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Claudin gene expression patterns do not associate with interspecific differences in paracellular nutrient absorption

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ABSTRACT

Bats exhibit higher paracellular absorption of glucose-sized molecules than non-flying mammals, a phenomenon that may be driven by higher permeability of the intestinal tight junctions. The various claudins, occludin, and other proteins making up the tight junctions are thought to determine their permeability properties. Here we show that absorption of the paracellular probe L-arabinose is higher in a bat (*Eptesicus fuscus*) than in a vole (*Microtus pennsylvanicus*) or a hedgehog (*Atelerix albiventris*). Furthermore, histological measurements demonstrated that hedgehogs have many more enterocytes in their intestines, suggesting that bats cannot have higher absorption of arabinose simply by having more tight junctions. We therefore investigated the mRNA levels of several claudins and occludin, because these proteins may affect permeability of tight junctions to macronutrients. To assess the expression levels of claudins per tight junction, we normalized the mRNA levels of the claudins to the constitutively expressed tight junction protein ZO-1, and combined these with measurements previously made in a bat and a rodent to determine if there were among-species differences. Although expression ratios of several genes varied among species, there was not a consistent difference between bats and non-flyers in the expression ratio of any particular gene. Protein expression patterns may differ from mRNA expression patterns, and might better explain differences among species in arabinose absorption.

1. Introduction

In the intestine, adjacent enterocytes are linked by tight junctions, which impede the movement of molecules across the epithelium and thereby form a barrier to solute flux. Tight junctions are complex structures composed of proteins such as claudins and occludin (OCLN) that span the cell membrane and interact in the extracellular space between cells, and also connect with intracellular scaffolding proteins via interactions with zonula occludens 1 (ZO-1) (Shen et al., 2011). These several proteins are thought to mediate the permeability characteristics of tight junctions, determining for example, the size and charge of solutes that can pass through the tight junction (Günzel and Yu, 2013). Many studies of tight junctions have focused on the movement of ions across the epithelium, although a few have investigated the permeability to larger, macronutrient-sized molecules. For example, overexpression and deletion studies of OCLN and claudin-1 (CLDN1) have shown that these genes are associated with increased permeability to mannitol (McCarthy et al., 2000; Van Itallie et al., 2001; Amasheh et al., 2002; Tamura et al., 2011), although this is not a consistent finding across all studies (see for example Schulzke et al., 2005). The effects of altered expression of any tight junction protein may be context-specific (depending on starting expression level, expression of other proteins, tissue type, etc.), and it is still not certain whether any claudins affect the permeability to macronutrients in a specific way (Günzel and Yu, 2013).

Variation in tight junction permeability might be able to provide a mechanistic explanation for the variation among species in their reliance on paracellular nutrient absorption. Whereas non-flying mammals such as rodents rely heavily on the transcellular, transporter-mediated pathway of glucose absorption, small birds and bats use the paracellular pathway (i.e., movement of glucose through tight junctions) for a majority of glucose absorption (Caviedes-Vidal et al., 2007; Karasov et al., 2012; Brun et al., 2014; Price et al., 2014). This has been hypothesized to help birds and bats compensate for their smaller intestines (Caviedes-Vidal et al., 2007; Price et al., 2015).

Although high paracellular glucose absorption has been documented in all bat species studied (Keegan, 1980, 1984; Tracy et al., 2007; Caviedes-Vidal et al., 2008; Fasulo et al., 2013a; Brun et al., 2014; Price et al., 2014), the mechanism by which this occurs is still unclear. Bats might simply have more tight junctions in their intestines, achieved either by greater villous amplification or smaller enterocytes. Alternatively, bats might have tight junctions that are more permeable to macronutrient-sized molecules. We recently suggested that both may be occurring

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(Price et al., 2014). Little brown bats (*Myotis lucifugus*) have a higher density of cells (and presumably tight junctions) than white-footed mice (*Peromyscus leucopus*), but the total number of enterocytes in the small intestine was lower. We suggested that the higher paracellular absorption of nutrients by little brown bats must instead be driven by characteristics of the tight junction, and we demonstrated differences in tight junction gene expression between the species. In particular, expression levels of CLDN1 and CLDN15 were higher, and the expression level of CLDN2 was lower, in little brown bat intestine compared to the white-footed mouse (Price et al., 2014).

This single species-pair comparison was intriguing, but begs further testing. For example, some of those expression differences might be related more to diet, phylogeny, or mere chance, than to the intestinal permeability characteristics associated with those species. In the present study, we therefore made integrated measurements on 3 more species: the insectivorous big brown bat (*Eptesicus fuscus*), the herbivorous meadow vole (*Microtus pennsylvanicus*), and the insectivorous hedgehog (*Atelerix albiventris*). These species provide a new comparison of a bat and a rodent, and the hedgehog provides a non-flying species that not only shares an insectivorous diet with the bat, but as part of the Laurasiatheria, is more closely related to the bat than to the vole (Nery et al., 2012). First, we demonstrate that the big brown bat has higher paracellular absorption of glucose-sized molecules than the non-flying species, thus confirming the pattern of high paracellular permeability in bats. Next we examine the anatomy and histology of the intestine, and show that it is unlikely that the high paracellular permeability in the bat can be explained by simply having more tight junctions than non-flyers. Finally, we measured tight junction gene expression in an attempt to understand how claudins and OCLN control paracellular permeability to glucose. For these gene expression measurements, we make comparisons among these three species and also with two previously measured species, the little brown bat (*M. lucifugus*) and the white-footed mouse (*P. leucopus*) (Price et al., 2014).

2. Methods

2.1. Animals

Big brown bats (*E. fuscus*) are common North American insectivorous bats (Kurta and Baker, 1990) (Table 1). We captured them in Dane County, Wisconsin, using mistnets placed near streams or over the exit of bats' day roosts in human habitations. Bats were used in experiments immediately following capture. We obtained domesticated hedgehogs (*A. albiventris*, all over 6 months age) from breeders in Wisconsin. Hedgehogs (order Erinaceomorpha) are primarily insectivorous (Santana et al., 2010), and were maintained on a diet of Purina Cat Chow Complete supplemented occasionally with mealworms (larvae of *Tenebrio molitor*). They were kept under 12 h:12 h L:D lighting conditions with food and water provided ad libitum between experiments. Meadow voles (*M. pennsylvanicus*) are common grassland rodents of the Midwest and northeastern United States and have a primarily herbaceous diet (Lindroth and Batzli, 1984). We captured meadow voles in the Biocore Prairie and community gardens of the Lakeshore Nature Preserve, University of Wisconsin-Madison. Voles were maintained under similar conditions as the hedgehogs except that their diet consisted of a commercial rodent chow (Purina 5010 Rodent Diet) supplemented daily with fresh produce (kale, carrots, and apples). No animals were obviously pregnant at the time of capture or testing. The sample sizes in Table 1 are for gene expression measurements. Smaller subsets of animals (noted in other tables and figures) were used for whole-animal and histology measurements so as to conserve resources. Experiments were approved by the University of Wisconsin-Madison Animal Care and Use Committee (#A1441). Bats and voles were caught with permission from the Wisconsin Department of Natural Resources (permits E/T 704 and SCP-SOD-2011).

Table 1
Animal attributes and gut measurements.

	Eptesicus fuscus	*Atelerix albiventris*	*Microtus pennsylvanicus*
N (#♂/#♀)	5/6	0/6	7/2
Body mass (g)	17.9 ± 1.1	439 ± 33	35.4 ± 4.1
Body length (cm; snout to base of tail)	7.1 ± 0.2	18.8 ± 0.8	10.86 ± 0.5
Small intestine length (cm)	12.4 ± 0.6[a]	54.1 ± 3.6[a]	26.3 ± 1.3
Small intestine circumference (mm)	6.58 ± 0.22	11.06 ± 0.19	7.59 ± 0.33
Cecum mass (g wet; including contents)	Absent	Absent	1.75 ± 0.17
Large intestine length (cm)	n.m.[a]	n.m.[a]	11.6 ± 0.73

[a] n.m. = not measured. In *E. fuscus* and *A. albiventris*, the large intestine is short and difficult to distinguish from the small intestine macroscopically. For these animals, the small intestine length listed in this table is the length of the whole intestine.

2.2. Measurement of paracellular nutrient absorption

We used two radiolabeled probes to assess nutrient absorption. L-arabinose (M_r 150) is a carbohydrate that is somewhat smaller than D-glucose (M_r 180) but its absorption is not transporter-mediated in bats or rodents (Lavin et al., 2007; Price et al., 2014). Its absorption was therefore used as an estimate of the paracellular component of D-glucose absorption. The difference between arabinose and glucose in molecular size likely causes this to be an overestimation, a point we consider further in our discussion. To estimate total glucose absorption, we used 3-O-methyl-D-glucose (3OMD-glucose), a molecule that is absorbed by both mediated and non-mediated mechanisms like D-glucose, but unlike glucose, is not substantially metabolized, and thus can be readily recovered. In the non-flying species, we also measured absorption of lactulose (M_r 342) and creatinine (Mr 113), and we present those data in Supplementary Table S1. Creatinine and lactulose absorptions were not assessed in the big brown bats due to a scarcity of experimental animals of that species.

Animals were dosed orally with [^{14}C]-L-arabinose and [^{3}H]-3OMD-glucose at the same time. The gavage vehicle was 50 mM glucose in water. Although Na^+ is required for Na^+-coupled glucose transport, Na^+ is secreted into the gut with bicarbonate and can readily diffuse from the blood (Brody, 1999). Thus, the lack of sodium in the gavage solution should not have affected our results, and indeed, glucose absorption was complete (see Results). After dosing, animals were placed in a metabolic chamber for collection of urine over the following several hours, where they had access to 50 mM glucose in water but no food. Voles and hedgehogs were placed in standard rat-sized wire-bottomed metabolic chambers, but for hedgehogs, we modified the chamber to have a smaller (5.7 mm) mesh size which seemed to make walking around the cage easier and more comfortable. Previously, we have favored a serial blood sampling technique in bats because of the difficulty of collecting urine (and separating it from feces) in bats (Tracy et al., 2007; Caviedes-Vidal et al., 2008; Brun et al., 2014). However, we tested and adopted a urine collection technique for bats in this study. We designed a small (15 × 15 × 5 cm) plastic metabolic chamber with metal screening glued to the top from which the bats could easily hang, and a sealable door near the bottom through which we could collect urine. The bats rarely produced feces during the experiments, and it was generally easy to separate from urine. Bats were occasionally offered water with or without glucose using a ball-tipped syringe, although they rarely drank. There were few urination events, and this likely led to some experimental variation, but this technique had some advantages over blood collection: 1) we were able to use the same calculation of fractional absorption for bats and the non-flying species, 2) we could avoid taking multiple blood samples from a small animal, and therefore, 3) we were able to use fewer bats because we could conduct two separate trials on individual bats.