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Original Research

Coping With Hypoxia: Adaptation of Glucose Transport Mechanisms Across Equine Jejunum Epithelium

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ABSTRACT

In horses, strangulation of the intestine often leads to hypoxia in jejunal loops resulting in irreversible lesions of the epithelium. This contrasts with the common view that this epithelium is able to adapt to different oxygenation conditions well. To get a better understanding of the pathomechanisms of hypoxic injury, our study aimed to investigate functional adaptation processes in equine jejunum epithelium. Therefore, we incubated jejunal epithelia of horses in Ussing chambers and simulated hypoxia by gassing with 1% oxygen or by incubation with the chemical hypoxia-mimetic $CoCl_2$. Because glucose is an important fuel for the cells' metabolism and thus its import is even more important under hypoxia, we assessed glucose transport mechanisms by measuring its electrogenic transport via sodium-coupled glucose transporter (SGLT1) and the transepithelial transport of ^{14}C-glucose and ^{14}C-ortho-methyl-glucose (OMG) as well as mRNA expression of glucose transporters. We could show that electrogenic uptake of glucose into the epithelium via SGLT1 was downregulated under hypoxia as was the mRNA expression of SGLT1 after $CoCl_2$ incubation. However, transepithelial transport of glucose and OMG was not altered. Thus, there must be alterations in transepithelial transport mechanisms securing an energy-independent uptake of glucose into the epithelial cells. We believe that these changes are probably mediated on protein level as we found only effects of prolonged $CoCl_2$ incubation on gene expression while the functional effects observed occurred much earlier.

1. Introduction

Colic is a common cause of death in horses. Because of the wide mesentery suspension of the jejunum, incarcerations, strangulations, or volvuli, that is, constriction of whole intestinal loops, often occur in this part of the equine gastrointestinal tract [1]. The constriction of loops is paralleled by a partial-to-complete occlusion of the supplying mesenterial blood vessels. The following hypoxia in the affected loops leads to an overall poor prognosis. Even after resection of the affected areas, complications like postoperative ileus, reperfusion damage, and/or endotoxemia occur [2,3]. The aim of medical treatment of colic patients is pain reduction on the one hand and inhibition or at least reduction of complications initiated by hypoxia and the following reperfusion on the other. It is under discussion whether hypoxia itself or reperfusion causes the more severe damage [4,5]. However, hypoxia is definitely the first noxa the epithelium has to cope with. Therefore, it is of crucial importance to know the pathomechanisms behind the intestinal tissue's reaction to hypoxia. Understanding the pathophysiology might as well indicate starting points for a more successful therapy. While a lot of attention has already been given to drugs modulating the intestinal motility and permeability to endotoxins after hypoxic insults in general [6,7], specific adaptation strategies of the epithelial cells are unknown yet. However, the intestinal epithelium plays a crucial role in the viability of the intestinal wall and thus the whole organism. The epithelial cells form the barrier to the outer environment and are responsible for the absorption of nutrients and maintenance of ion homoeostasis. Thus, their integrity is a prerequisite for the animal's survival. Epithelial cells are able to adapt to a varying oxygen supply under physiological circumstances, ranging from 30% of the total blood volume during food intake to 5% in the interdigestive period [8]; thus, they should be considered to cope with hypoxia well. Their

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adaptation potential should be of use under pathological conditions like hypoxia after strangulation ilei as well, but its initiation, mediation, and targets remain to be identified yet.

The so-called "master regulator of hypoxia signaling" [9], hypoxia inducible factor (HIF) [10], mediates an adaptation to hypoxia in most mammalian cells and is a transcription factor that consists of an α and a β subunit. While the β subunit is expressed constitutively in the nucleus of the cells, the α subunit is located in the cytoplasm, where it is marked for proteasomal degradation by oxygen-dependent hydroxylation by prolyl hydroxylases (PHD) [11,12]. Thus, HIF 1α is continuously degraded at physiological oxygen levels. With decreasing oxygen levels, HIF 1α is no longer degraded and can translocate into the nucleus where it forms a dimer and thus active transcription factor with the β subunit [9]. Many target genes of HIF that have been identified so far focus on the adaptation of the cells' energy metabolism. Under hypoxia, the cells have to switch from a predominant aerobic to an anaerobic metabolism, which is by far less efficient and thus needs more glucose for the same adenosine triphosphate (ATP) output [13,14]. Thus, a major adaptation mechanism to hypoxia could be an efficient uptake of glucose into the enterocytes.

Under physiologic conditions, there is a net transfer of glucose from the lumen to the blood. Luminal glucose import in the equine intestinal epithelium has been shown to be mediated by a sodium-coupled transport mechanism (SGLT1), which is driven by the sodium gradient established by the Na^+/K^+-ATPase, that is, secondary active [15,16]. Once inside the cell, glucose follows its concentration gradient into the blood and is exported via facilitated diffusion by GLUT2 [15,16]. Especially, the energy expense for the uptake of glucose from the gut lumen is a critical point under hypoxia. At the same time, glucose as the epithelial cells' main fuel besides glutamine must be available to a sufficient amount to secure the cells' energy supply and thus viability [17,18]. Therefore, it suggests itself that glucose transport is a core component to be adapted to hypoxia in intestinal epithelial cells similar to tumor cells. Thus, we wanted to elucidate modifications in glucose transport across the equine jejunum epithelium under hypoxia on the functional level using the Ussing chamber technique and on the mRNA level investigating the expression of (potential) HIF target genes with quantitative polymerase chain reaction (qPCR).

2. Material and Methods

2.1. Animals and Tissue Sampling

Horses (*Equus caballus*) of different age (mean age 11 years, ranging from 2 to 19 years), sex, and breed (standardbred, warmblood, and pony) were used. The horses were euthanized for clinical reasons unrelated to the gastrointestinal tract and donated for research or they were euthanized for studies conducted by other groups who did not need the gastrointestinal tract. The animals were first sedated with detomidine (60 μg/kg iv, Sedivet, Boehringer) and butorphanol (30 μg/kg iv, Alvegesic, CP Pharma). Then, anesthesia was induced with a mixture of diazepam (80 μg/kg iv, B Braun) and ketamine hydrochloride (2.5 mg/kg iv, Ursotamin Serumwerk Bernburg AG). To euthanize the horses, pentobarbital was administered iv to effect after a deep anesthesia was achieved (approximately 6 mL/50 kg iv, Intervet, Germany).

Directly after the death of the animal, an incision of approximately 15 cm was made in the *linea alba*, and the *lig. ileocecale* was located by palpation. Approximately 3 m proximal from there, ~1 m distal jejunum was excised, opened along the mesenteric border, and rinsed and submerged in 37°C warm oxygenated buffer solution before it was transported to the laboratory in the buffer solution gassed with oxygen. There, the mucosa was stripped off the underlying muscle and mounted in Ussing chambers as described by Gäbel et al [19]. The area exposed accounted for 1.1 cm^2. Before conducting the experiments, the epithelia were allowed to equilibrate in the system for at least 30 minutes.

2.2. Buffer Solutions and Gassing

The buffer solutions were prepared with chemicals obtained from Sigma-Aldrich (Darmstadt, Germany), Carl Roth (Karlsruhe, Germany), VWR (Darmstadt, Germany) or Merck (Darmstadt, Germany) unless stated otherwise. The gasses were procured from Linde Gas (München, Germany).

In all of the experiments, a basal buffer solution consisting of 120 mM NaCl, 5.5 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM Mg Cl, 0.6 mM NaH_2PO_4, 2.4 mM Na_2HPO_4, 10 mM glucose, 5 mM L-glutamine, and 10 mM HEPES was used for rinsing, preparation and transport of the epithelia. All other buffer solutions were based on this basal buffer solution, except that glucose was substituted by mannitol completely or in part for the glucose-free and the 3 mM glucose buffer solution, respectively, or by 3 mM ortho-methyl-glucose (OMG) in the respective buffer solutions. Mannitol was used to adjust the osmolarity to 280 ± 5 mOsm/L, except in the experiments to determine the transepithelial flux rate of mannitol. In these experiments, the concentration of mannitol amounted to 5 mM. The pH was adjusted to 7.4 using HCl or NaOH. All buffer solutions were gassed with 100% oxygen, except during the simulation of hypoxia by gassing.

2.3. Simulation of Hypoxia

We used two different approaches to simulate hypoxia *in vitro*. One was to switch gassing from 100% oxygen to 1% oxygen plus 99% nitrogen for one hour. In a second setup, we incubated the epithelia with 1 mM $CoCl_2$, an inhibitor of PHD and therefore stabilizer of HIF [20,21], to induce a so-called "chemical hypoxia." For practical reasons, epithelia were incubated with 1 mM $CoCl_2$ not only for 1 hour but for the complete incubation period after its addition (simultaneously to the change of gassing). To test for additional effects, we used all possible combinations of gassing and $CoCl_2$ incubation and denominated them as follows: 100% oxygen ("100%"), 100% oxygen plus incubation with $CoCl_2$ ("100% + $CoCl_2$"), 1% oxygen for 1 hour ("1%"), and also 1% oxygen for 1 hour plus incubation with $CoCl_2$ ("1% + $CoCl_2$").

2.4. Electrical Measurements

Electrical measurements were taken continuously with the aid of a computer-controlled voltage clamp device (Ingenieurbüro für Mess- und Datentechnik, Dipl.-Ing. K. Mußler, Aachen, Germany). The short-circuit current (I_{sc}) and transepithelial tissue conductance (G_t) were calculated computationally as described by Gäbel et al [19]. Changes of the transepithelial potential difference (PD_t) due to the application of bipolar current pulses (100 μA, 200 ms) were used to calculate transepithelial tissue conductance (G_t) according to Ohm's law. Based on the measured PD_t and the calculated G_t, active charge transfer across the epithelia was calculated ($I_{sc} = PD_t \times G_t$).

After an equilibration of approximately 30 minutes run under open-circuit conditions, an external current, equal to I_{sc} but directed oppositely, was continuously applied to clamp PD_t to 0 mV (short circuit). All experiments were conducted under short-circuit conditions.

The different treatments in each experiment were assigned to the individual epithelia within one animal according to their G_t so