saturated (Wei et al., 2011; Gorboulev et al, 2012; Zheng and Sarr, 2012).

Two theories have been proposed to explain the mechanism behind this other component of glucose absorption, when the glucose concentration in the intestine is beyond the transport saturation of SGLT1. The first, proposed by Pappenheimer & Reiss (1987), has been termed "paracellular flow" or "solvent drag". It postulates that enterocytes undergo a conformational change in response to a greater luminal concentration of glucose. Thus, tight junctions coupled with large water and solute shifts, open between enterocytes and lead to an augmented influx of glucose into the paracellular space where it is transported into the cell along a concentration gradient.

The second, proposed by Kellett and colleagues (2001), suggests that under conditions of high luminal glucose concentrations, SGLT1 becomes saturated and promotes GLUT2 recruitment to the enterocyte apical membrane. There, it can scavenge more glucose from the lumen via a signaling mechanism initiated by activation of SGLT1 through protein kinase C (PKC) and thus can contribute significantly to glucose absorption (Göstemeyer 2010; Wei et al., 2011; Scow et al., 2011; Chaudhry et al., 2011; Alzaid et al., 2013). Some studies demonstrated the presence of apical GLUT2 in perfused, isolated intestinal segments but not in everted intestinal sleeves (Röder et al., 2014).

Several investigators have confirmed, however, (Barone et al., 2009; Dyer et al., 2009; Moran et al., 2010; Batchelor et al., 2011; Gorboulev et al., 2012) that GLUT2 is involved in glucose efflux across the basolateral membrane only, where the protein is located exclusively. In addition, evidence is presented that SGLT1 mediates the majority of glucose transport across the BBM of enterocytes independent of the glucose load (Röder et al., 2014).

2. Gastrointestinal Regulation of Glucose Homeostasis

Intestinal absorption is controlled by neural and hormonal mechanisms. There is extensive interaction with the higher brain centers (brainstem and hypothalamus), via autonomic nervous system. The nervous system, also, influences the release of hormones from gastrointestinal endocrine cells (Bird et al., 1996).

a. Neural Control

The autonomic nervous system plays an important role in the control of gastrointestinal function. It consists of three branches: two of them constitute the extrinsic supply made of the sympathetic and the parasympathetic divisions; the third branch is the enteric nervous system, which constitutes the main intrinsic neural supply of the GI tract. Both intrinsic and extrinsic innervations control the functions of the GI tract, including sensing nutrients, absorption, secretion, motility and regulation of the blood flow (Goyal and Hirano, 1996; Benarroch, 2007, Mourad and Saadé, 2011)

The enteric nervous system is composed of myenteric and submucosal plexuses located in the muscularis and under the mucosa parts of the GI tube, respectively. The parasympathetic efferent supply to the upper GI tract is made essentially of the vagal nerve and it exerts, usually, excitatory effects on the functions of the GI. Preganglionic parasympathetic fibers are cholinergic and synapse in the myenteric and submucosal plexuses. They send axonal terminals to the smooth muscles, secretory and endocrine cells of the GI tract (Aziz and Thompson, 1998; Goyal and Hirano, 1996).

The sympathetic efferents exert, usually, inhibitory effects on the functions of the GI tract. Preganglionic sympathetic fibers synapse in the prevertebral ganglia. Postganglionic sympathetic adrenergic fibers leave the prevertebral ganglia and synapse in the myenteric and submucosal plexuses. Direct postganglionic adrenergic innervation of blood vessels and some smooth muscles also occurs (Goyal and Hirano, 1996; Aziz and Thompson, 1998).

Glucose uptake by enterocytes evokes enteric (Raybould and Zittel, 1995) and enteropancreatic reflexes (Kirchgessner et al., 1996). Intestinal vagal and spinal afferent nerve fibers are sensitive to glucose (Grundy and Scratcherd 1989; Mei 1978). As illustration, infusion of glucose activates myenteric neurons and increases the number of Fos-immunoreactive neurons in the submucosal plexus of duodenum and jejunum. Thus, these neurons participate in monitoring the chemical milieu of the intestine and initiating reflexes in response to nutrient stimuli (Sayegh et al., 2004; Mourad and Saadé 2011).

Functional evidence has demonstrated that the regulation of glucose transport into enterocytes is induced by the sensing of sugar by the enteroendocrine cells through activation of sweet taste receptors (T1R2 and T1R3) and their associated elements of G-protein-linked signaling pathways (e.g. α-gustducin, phospholipase C β type 2 and transient receptor potential channel M5) (Merigo et al., 2011).

b. Capsaicin Sensitive Primary Afferents

Sensory afferents innervating the GI tract run along sympathetic and parasympathetic fibers. They are made essentially of unmyelinated or thinly myelinated fibers, which belong to the group of capsaicin sensory primary afferents (CSPA). Part of

these fibers run through the vagal nerve to project at the level of the medulla oblongata; the remaining fibers enter the spinal cord through the dorsal roots.

In the duodenum and small intestine, the CSPA fibers provide sensory feedback to regulate several gut functions including motility, secretion and absorption (Lloyd et al, 1993).

CSPA fibers play an important role in the ability of the small intestine to adapt to changes in carbohydrate dietary composition. Such an adaptive response takes a few days to become evident as the change in the nutrient transporter expression occurs in crypt cells and therefore the alteration becomes evident after the migration of these cells to the villi (Ferraris 2001; Ferraris and Diamond, 1997).

Resiniferatoxin (RTX) is a naturally occurring chemical found in resin spurge (Euphorbia resinifera), a cactus-like plant commonly found in Morocco; it is also found in Euphorbia poissonii located largely in northern Nigeria. It is an ultrapotent analog of capsaicin (8 methyl-N-vanillyl-6-nonenamide), the pungent algesic substance contained in red pepper plants belonging to the genus Capsicum. Resiniferatoxin is 500-1000 times more potent than capsaicin in desensitizing the capsaicin-sensitive primary afferents to a subsequent challenge with capsaicin itself (Maggi 1990).

CSPA fibers ablation decreases basal jejunal glucose absorption in vivo whereas capsaicin inhibits, in a dose dependent manner, SGLT1 mediated uptake of glucose by jejunal strips in vitro (Barada et al., 2008).

c. Vagal Innervation

Vagal nerves regulate the physiological functions of the upper gastrointestinal (GI) tract. The vagal nerve and its branches innervate the GI tract from the lower esophageal sphincter to the upper GI tract, and the extensive distribution of fibers to the gut plays an important role in the regulation of many digestive functions including food intake, gastric accommodation and GI motility. The vagal nerves predominantly act on the stomach and small intestine rather than the lower GI tract, and subdiaphragmatic vagotomy results in a transient slowing of the rate of gastric emptying and transit of the upper small intestine (Lee et al., 2012). Furthermore, subdiaphragmatic vagotomy and vagal deafferentiation abolish the diurnal increase in SGLT1 protein expression that is seen in normal rats (Stearns et al., 2008). The reduced gastric activity and food intake following vagotomy is dependent on the digestibility and/or composition of the diet. Vagotomy reduces thermogenic responses to carbohydrate, probably as a result of impaired insulin release (Andrews et al., 1985).

d. Flavonoids Inhibitory Effects

Flavonoids are a large group of natural polyphenols that are widely distributed in foods, especially fruits and vegetables. In general, dietary flavonoids are attached to sugar residues, which affect the mechanism of absorption by altering their physico-chemical properties and thus their ability to enter cells (Day et al., 2003).

Phlorizin belongs to the group of dihydrochalcones, a type of flavonoids, and it has been shown to induce experimental glycosuria. The entry step of glucose across the apical membrane via SGLT1 is inhibited by phlorizin. Addition of phlorizin (an inhibitor

of SGLT1) from mucosal side inhibited both glucose accumulation in the tissue and its release into serosal fluid (AU et al., 2002).

Phloretin is another member of dihydrochalcones found in apple leaves and the Manchurian apricot. Phloretin is an inhibitor of basolateral glucose GLUT2 transporter. It decreases release of glucose from enterocytes into serosal fluid (AU et al., 2002).

Quercetin is relatively more abundant than phloretin, high concentrations of which can be found, for instance, in tea, apples and onions (Cermak et al., 2004). Quercetin has been shown to inhibit non-competitively GLUT2-mediated glucose and fructose transport in injected oocyte (Kwon et al., 2007).

Finally, flavonoids represent a therapeutic method for reducing sugar absorption in metabolic syndrome and type II diabetes.

D. Purpose of the Study

The study aims to characterize the relative contribution of SGLT1 and GLUT2 to glucose absorption under various glucose concentrations by using the method of intraluminal perfusion of a jejunal loop, *in-vivo,* and during the first minute of incubation of jejunal strips *in-vitro*. Moreover, the study aims to elucidate the neural pathways that are involved in modulating the expression and/or the function of the glucose transporters SGLT1 and GLUT2 in jejunal epithelial cells in response to changing intraluminal glucose concentrations. This investigation was performed on rats subjected to various forms of chemical or surgical ablation of gut innervations, and in the jejunum of control rats. Furthermore, the study aims to determine if CSPA fibers exert their regulatory effect on the mRNA expression of SGLT1, GLUT2, both or none in the jejunum of rats.

CHAPTER II

MATERIALS AND METHODS

A. Animal groups

Adult Sprague-Dawley rats (n=200) weighing 220-250g fed regular lab chow ad libithium were used in all experiments. Rats were fasted for 18 hours before the day of the experiment, with free access to water. Before any surgery, rats were anesthetized by an intraperitoneal (IP) injection of ketamine (75-100mg/kg) and xylazine (10mg/kg). Chemical or surgical denervation of various components of the nervous system will be performed as detailed below. Control rats were treated with the vehicle or subjected to sham surgery.

B. Determination of Glucose Uptake

1. Glucose Absorption in-vivo

Glucose absorption was measured using the single pass intraluminal perfusion technique. The anesthetized rat was tracheostomized in order to allow for adequate ventilation. The abdomen was opened longitudinally by a midline incision and the jejunum was exposed with its blood supply kept intact. A jejunal loop was isolated and cannulated by an inlet inserted 5 cm distal to the ligament of Treitz, and an outlet inserted 15-20 cm distal to the inlet and fixed by ligation (Nassar et al, 1995). The contents of the loop were emptied by flushing the perfused intestine with warm phosphate buffered saline (PBS). The intestine was returned to the abdominal cavity that was covered by gauze soaked in phosphate buffered saline (PBS) in order to avoid tissue dehydration.

The cannulated intestinal loop was perfused using a peristaltic pump at a flow rate of 0.7 ml/min for a period of 3 hours. The perfusate consisted of Ringer solution (phosphate buffered solution composed of 0.14M NaCl and 0.01M K_2HPO_4, pH=7.4) containing various concentrations of D-glucose (1, 5, 20, 50 or 100 mM), trace amount of radioactive [^{14}C]-D-glucose (0.125μCi/L) and 17mg/L phenol red that is a non-absorbable indicator used to correct for changes in glucose concentration as a result of variation in fluid transport.

The first twenty minutes of perfusion ensured the establishment of a steady state in glucose absorption and equilibration of the jejunal lumen, after which 10 min collections of the effluent were made for three hours. After an hour from starting the jejunal perfusion, phloretin or phloridzin dihydrate (1mM) dissolved in 100% ethanol, or quercetin dissolved in 1M NaOH, were added to the perfusion solution and maintained for one hour, then removed during the third hour. The rat was euthanized by an overdose of anesthesia and the length of the perfused intestinal segment was measured.

The eighteen samples of effluent were analyzed immediately. One-milliliter aliquots of the initial and effluent solutions were assayed for phenol red concentration and radioactivity content according to the method described by Schedl, 1966; Bierhioff et al, 1988; Nassar et al, 1995. Glucose absorption in the different groups was calculated from the rate of disappearance of radiolabeled D-glucose in the initial perfusate, taking into consideration fluid secretion measured by any change in phenol red absorbance according to the equation:

$$Glucose\ absorption = \left(G_{p} - \frac{G_{pe} \times \lambda_{p}}{\lambda_{e}}\right) \times \frac{C \times rate\ of\ Perfusion}{G_{p} \times Length}$$

Where, $\mathbf{G_p}$ and $\mathbf{G_e}$ represent the measured counts per minute (cpm) of the radioactive carbon in the perfusate and the effluent solutions, respectively; $\boldsymbol{\lambda_p}$ & $\boldsymbol{\lambda_e}$ represent the absorbance at 560nm of phenol red in the perfusate and effluent solutions, respectively; and **C** represents the glucose concentration in mM. Glucose absorption is expressed as µmol/ 20min-cm.

2. Glucose Transport in-vitro

Experiments (n=100 rats) were performed to study glucose uptake in the presence or absence of various concentrations of phlorizin, the selective inhibitor of SGLT1, or the selective inhibitors of GLUT2, phloretin or quercetin. Three different groups of rats were used: Control rats, sham rats treated with the vehicle and rats subjected to CSPA fibers ablation.

The abdominal cavity of an anaesthetized rat was opened, and the jejunum was excised and placed in ice-cold PBS solution. It was freed from its mesenteric and fat attachments, opened along the mesenteric line, and cut longitudinally into strips each 1 cm in length. Isolated jejunal strips (weighing 50-80 mg) from control rats and rats subjected to ablation of CSPA fibers were pre-incubated for five minutes in oxygenated PBS containing phlorizin, phloretin, quercetin or PBS. Various concentrations of D-Glucose (1 mM, 20 mM and 50 mM) and a trace amount of [^{14}C]-D-glucose were added for 1-minute in the medium and shaken in a water bath at a temperature of 37^{o}C. 1-minute

uptake by jejunal strips was determined because this reflects mostly unidirectional uptake of glucose across the brush border membrane.

After 1-minute incubation, the strips were removed and immediately dipped into ice-cold isotonic mannitol solution. Each strip was blotted with whatman no.1 filter paper, its wet weight was determined, and then the tissue was extracted in 2 ml of 0.1 M HNO_3 for at least four hours. Aliquots of the tissue extracts and incubation media were counted for their [^{14}C]-glucose content in a liquid scintillation counter. The intracellular concentration of glucose was calculated as follows:

$$\text{Intracellular Concentration} = \frac{1000 \text{ X } D \text{ X } \frac{G_e}{G_i}}{dw}$$

Where, G_i and G_e represent the measured counts per minute (cpm) of the radioactive carbon in the incubation solution and the aliquots of the tissue extracts, respectively; 1000 represent the volume in μL taken from extracted tissue in HNO_3; dw represents the dry weight of the tissue (wet weight*0.2); and D represents the diffusion factor: for 1, 20 and 50 mM Glucose, factors are 0.192, 3.84 and 9.6 respectively.

3. Determination of SGLT1 and GLUT2 mRNA Expression in Intestinal Mucosal Scrapings

RNA Extraction

RNA was extracted using RNeasy Plus Mini Qiagen kit. Mucosal scraping obtained from the jejuna of the rats (n=12) were immediately frozen in liquid nitrogen and stored at -80˚C for further experiments (Nassar et al.,1995). Following the

manufacturer's instructions of the kit, about 20-30 mgs of the mucosal scraping were transferred to a conical tube and 400 µL of the lysis buffer were added. The mix was dounced 100-150 times, vortexed, and then centrifuged at 13,200 rpm for 3 minutes. The supernatant was transferred to a gDNA eliminator spin column followed by a centrifugation at 10,400 rpm for 30 seconds. 400 µL of cold 70% ethanol was added to the flow and 700 µL of the mix were transferred to an RNeasy spin column and centrifuged at 10,400 rpm for 30 seconds. The same column was transferred to new collection tubes and the washes were added: 700 µL RWI then 500 µL RPE were added and the mix was centrifuged at 10,400 rpm for 30 seconds each time. Then 500 µL RPE were added and the mix was centrifuged at 10,400 rpm for 2 minutes. The mix is transferred to a new collection tube with the addition of 30 µL RF water, left for 5 mins on ice, then centrifuged at 10,400 rpm for 1 minute.

RNA was quantified in the Nanodrop ND1000-(Spectrophotometer) (Bio-Rad Laboratories Ltd. Hemel Hempstead, UK) using a 260/280nm absorbance ratio method to assess the purity of RNA. Pure RNA is stored at -80˚C for further experiments.

RT- PCR

Prior to Real Time PCR, mRNA was reversed transcribed to cDNA. cDNA was synthesized from 1 µg of the total RNA using RevertAid First strand cDNA synthesis Kit #K1621 (Thermo Scientific) samples. According to the manufacturer's instructions, the components of the kit were thawed, mixed and centrifuged then stored on ice. Template RNA (total RNA) (1 µg) and primer (oligo $(dT)_{18}$ primer) (1 µl) were added into a sterile nuclease-free tube and a total volume of 12 µl was obtained by adding nuclease-free

water. The vial was gently mixed, centrifuged at 7,500 RPM for 5 minutes, incubated at 65˚C for another 5 minutes and chilled on ice. After spinning down the vial, the following components were added in the indicated order: 5x Reaction buffer (4 µl), Ribolock RNase inhibitor (20u/µl) (1 µl), 10mM dNTP mix (2 µl), RevertAid MuLV Reverse transcriptase (200u/ µl) (1 µl), thus a total volume of 20 µl was obtained. The mix was incubated at 42°C for one hour for the reaction to take place, and then incubated at 70°C for 5 minutes to stop the reaction.

Real-time PCR was performed using Real-time PCR biorad CFX96. The expressions of both SGLT1 and GLUT2 genes were evaluated in the samples of mucosal scrapings. For the housekeeping gene, the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was assessed. The sequences of the primers used in this study are listed below:

- Fw SGLT1: 5’TCTTCGCTATCAGCGTCGTC3’
- Rw SGLT1: 5’GTAGACTCCAGCACAGACGG3’
- Fw GLUT2: 5’ATGTCAGAAGACAAGATCACCG3’
- Rw GLUT2: 5’ACACCGATGTCATATCCGAACT3’
- Fw GAPDP: 5'GGGGCTCTCTGCTCCTCCCTG3'
- Rv GAPDH: 5'CGGCCAAATCCGTTCACACCG3'

The reaction mix was prepared in a single tube to ensure homogenous distribution of reagents in the wells. Amplification of cDNA was performed using SYBR green Qiagen kit. For the real time PCR, 12.5 µL 2x QuantiFast SYBR Green PCR

Master Mix, 1 µL reverse primer, and 1 µL forward primer were added to each PCR tube and the final volume was adjusted to 23 µL by adding RNase-free water. 23 µL of the mix were loaded in the wells, and 2 µL of the template cDNA samples containing the reaction mix (the volume of cDNA added from the undiluted reverse-transcription reaction should not exceed 10% of the final PCR volume) were added later. The plate was very well sealed then a short spin down was done.

The real-time cycler was programmed as follow:

Step	**Time**	**Temperature**
PCR initial heat activation	5 min	95˚C
Denaturation	10 sec	95˚C
Combined annealing/extension	30 sec	54˚C
Number of cycles	35-40 cycle	

The annealing temperature was adjusted according to the melting temperature (T_m) of the primers used, then the PCR plate was placed in the cycler and the cycling program was started. Relative RNA expression was calculated using the delta–delta threshold cycle method and normalized to GAPDH expression in the same cDNA sample (Yan S et al, 2013).

C. Extrinsic Denervation Procedures

1. Chemical ablation of Capsaicin-Sensitive Primary Afferents

Ablation of the CSPA fibers in adult rats was achieved by repetitive subcutaneous injections of capsaicin according to the following sequence: 50mg/kg day 1, and 30mg/kg day 2 and 3. Capsaicin was dissolved in 10% Tween 80, 10% olive oil and 80 % PBS. Sham control rats received a vehicle injection (McCafferty et al, 1997).

In some groups of rats, resiniferatoxin (RTX), an ultrapotent analog of capsaicin, was used to ablate CSPA fibers, because it was more potent and cheaper than capsaicin. RTX (Sigma, St. Louis, MO) was dissolved in 100% ethanol to the concentration of 1 mg/mL (Hang et al., 2011). Rats received single intraperitoneal (IP) injection of RTX (100 μg/kg/ml) under light anesthesia. RTX was dissolved in a mixture of 10% Tween 80, 10% pure ethanol (100%) and 80 % normal saline (Pan et al., 2003; Pan et al, 2003; Hang et al., 2011).

Two weeks post-treatment, verification of desensitization to capsaicin and RTX was done by using the eye-wiping test in response to capsaicin (Hammond and Ruda, 1991; Holzer 1991). All capsaicin-treated rats that did not react to topical corneal application of capsaicin (negative eye-wipe response), confirming effective capsaicin-mediated desensitization, were included in the experiments (Gram et al., 2005).

2. Subdiphragmatic Vagotomy

The abdominal cavity (n=32) was exposed through a midline incision exposing the intestine, the stomach and the lower part of the esophagus was exposed by pulling the stomach. Using an operating microscope, a subdiaphragmatic vagotomy was performed by isolating and cutting the anterior and lateral branches of the descending vagus nerves running on the wall of the esophagus. After completion of the surgical procedure, the abdominal cavity was closed and the rats were left to recover for one to two weeks.

D. Data Collection and Statistical Analysis

The rates of glucose absorption, in *in-vivo* experiments, are expressed as mean ±SEM in micromoles per centimeter per 20 min of intestinal segment length at different time intervals (every 20 min). Intracellular concentrations in jejuna strips, in *in-vitro* experiments, are expressed as mean ±SEM in micromoles per gram of dry-weight per 1 minute of incubation. Comparisons between groups were made by unpaired-t test depending on experimental protocols. In multiple comparisons, ANOVA was used followed by Bonferroni post hoc test as appropriate. GraphPad Prism 6 and Instat 3 software were used for statistics and graphics (GraphPad Software, San Diego, CA).

qPCR results are analyzed using the ΔΔCt method. For each sample, the difference is calculated between the threshold cycle (Ct) for each gene of interest GOI (SGLT1 and GLUT2) and the housekeeping gene HKG (GAPDH): ΔCt = Ct (GOI) – Ct (HKG). Then, for each pair-wise set of samples to be compared, the ΔΔCt is calculated as follows: ΔΔCt = ΔCt (experimental) – ΔCt (control). The fold change is calculated in gene expression as follows: Fold change = 2^(- ΔΔCt).

E. Materials

Ketamine was obtained from Panpharma and xylazine was obtained from Interchemie Werken. Capsaicin, RTX, phloretin, phloridzin dihydrate and quercetin were obtained from Sigma Chemical Company (St Louis, Missouri, USA). Radiolabelled [^{14}C]-D-glucose was obtained from Amersham International (Buckinghamshire, UK).

CHAPTER III

RESULTS

A. In-vitro study: Dose-dependent Influx in Strips from Normal Rats and Rats Subjected to Chronic CSPA Ablation

A series of experiments were performed to determine the relative contribution of SGLT1 and GLUT2 transporters to glucose uptake *in-vitro* for 1-minute because this reflects more unidirectional uptake of glucose across the brush border membrane.

1. Effect on 1 mM Glucose Uptake

a. Role of SGLT1

Phlorizin, the selective SGLT1 antagonist, reduced 1 mM glucose uptake by jejunal strips in a dose dependent manner (n=6). At concentrations of 0, 10, 25 and 100 μM phlorizin, uptake was 2.99±0.02, 0.67±0.05, 0.50±0.05, and 0.49±0.07 μmol/ min-gdw (n=6 rats), respectively. Compared to control, this inhibition varied between 72 up to 90%, with an average of 78% by 10 μM phlorizin (Fig 2).

In rats subjected to CSPA fibers ablation, basal glucose absorption was reduced by about 55% ($P<0.001$). As shown in fig. 2, in the presence of 10, 25 and 100 μM phlorizin, the uptake of 1 mM glucose was reduced from 1.35±0.03 to 0.46±0.01, 0.41±0.01 and 0.37±0.01 μmol/ min-gdw, respectively (n=10 rats).

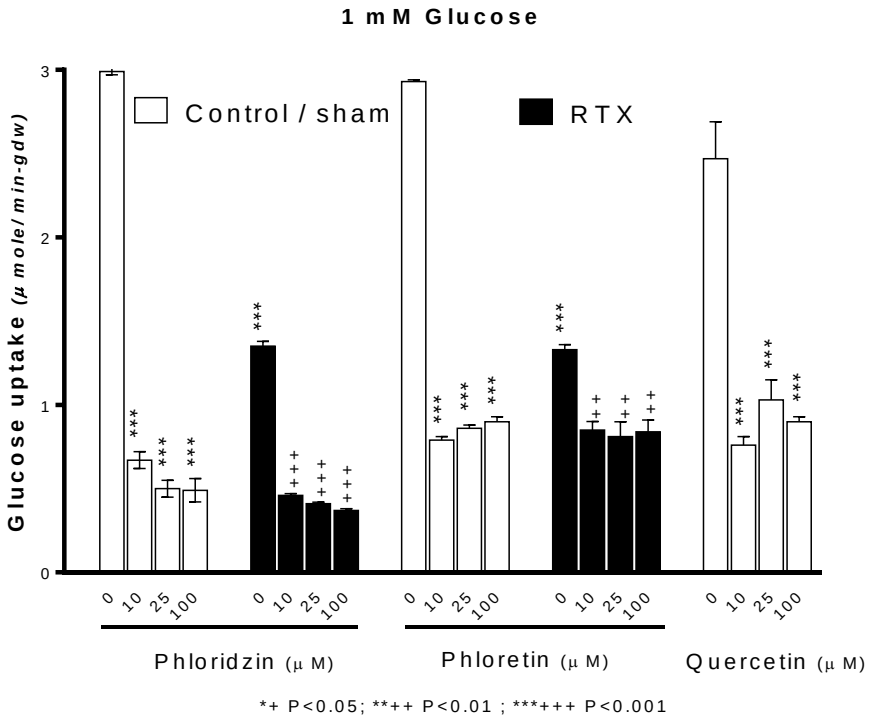


Figure 2: Effect of various concentrations of phlorizin, phoretin and quercetin on 1 mM glucose uptake in sham or in rats subjected to CSPA ablation.

Jejunal strips isolated from sham (n=6) or CSPA ablated (n=10) were pre-incubated in saline or in saline containing different concentrations of the antagonists. Note the significant alteration of the effects of low concentrations of phlorizin, phloretin and Quercetin (10 to 25 μM) on 1 mM glucose uptakes. The significance of difference was determined with reference to sham (*) or RTX treated (+) values by ANOVA followed by Bonferroni post hoc.

b. Role of GLUT2

In the presence of phloretin, the selective GLUT2 antagonist, glucose uptake was almost equally reduced by about 72%. At the concentrations of 0, 10, 25 and 100 μM

phloretin, the uptake was 2.95±0.01, 0.79±0.02, 0.86±0.02 and 0.90±0.03 μmol/ min-gdw, respectively (n=7 rats). In rats subjected to CSPA fibers ablation, this effect was relatively attenuated, but still significant (n=7) (fig. 2).

Similarly, Quercetin, another selective GLUT2 antagonist, reduced glucose uptake in vitro in normal rats. At concentrations of 0, 10, 25 and 100-μM quercetin uptake was 2.72±0.17, 0.70±0.03, 0.94±0.07, and 0.97±0.02 μmol/ min-gdw, respectively (n=6 rats), (fig. 2).

2. Effect on 20 mM Glucose Uptake

a. Role of SGLT1

In sham rats, the addition of 10-μM phlorizin reduced 20 mM glucose uptake by about 67% (from 28.3 ± 0.26 to 14.56 ± 0.61, n= 6 rats). At the concentrations of 25 and 100-μM phlorizin, uptake was reduced to 9.80±0.70 and 10.39±0.28 μmol/min-g.dw, respectively (n=6 rats).

As shown in figure 3, the uptake of 20 mM glucose was reduced by about 30% in rats subjected to CSPA fibers ablation ($P<0.001$). The effect of phlorizin on 20 mM glucose uptake was moderately attenuated by CSPA ablation, with an average inhibition of ~50% (n=8).

b. Role of GLUT2

In sham rats, when GLUT2 inhibitors, phoretin or quercetin, were added to the incubation medium, 20 mM glucose uptake was reduced by 48% (n=6) and 69% (n=7), respectively. At concentrations of 25 and 100-μM phloretin, uptake was 13.30±0.61 and

10.51±0.60 μmol/min-g.dw (n=6 rats). When rat jejunal strips were incubated with glucose in the presence of quercetin, there was a more significant decrease (67%) than that observed with phloretin; the maximum effect was observed with lowest concentration of quercetin (10 μM) (Fig. 3).

In rats subjected to chemical ablation of their CSPA, the effect of phloretin on 20 mM glucose uptake was negligeable for the lowest concentrations, reaching significance level at 100 μM phloretin (n=7) (Fig. 3).

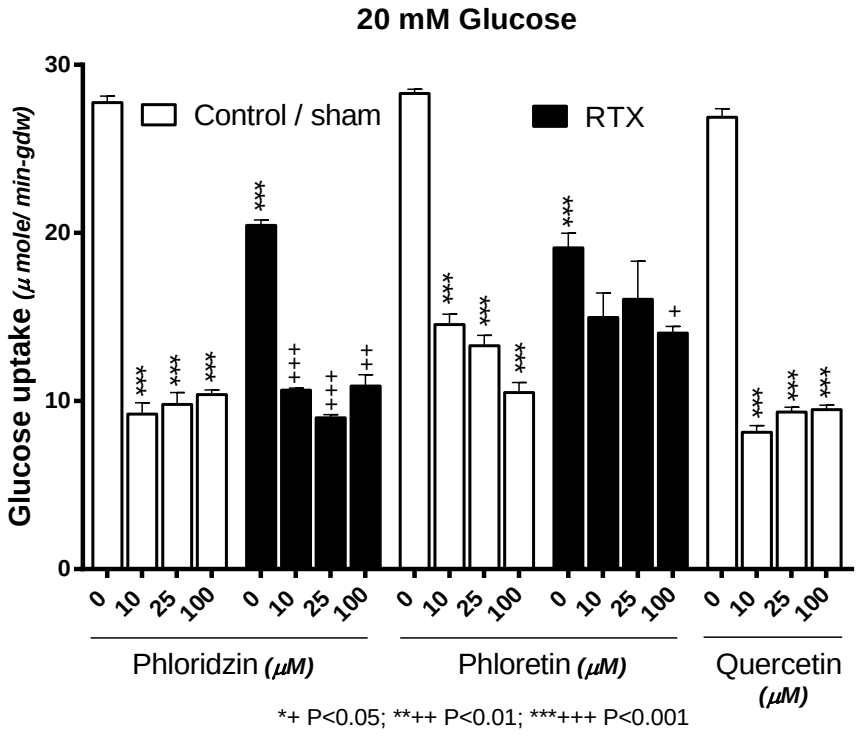


Figure 3: Effect of various concentrations of phlorizin, phoretin and quercetin on 20 mM glucose uptake in sham or in rats subjected to CSPA ablation.

Jejunal strips were pre-incubated in saline or in saline containing different concentrations of the antagonists. 20 mM glucose was added for 1 minute to the medium before removing the strips and determining their content in glucose.

Results are means + SEM from 4-8 rats in each set of experiments. Each bar represents the average + SEM of determinations made on strips isolated from the rats. The significance of difference was determined with reference to sham(*) or RTX treated(+) values by ANOVA followed by Bonferroni post hoc.

3. Effect on 50 mM Glucose Uptake

a. Role of SGLT1

One-minute uptake of 50 mM glucose was 38.42±0.60 (n=13) μmol/min-gdw (n=13) in control strips and was reduced to 26.63±1.71 μmol/min-gdw by 10-μM phlorizin, a 31% decrease from control level (fig. 4).

However, ablation of CSPA fibers by capsaicin in adult rats had no effect on one-minute uptake of 50 mM glucose absorption (n=9) (<0.01). The significance between sham and CSPA ablated fibers rats was only pronounced at 25 μM Phlorizin, a 25% inhibition of the glucose absorption.

b. Role of GLUT2

In the presence of 10-μM phloretin (n=5), uptake of 50 mM glucose in sham rats strips was 28.61±1.95 and that of quercetin (n=7) was 21.90±0.93 μmol/min-gdw, a 38% and 42% decrease respectively (Fig. 4).

Ablation of CSPA fibers by capsaicin in adult rats, a significant effect was only pronounced at 10 and 100-μM phloretin, a 38% and 14% inhibition, respectively (n=5) (Fig. 4).

Further confirmation of the action of capsaicin on the active and passive transport mechanisms is provided on high glucose concentration uptake in jejunal strips

isolated from rats subjected to chemical ablation of their CSPA. The reduction was most evident on the uptake of 1 mM glucose (Fig. 2), while the effect of transporters antagonists on 20 and 50 mM glucose uptake was reduced, moderately altered by CSPA ablation (Fig.3 and 4).

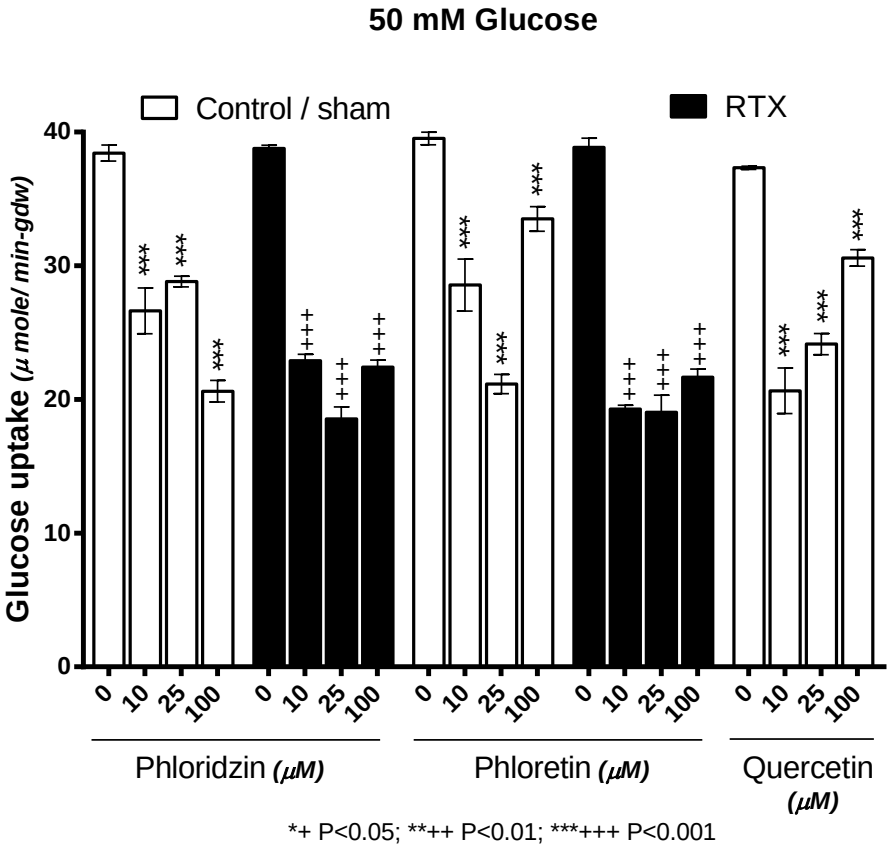


Figure 4: Effect of various concentrations of phlorizin, phoretin and quercetin on 50 mM glucose uptake in sham or in rats subjected to CSPA ablation.

Jejunal strips were pre-incubated in saline or in saline containing different concentrations of the antagonists. 50 mM Glucose was added for 1 minute to the medium before removing the strips and determining their content in glucose. Results are means + SEM from 5-8 rats in each set of experiments. Each bar represents the average + SEM of determinations made on jejunal strips. The significance of difference was determined with reference to sham(*) or RTX treated(+) values by ANOVA followed by Bonferroni post hoc.

B. In-vivo Absorption of Glucose at Various Intrajejunal Concentrations and the effect of Transporters Inhibitors

1. Jejunal Absorption of 20 mM glucose in normal rats and rats subjected to chronic CSPA ablation

a. Role of SGLT1

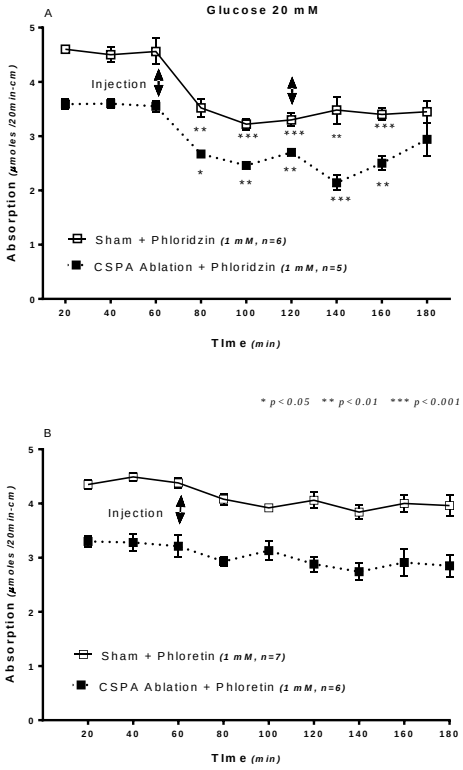
In sham rats, when 1 mM phlorizin was added to the perfusate in the second hour, 20 mM glucose absorption was reduced by 24% (n=6) (fig. 5b). The antagonist's effect persisted for another 60 min despite stopping perfusion with a solution containing phloridzin. Ablation of CSPA fibers by capsaicin in adult rats decreased basal absorption of 20 mM glucose from 13.21±0.22 to 10.07±0.25 μmoles/20min-cm (fig. 5a), a 24% inhibition (n=6, $p<0.01$). When 1 mM phlorizin (n=6) was added to the perfusate for one hour in those rats, the glucose absorption was almost equally reduced compared to sham rats.

b. Role of GLUT2

Intraluminal perfusion of 1 mM phloretin in the second hour in normal rats reduced glucose uptake by 9% (n=7) (fig. 5B). Although not significant, this reduction became evident within minutes of starting the antagonist perfusion and reached significance during the second hour, after stopping its perfusion. Adding 1 mM phloretin to the perfusate for one hour, in rats subjected to CSPA fibers ablation, elicited comparable effects to those observed in sham (n=10) (fig. 5B).

Figure 5: Absorption of 20 mM glucose and effect of intraluminal perfusion of 1 mM phloretin (a) or 1 mM phloridzin (b) in vivo in sham rats and rats subjected to CSPA fibers ablation.

Glucose absorption was determined every 20 min, averaged for each time interval and plotted as absorption per 20 min per cm of jejunum. Each bar represents the means + SEM of measurements made on a different group of rats. Phlorizin and phloretin perfusion was started at 60 min and maintained for 1 hour, as illustrated by arrows. The significance of variations was measured with reference to the control value (*) or RTX treated (+) in each experimental group.



2. Jejunal Absorption of 50 mM glucose in normal rats and rats subjected to chronic CSPA ablation

a. <u>Role of SGLT1</u>

Intraluminal perfusion of 1 mM phlorizin for 1 hour in sham rats reduced 50 mM glucose absorption by about 29% (n=5) (Fig. 6A). This reduction started within minutes of adding the antagonist, and persisted for another 60 min despite stopping its perfusion. In rats subjected to CSPA fibers ablation, 50 mM glucose absorption was reduced from 33.92±0.68 to 19.08±0.38 µmoles/h - 20min-cm, a 40% inhibition (n=5, $p<0.001$, Fig. 6B). Perfusion of 1 mM phlorizin for one hour in those rats reduced further glucose absorption by 38%, an effect that lasted for a third hour despite stopping phlorizin perfusion (Fig. 6B).

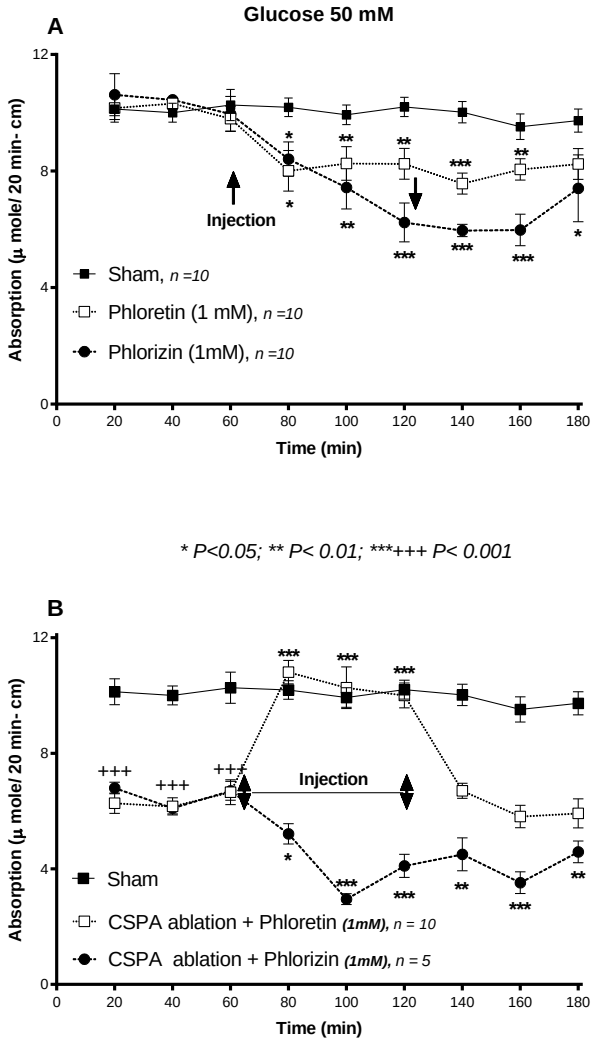


Figure 6: Time course of the absorption of 50 mM glucose and effect of intraluminal perfusion of 1 mM phlorizin or 1 mM phloretin in sham rats (A) and rats subjected to CSPA fibers ablation (B).

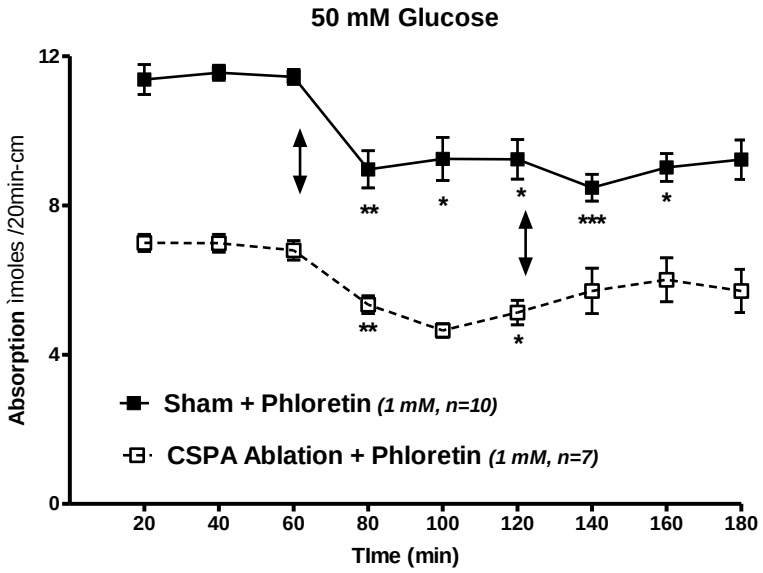
Glucose absorption was determined every 20 min, averaged for each time interval and plotted as absorption per 20 min per cm of jejunum. Data in each panel are means + SEM of measurements made on 3 different groups of rats. Phlorizin or phloretin perfusion was started at 60 min and maintained for 1 hour, as illustrated by arrows. The significance of variations was determined with reference to the control p in each experimental group.

b. Role of GLUT2

When 1 mM phloretin was added to the perfusate for 1 hour in sham rats, 50 mM glucose absorption was reduced by about 18% for a period outlasting the perfusion time (Fig. 6A). However, in rats subjected to CSPA fibers, intraluminal perfusion of 1 mM phloretin elicited contradictory effects that were observed in, a relatively, two large samples of treated animals. In the first group of CSPA ablated rats (n=10), intraluminal phloretin (1mM) for one hour induced a full recovery of glucose absorption to the levels

observed in sham controls. This recovery persisted during the perfusion time and vanished immediately after stoping intraluminal phloretin (Fig 6B).

In a second group of treated rats (n=7), intraluminal phloretin (1mM) for one hour induced significant decrease in glucose absorption that started within 20 min ($P<0.01$) and stopped at the end of perfusion.



* P<0.05; ** P<0.01; *** P<0.001

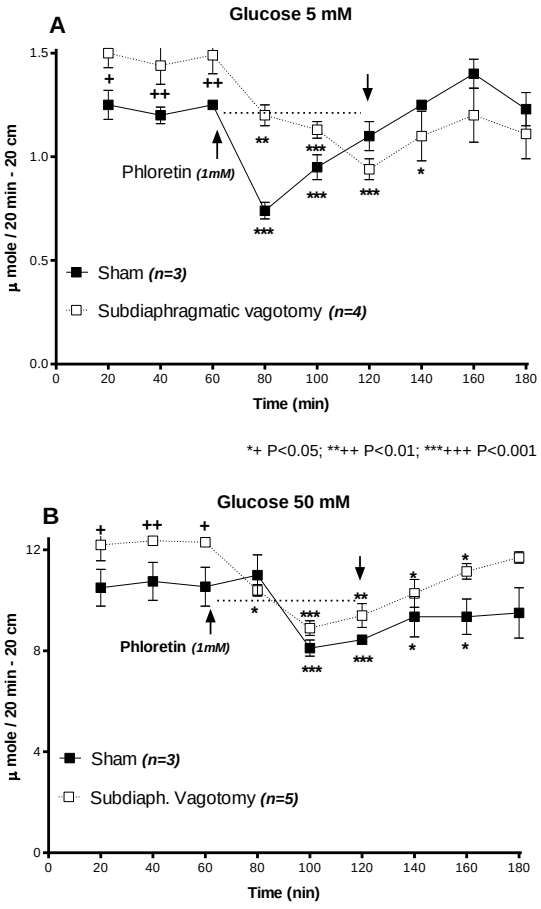
Figure 7: Time course of the of intraluminal perfusion of 1 mM phloretin on the absorption of 50 mM glucose in sham rats and rats subjected to CSPA fibers ablation.

Glucose absorption was determined every 20 min, averaged for each time interval and plotted as absorption per 20 min per cm of jejunum. Data in each curve are means + SEM of measurements made on 2 different groups of rats. Phloretin perfusion was started at 60 min and maintained for 1 hour, as illustrated by arrows. The significance of variations was determined with reference to the control in each experimental group.

3. Effect of Subdiaphragmatic Vagotomy on glucose absorption

Subdiaphragmatic vagotomy induced 35% and 27% increase in glucose absorption following intraluminal 5 mM (n=8) and 50 mM (n=11) glucose perfusion, respectively (Fig.8). Adding 1 mM phloretin to the intraluminal perfusate, over 1 h period, induced sustained decrease of glucose absorption, which was comparable to that observed in sham treated rats (Fig.8).

Figure 8. Time course of glucose absorption and the effect of intraluminal perfusion of 1 mM phloretin in sham rats and rats subjected to subdiaphragmatic vagotomy. *Glucose absorption was determined every 20 min, averaged for each time interval and plotted as absorption per 20 min per cm of jejunum. Data are means + SEM of measurements made on 2 different groups of rats. Phlorizin and phloretin perfusion was started at 60 min and maintained for 1 hour, as illustrated by arrows. The significance of variations was measured with reference to the control value in each experimental group.*



C. mRNA Expression of SGLT1 and GLUT2 in Normal and CSPA Ablated Rats

Experiments were performed to determine the effect of CSPA fibers on mRNA expression of glucose transporters. mRNA expressions of SGLT1 and GLUT2 were determined in mucosal scraping of jejunal samples by real-time PCR with GAPDH as the housekeeping gene. Preliminary results of the first trials demonstrate a 52 % decrease in SGLT1 and 47% decrease in GLUT2 mRNA expression in capsaicin-treated rats as compared to sham (fig. 9).

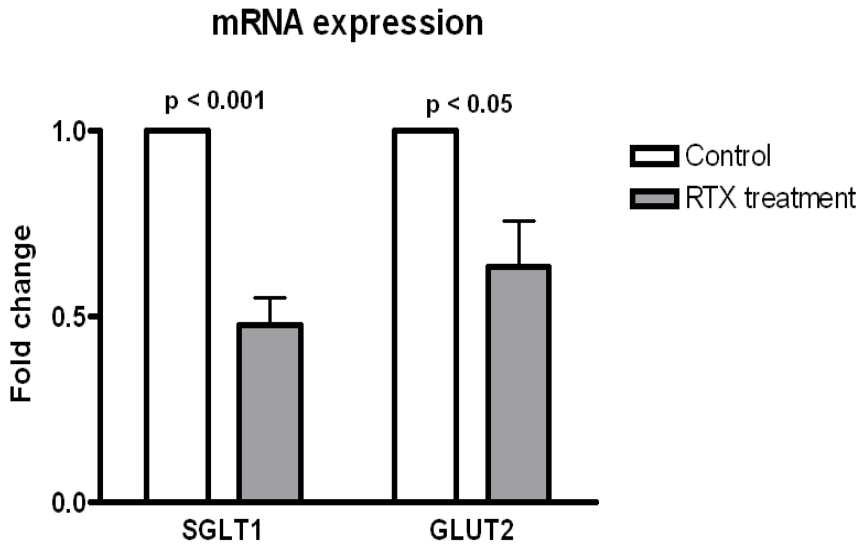


Figure 9. Relative changes in the mRNA expression of SGLT1 and GLUT2 determined by real-time PCR in normal and CSPA ablated rats (n=6).

The expression of each gene was normalized to that of GAPDH. Data are presented as fold change in RTX treated rats compared with the expression level in normal rats. Bars represent the mean ± SE.

The mean fold change was calculated in each sample by the $2^{-\Delta\Delta CT}$ *method. mRNA expression was significantly lower for SGLT1 and GLUT2 by 0.48 and 0.53-fold, respectively.*

CHAPTER IV

DISCUSSION

Intestinal glucose absorption is an integral component of overall glucose homeostasis in health and disease and depends on a complex network of neural and humoral interactions. Our study suggests that both glucose transporters GLUT2 and SGLT1 play a key role in glucose absorption in-vitro and in-vivo, at both low and high glucose concentrations. This role is modulated by CSPA fibers.

Our *in-vitro* data suggest that SGLT1 and GLUT2 contribute almost equally to glucose uptake across the brush border membrane of jejunal epithelial cells at very low glucose concentrations. As the concentration of glucose in the incubation medium is increased, the contribution of both SGLT1 and GLUT2 decreases. Our findings of glucose uptake by GLUT2, at low concentration in the incubation medium, is a new observation and does not appear to be in line with traditionally held views on the role of GLUT2. The data may not be explained by the inhibitory effect of phloretin on basolateral GLUT2, which would be expected to increase -rather than decrease - intracellular glucose concentration. Similarly to Phloretin, Quercetin - another GLUT2 antagonist - was equally effective in reducing unidirectional glucose uptake across the jejunal strips. This decrease in intracellular glucose concentration in response to Phloretin or Quercetin supports the proposition that GLUT2 has been recruited to the BBM.

In rats that were desensitized to capsaicin, basal jejunal uptake of low glucose concentrations was substantially reduced. As the concentration of glucose was increased, this desensitization effect decreased, and at 50 mM glucose, it was completely abolished.

Ablation of CSPA fibers altered mostly the effects of GLUT2 antagonist at 1 and 20 mM glucose. However, at 50 mM, CSPA ablation did not alter glucose influx or the effects of SGLT1 and GLUT2 antagonists (Fig. 2, 3 and 4). This suggests the involvement of a CSPA-independent transport mechanism at work at high concentrations of glucose.

Overall, the in vitro data suggest that both SGLT1 and GLUT2 contribute to jejunal glucose uptake at low glucose concentrations. On the other hand, ablation of CSPA fibers seems to be associated with reduced activity of SGLT1 and minimal effect on GLUT2. This suggests that CSPA fibers have a basal glucose pro-absorptive tone. This appears to be in contrast to *in-vivo* data in terms of the effects of the transporter antagonists and CSPA fiber ablation on jejunal glucose absorption.

In vivo studies of the effect of antagonists on glucose [20 mM] absorption showed that phlorizin reduced absorption by ~25%, whereas phloretin reduced it by less than 10%. Similarly, absorption of 50 mM glucose was reduced more by phlorizin (29%) than by phloretin (18%). Thus, the contribution of SGLT1 to glucose absorption seems to be more than that of GLUT2 when intraluminally perfused with glucose at low and high glucose concentrations. This discrepancy is further enhanced by the results of CSPA fiber ablation. This showed a more important decrease of absorption with high glucose concentrations.

Furthermore, ablation of CSPA fibers reduced significantly basal jejunal glucose absorption *in-vivo*. The effect was more pronounced with 50 mM glucose (40% inhibition) than with 20 mM glucose (24% inhibition). Preliminary data from our lab showed that capsaicin ablation had no significant effect on the *in-vivo* absorption of lower glucose concentrations (1 mM).

The inhibitory effect of SGLT1 antagonists was not altered by CSPA ablation, while that of GLUT 2 elicited contradictory effects with intraluminal 50 mM glucose, with a steady inhibition in one experimental set (n=7 rats) and a reversal of CSPA decreasing absorption (proabsorptive role) in another experimental set (n=10 rats). The overall results suggest that ablation of CSPA fibers reduces the activity of SGLT1. Clarification of the effect on GLUT 2 requires further testing, but it is likely that GLUT2 activity is also reduced in rats that are desensitized to capsaicin.

The results of subdiaphragmatic vagotomy can illustrate the ambiguity in the role of extrinsic innervation: CSPA afferents seem to have a proabsorptive tone, removed by CSPA ablation (decrease in absorption in-vitro and in-vivo) and efferent or reflex anti-absorptive tone revealed by increased glucose uptake following vagotomy. These observations can be correlated with preliminary results of decrease of SGLT1 and GLUT2 mRNA expression following CSPA ablation.

In conclusion, this study provides new findings about the role of extrinsic innervation and modulation of glucose transport and transporters. The regulation of glucose transporters implies a role of CSPA fibers in sensing glucose concentrations. Decreased glucose absorption following ablation of CSPA fibers is supported by alterations of glucose transporters mRNA expressions. Furthermore, GLUT2 plays a key role in glucose influx into enterocytes at low glucose concentrations. Whether this process occurs through an increase in the intrinsic activity of GLUT2 expressed basally, or through activation of an intracellular secondary messenger system, which causes rapid insertion of GLUT2 transporters to the apical membrane, cannot be determined based on

our data. The complex regulatory mechanisms of glucose homeostasis as revealed by some contradictory findings from in-vitro and in-vivo experiments need further investigations.

REFERENCES

Ait-Omar, A., Monteiro-Sepulveda, M., Poitou, C., Le Gall, M., Cotillard, A., Gilet, J., et al. (2011). GLUT2 accumulation in enterocyte apical and intracellular membranes: A study in morbidly obese human subjects and ob/ob and high fat-fed mice. *Diabetes, 60*(10), 2598-2607.

Alzaid, F., Cheung, H., Preedy, V. R., & Sharp, P. A. (2013). Regulation of glucose transporter expression in human intestinal caco-2 cells following exposure to an anthocyanin-rich berry extract. *PloS One, 8*(11), e78932.

Andrews, P. L., Rothwell, N. J., & Stock, M. J. (1985). Effects of subdiaphragmatic vagotomy on energy balance and thermogenesis in the rat. *The Journal of Physiology, 362*, 1-12.

Aschenbach, J., Steglich, K., Gäbel, G., & Honscha, K. (2009). Expression of mRNA for glucose transport proteins in jejunum, liver, kidney and skeletal muscle of pigs. *Journal of Physiology and Biochemistry, 65*(3), 251-266.

Au, A., Gupta, A., Schembri, P., & Cheeseman, C. (2002). Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. *Biochem.J, 367*, 247-254.

Aziz, Q., & Thompson, D. G. (1998). Brain-gut axis in health and disease. *Gastroenterology, 114*(3), 559-578.

Bajaj-Elliott, M., & Sanderson, I. R. (2008). Physiology and pathophysiology ii.

Barada, K., Karrowni, W., Abdallah, M., Shamseddeen, W., Sharara, A. I., & Dakik, H. A. (2008). Upper gastrointestinal bleeding in patients with acute coronary syndromes: Clinical predictors and prophylactic role of proton pump inhibitors. *Journal of Clinical Gastroenterology, 42*(4), 368-372.

Barone, S., Fussell, S. L., Singh, A. K., Lucas, F., Xu, J., Kim, C., et al. (2009). Slc2a5 (Glut5) is essential for the absorption of fructose in the intestine and generation of fructose-induced hypertension. *The Journal of Biological Chemistry, 284*(8), 5056-5066.

Batchelor, D. J., Al-Rammahi, M., Moran, A. W., Brand, J. G., Li, X., Haskins, M., et al. (2011). Sodium/glucose cotransporter-1, sweet receptor, and disaccharidase expression in the intestine of the domestic dog and cat: Two species of different dietary habit. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology, 300*(1), R67-75.

Benarroch, E. E. (2007). Enteric nervous system: Functional organization and neurologic

implications. *Neurology, 69*(20), 1953-1957.

Bierhoff, M. L., & Levine, G. M. (1988). Luminal and metabolic regulation of jejunal amino acid absorption in the rat. *Gastroenterology, 95*(1), 63-68.

Bird, A. R., Croom, W. J.,Jr, Fan, Y. K., Black, B. L., McBride, B. W., & Taylor, I. L. (1996). Peptide regulation of intestinal glucose absorption. *Journal of Animal Science, 74*(10), 2523-2540.

Bourzac, J., L'eriger, K., Larrivée, J., Arguin, G., Bilodeau, M. S., Stankova, J., et al. (2013). Glucose transporter 2 expression is down regulated following P2X7 activation in enterocytes. *Journal of Cellular Physiology, 228*(1), 120-129.

Cermak, R., Landgraf, S., & Wolffram, S. (2004). Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. *British Journal of Nutrition, 91*(06), 849-855.

Chaturvedi, L. S., & Basson, M. D. (2013). Glucagonlike peptide 2 analogue teduglutide: Stimulation of proliferation but reduction of differentiation in human caco-2 intestinal epithelial cells. *JAMA Surgery, 148*(11), 1037-1042.

Chaudhry, R. M., Scow, J. S., Madhavan, S., Duenes, J. A., & Sarr, M. G. (2011). Acute enterocyte adaptation to luminal glucose: A posttranslational mechanism for rapid apical recruitment of the transporter GLUT2. *Gastroenterology, 140*(5), S-997-S-998.

Chichger, R. F. (2011). *Regulation of Renal Brush-Border Glucose Transport in Response to Metabolic Dysregulation,*

Day, A. J., Gee, J. M., DuPont, M. S., Johnson, I. T., & Williamson, G. (2003). Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: The role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochemical Pharmacology, 65*(7), 1199-1206.

Dyer, J., Al-Rammahi, M., Waterfall, L., Salmon, K. S., Geor, R. J., Bouré, L., et al. (2009). Adaptive response of equine intestinal na /glucose co-transporter (SGLT1) to an increase in dietary soluble carbohydrate. *Pflügers Archiv-European Journal of Physiology, 458*(2), 419-430.

Ferraris, R. P. (2001). Dietary and developmental regulation of intestinal sugar transport. *Biochemical Journal-London-, 360*(2), 265-276.

Ferraris, R. P., & Diamond, J. (1997). Regulation of intestinal sugar transport. *Physiological Reviews, 77*(1), 257-302.

Gorboulev, V., Schurmann, A., Vallon, V., Kipp, H., Jaschke, A., Klessen, D., et al.

(2012). Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes, 61*(1), 187-196.

Göstemeyer, A. (2010). Glucose transport at the renal brush border membrane: GLUT2 and the involvement of PKC-βI.

Goyal, R. K., & Hirano, I. (1996). The enteric nervous system. *New England Journal of Medicine, 334*(17), 1106-1115.

Gram DX, Hansen AJ, Wilken, M., Elm T, Svendsen O, Carr RD, Ahren B. (2005). Plasma calcitonin gene-related peptide is increased prior to obesity, and sensory nerve desensitization by capsaicin improves oral glucose tolerance in obese Zucker rats. Eur. J. Endocrinol. **153**: 963-969.

Grundy, D., & Scratcherd, T. (1989). Food for thought--nutritional problems in space. *Journal of the British Interplanetary Society, 42*(7), 374-377.

Gruzdkov, A. A., Gromova, L. V., Grefner, N. M., & Komissarchik, Y. Y. (2012). Kinetics and mechanisms of glucose absorption in the rat small intestine under physiological conditions. *Journal of Biophysical Chemistry, 3*(2).

Hamilton, K. L., & Butt, A. G. (2013). Glucose transport into everted sacs of the small intestine of mice. *Advances in Physiology Education, 37*(4), 415-426.

Hammond, D. L., & Ruda, M. (1991). Developmental alterations in nociceptive threshold, immunoreactive calcitonin gene- related peptide and substance P, and fluoride- resistant acid phosphatase in neonatally capsaicin- treated rats. *Journal of Comparative Neurology, 312*(3), 436-450.

Herrmann, J., Schröder, B., Klinger, S., Thorenz, A., Werner, A., Abel, H., et al. (2012). Segmental diversity of electrogenic glucose transport characteristics in the small intestines of weaned pigs. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 163*(1), 161-169.

Holzer, P. (1991). Capsaicin: Cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol Rev, 43*, 143-201.

Hummel, C. S., & Wright, E. M. (2011). Glucose reabsorption in the kidney.

Iqbal, C. W., Fatima, J., Duenes, J., Houghton, S. G., Kasparek, M. S., & Sarr, M. G. (2009). Expression and function of intestinal hexose transporters after small intestinal denervation. *Surgery, 146*(1), 100-112.

Jodal, M., Holmgren, S., Lundgren, O., & Sjöqvist, A. (1993). Involvement of the myenteric plexus in the cholera toxin-induced net fluid secretion in the rat small intestine. *Gastroenterology-Baltimore then Philadelphia-, 105*, 1286-1286.

Kellett, G., & Helliwell, P. (2000). The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. *Biochem.J, 350*, 155-162.

Kellett, G. L. (2001). The facilitated component of intestinal glucose absorption. *The Journal of Physiology, 531*(3), 585-595.

Kellett, G. L., Brot-Laroche, E., Mace, O. J., & Leturque, A. (2008). Sugar absorption in the intestine: The role of GLUT2. *Annu.Rev.Nutr., 28*, 35-54.

Khachab, M. Y., Chakass, D. N., Al Jeitani, S., & Nassar, C. F. (2011). Capsaicin-sensitive primary afferent (CSPA) fibers are involved in the regulation of glucose transport and the expression of its transporters in the small intestine in rats. *The FASEB Journal, 25*, 1065.5.

Kirchgessner, A. L., Liu, M., & Gershon, M. D. (1996). In situ identification and visualization of neurons that mediate enteric and enteropancreatic reflexes. *Journal of Comparative Neurology, 371*(2), 270-286.

Koeppen, B. M., & Stanton, B. A. (2009). *Berne & levy physiology* Elsevier Health Sciences.

Kwon, O., Eck, P., Chen, S., Corpe, C. P., Lee, J. H., Kruhlak, M., et al. (2007). Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology, 21*(2), 366-377.

Lam, C. K., Chari, M., & Lam, T. K. (2009). CNS regulation of glucose homeostasis. *Physiology (Bethesda, Md.), 24*, 159-170.

Lee, J. H., Kwon, O. D., Ahn, S. H., Choi, K. H., Park, J. H., Lee, S., et al. (2012). Reduction of gastrointestinal motility by unilateral thyroparathyroidectomy plus subdiaphragmatic vagotomy in rats. *World Journal of Gastroenterology: WJG, 18*(33), 4570.

Li, Y., Ji, D., Zhong, S., Lin, T., Lv, Z., Hu, G., et al. (2013). 1-deoxynojirimycin inhibits glucose absorption and accelerates glucose metabolism in streptozotocin-induced diabetic mice. *Scientific Reports, 3*

Liu, L., Yu, Y., Liu, C., Wang, X., Liu, X., & Xie, L. (2011). Insulin deficiency induces abnormal increase in intestinal disaccharidase activities and expression under diabetic states, evidences from in vivo and in vitro study. *Biochemical Pharmacology, 82*(12), 1963-1970.

Liu, R., Wei, N., Guo, W., Qiang, O., Li, X., Ou, Y., et al. (2013). Octreotide alleviates obesity by reducing intestinal glucose absorption and inhibiting low-grade

inflammation. *European Journal of Nutrition, 52*(3), 1067-1075.

Lloyd KCK, Holzer HH, Zittel TT, et al. (1993). Duodenal lipid inhibits gastric acid secretion by vagal, capsaicin-sensitive afferent pathways in rats. Am J Physiol; 264: G659-663.

Maggi, C., Theodorsson, E., Santicioli, P., Patacchini, R., Barbanti, G., Turini, D., et al. (1990). Motor response of the human isolated colon to capsaicin and its relationship to release of vasoactive intestinal polypeptide. *Neuroscience, 39*(3), 833-841.

Marks, J., Carvou, N. J., Debnam, E. S., Srai, S. K., & Unwin, R. J. (2003). Diabetes increases facilitative glucose uptake and GLUT2 expression at the rat proximal tubule brush border membrane. *The Journal of Physiology, 553*(1), 137-145.

McCafferty, D. M., Wallace, J. L., & Sharkey, K. A. (1997). Effects of chemical sympathectomy and sensory nerve ablation on experimental colitis in the rat. *The American Journal of Physiology, 272*(2 Pt 1), G272-80.

Mei, N. (1978). Vagal glucoreceptors in the small intestine of the cat. *The Journal of Physiology, 282*, 485-506.

Merigo, F., Benati, D., Cristofoletti, M., Osculati, F., and Sbarbati, A. (2011). Glucose transporters are expressed in taste receptor cells. *Journal of Anatomy*, 219: 243-252.

Migrenne, S., Marsollier, N., Cruciani-Guglielmacci, C., & Magnan, C. (2006). Importance of the gut–brain axis in the control of glucose homeostasis. *Current Opinion in Pharmacology, 6*(6), 592-597.

Moran, A. W., Al-Rammahi, M. A., Arora, D. K., Batchelor, D. J., Coulter, E. A., Ionescu, C., et al. (2010). Expression of na /glucose co-transporter 1 (SGLT1) in the intestine of piglets weaned to different concentrations of dietary carbohydrate. *British Journal of Nutrition, 104*(05), 647-655.

Mourad, F. H., & Saadé, N. E. (2011). Neural regulation of intestinal nutrient absorption. *Progress in Neurobiology, 95*(2), 149-162.

Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., et al. (1985). Sequence and structure of a human glucose transporter. *Science (New York, N.Y.), 229*(4717), 941-945.

Naftalin, R. J. (2012). Intestinal incretin responses to increased GLUT2 expression--chacun a son gout. *The Journal of Physiology, 590*(Pt 12), 2825-2826.

Nassar, C. F., Barada, K. A., Abdallah, L. E., Hamdan, W. S., Taha, A. M., Atweh, S. F.,

et al. (1995). Involvement of capsaicin-sensitive primary afferent fibers in regulation of jejunal alanine absorption. *The American Journal of Physiology, 268*(4 Pt 1), G695-9.

Pan, H. L., Khan, G. M., Alloway, K. D., & Chen, S. R. (2003). Resiniferatoxin induces paradoxical changes in thermal and mechanical sensitivities in rats: Mechanism of action. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 23*(7), 2911-2919.

Penicaud, L., Leloup, C., Fioramonti, X., Lorsignol, A., & Benani, A. (2006). Brain glucose sensing: A subtle mechanism. *Current Opinion in Clinical Nutrition and Metabolic Care, 9*(4), 458-462.

Raybould, H., & Zittel, T. (1995). Inhibition of gastric motility induced by intestinal glucose in awake rats: Role of na - glucose co- transporter. *Neurogastroenterology & Motility, 7*(1), 9-14.

Röder, P. V., Geillinger, K. E., Zietek, T. S., Thorens, B., Koepsell, H., & Daniel, H. (2014). The role of SGLT1 and GLUT2 in intestinal glucose transport and sensing. *PloS One, 9*(2), e89977.

Sabino-Silva, R., Mori, R., David-Silva, A., Okamoto, M., Freitas, H., & Machado, U. (2010). The na /glucose cotransporters: From genes to therapy. *Brazilian Journal of Medical and Biological Research, 43*(11), 1019-1026.

Sakar, Y., Meddah, B., Faouzi, M., Cherrach, Y., Bado, A., & Ducroc, R. (2010). Metformin-induced regulation of the intestinal D-glucose transporters. *Journal of Physiology and Pharmacology, 61*(3), 301.

Sayegh, A. I., Covasa, M., & Ritter, R. C. (2004). Intestinal infusions of oleate and glucose activate distinct enteric neurons in the rat. *Autonomic Neuroscience, 115*(1), 54-63.

Schedl, H. P. (1966). Use of polyethylene glycol and phenol red as unabsorbed indicators for intestinal absorption studies in man. *Gut, 7*(2), 159-163.

Schneeman, B. O. (2002). Gastrointestinal physiology and functions. *British Journal of Nutrition, 88*(S2), S159-S163.

Scow, J. S., Iqbal, C. W., Jones III, T. W., Qandeel, H. G., Zheng, Y., Duenes, J. A., et al. (2011). Absence of evidence of translocation of GLUT2 to the apical membrane of enterocytes in everted intestinal sleeves. *Journal of Surgical Research, 167*(1), 56-61.

Sellin, J. H. (2001). The pathophysiology of diarrhea. *Clinical Transplantation, 15*(s4), 2-10.

Serhan, M. F., & Kreydiyyeh, S. I. (2010). Insulin down-regulates the na< sup> </sup>/K< sup> </sup> ATPase in enterocytes but increases intestinal glucose absorption. *General and Comparative Endocrinology, 167*(2), 228-233.

Sharp, P. A., Debnam, E. S., & Srai, S. K. (1996). Rapid enhancement of brush border glucose uptake after exposure of rat jejunal mucosa to glucose. *Gut, 39*(4), 545-550.

Shirazi-Beechey, S., Moran, A., Batchelor, D., Daly, K., & Al-Rammahi, M. (2011). Glucose sensing and signalling; regulation of intestinal glucose transport. *Proceedings of the Nutrition Society, 70*(02), 185-193.

Shirazi-Beechey, S. P., Moran, A. W., Bravo, D., & Al-Rammahi, M. (2011). Nonruminant nutrition symposium: Intestinal glucose sensing and regulation of glucose absorption: Implications for swine nutrition. *Journal of Animal Science, 89*(6), 1854-1862.

Song, J., Kwon, O., Chen, S., Daruwala, R., Eck, P., Park, J. B., et al. (2002). Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. *The Journal of Biological Chemistry, 277*(18), 15252-15260.

Stearns, A. T., Balakrishnan, A., Rounds, J., Rhoads, D. B., Ashley, S. W., & Tavakkolizadeh, A. (2008). Capsaicin-sensitive vagal afferents modulate posttranscriptional regulation of the rat Na+/glucose cotransporter SGLT1. *American Journal of Physiology.Gastrointestinal and Liver Physiology, 294*(4), G1078-83.

Stumpel, F., Burcelin, R., Jungermann, K., & Thorens, B. (2001). Normal kinetics of intestinal glucose absorption in the absence of GLUT2: Evidence for a transport pathway requiring glucose phosphorylation and transfer into the endoplasmic reticulum. *Proceedings of the National Academy of Sciences of the United States of America, 98*(20), 11330-11335.

Uldry, M., Ibberson, M., Hosokawa, M., & Thorens, B. (2002). GLUT2 is a high affinity glucosamine transporter. *FEBS Letters, 524*(1), 199-203.

Wei, N., Liu, R., Ou, Y., Li, X., Qiang, O., Guo, W., et al. (2011). Effects of octreotide on glucose transporter type 2 expression in obese rat small intestine. *World Journal of Gastroenterology : WJG, 17*(39), 4434-4439.

Wilson-O'Brien, A. L., Patron, N., & Rogers, S. (2010). Evolutionary ancestry and novel functions of the mammalian glucose transporter (GLUT) family. *BMC Evolutionary Biology, 10*, 152-2148-10-152.

Wright, E., Loo, D., Hirayama, B., & Turk, E. (2006). Sugar absorption. *Physiology of*

the Gastrointestinal Tract (4th Ed.).New York: Elsevier, , 1653-1666.

Wright, E. M., Hirayama, B., Loo, D., Turk, E., & Hager, K. (1994). Intestinal sugar transport. *Physiology of the Gastrointestinal Tract, 2*, 1751-1772.

Wright, E. M., Martín, M. G., & Turk, E. (2003). Intestinal absorption in health and disease—sugars. *Best Practice & Research Clinical Gastroenterology, 17*(6), 943-956.

Wright, E. M., Loo, D. D., Panayotova-Heiermann, M., Lostao, M. P., Hirayama, B. H., Mackenzie, B., et al. (1994). 'Active' sugar transport in eukaryotes. *The Journal of Experimental Biology, 196*, 197-212.

Yan, S., Sun, F., Li, Z., Xiang, J., Ding, Y., Lu, Z., et al. (2013). Reduction of intestinal electrogenic glucose absorption after duodenojejunal bypass in a mouse model. *Obesity Surgery, 23*(9), 1361-1369.

Yoshikawa, T., Inoue, R., Matsumoto, M., Yajima, T., Ushida, K., & Iwanaga, T. (2011). Comparative expression of hexose transporters (SGLT1, GLUT1, GLUT2 and GLUT5) throughout the mouse gastrointestinal tract. *Histochemistry and Cell Biology, 135*(2), 183-194.

Zheng, Y., & Sarr, M. G. (2012). Translocation of transfected GLUT2 to the apical membrane in rat intestinal IEC-6 cells. *Digestive Diseases and Sciences, 57*(5), 1203-1212.