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Intestinal perfusion indicates high reliance on paracellular nutrient absorption in an insectivorous bat *Tadarida brasiliensis*

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ABSTRACT

Flying vertebrates have been hypothesized to have a high capacity for paracellular absorption of nutrients. This could be due to high permeability of the intestines to nutrient-sized molecules (i.e., in the size range of amino acids and glucose, MW 75–180 Da). We performed intestinal luminal perfusions of an insectivorous bat, *Tadarida brasiliensis*. Using radio-labeled molecules, we measured the uptake of two nutrients absorbed by paracellular and transporter-mediated mechanisms (L-proline, MW 115 Da, and D-glucose, MW 180 Da) and two carbohydrates that have no mediated transport (L-arabinose, MW 150 Da, and lactulose, MW 342 Da). Absorption of lactulose (0.61 ± 0.06 nmol min^{-1} cm^{-1}) was significantly lower than that of the smaller arabinose (1.09 ± 0.04 nmol min^{-1} cm^{-1}). Glucose absorption was significantly lower than that of proline at both nutrient concentrations (10 mM and 75 mM). Using the absorption of arabinose to estimate the portion of proline absorption that is paracellular, we calculated that $25.1 \pm 3.0\%$ to $66.2 \pm 7.8\%$ of proline absorption is not transporter-mediated (varying proline from 1 mM to 75 mM). These results confirm our predictions that 1) paracellular absorption is molecule size selective, 2) absorption of proline would be greater than glucose absorption in an insectivore, and 3) paracellular absorption represents a large fraction of total nutrient absorption in bats.

1. Introduction

Water-soluble nutrients (e.g., glucose and amino acids) are absorbed at the intestine via the transcellular and paracellular pathways. The transcellular pathway, in which there is transporter-mediated absorption of nutrients through enterocytes, is thought to be dominant in humans and many other mammals. The paracellular pathway, in which nutrients move passively through the tight junctions between enterocytes, might be thought to be of minimal importance so that the intestinal epithelium can be a better barrier to the absorption of small water-soluble toxins (Diamond, 1997; Karasov, 2011). Nonetheless, there are several species in which the paracellular pathway is an important, even dominant, route of absorption (Caviedes-Vidal et al., 2007; Caviedes-Vidal et al., 2008; McWhorter et al., 2010). In particular, small birds (Chang and Karasov, 2004; McWhorter et al., 2010) and some bats (Tracy et al., 2007; Caviedes-Vidal et al., 2008; Fasulo et al., 2012) have a high capacity for paracellular nutrient absorption. In experiments in which bats were orally dosed with nutrient-sized molecules (i.e., in the size range of amino acids and glucose, approximately MW 75–180 Da) that can only be absorbed paracellularly (e.g., rhamnose, MW 164 Da, or arabinose, MW 150 Da), 62–100% of the dose was absorbed. Absorption of arabinose and rhamnose is comparatively lower in non-flying mammals (Lavin et al., 2007; McWhorter and Karasov, 2007; Karasov et al., 2012). It should be noted that paracellular absorption in these and other species is size-selective; larger molecules (e.g. cellobiose, MW 342) have much lower absorption (Tracy et al., 2007; Caviedes-Vidal et al., 2008; Fasulo et al., 2012).

This variation among species with regard to the capacity for paracellular absorption could arise from several sources. Higher paracellular absorption of nutrients could be due to greater contact time with the intestinal epithelium (Lannernäs, 1995) or differences in gastric evacuation rates. High paracellular absorption could also be a result of differences in epithelial permeability, that is, bats may simply have "leakier" tight junctions than other mammals. Lavin et al. (2007) found that high absorption of paracellularly-absorbed probes was still evident in isolated duodenal loops of birds, indicating that there may indeed be differences among species with regard to epithelial permeability.

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To determine the epithelial permeability to nutrients in bats, we conducted luminal perfusions of the intestine in the insectivorous Brazilian free-tailed bat (*Tadarida brasiliensis*). These experiments represent the first measurements of amino acid absorption in a bat. We predicted that the size selectivity observed in vivo in mammals and birds for paracellular probes would be evident in our isolated intestinal preparations. Therefore, the absorption of arabinose (MW 150) should be greater than that of lactulose (MW 342). Because previous in vivo experiments demonstrated complete absorption of orally dosed arabinose in this species (Fasulo et al., 2012), we also predicted that paracellular absorption would represent a substantial fraction of nutrient uptake. Finally, because *T. brasiliensis* is an insectivore we predicted that glucose absorption would be relatively low compared to proline absorption (Diamond and Buddington, 1987; Karasov and Diamond, 1988).

2. Materials and methods

Adult Brazilian free-tailed bats (*T. brasiliensis*) were captured on the campus of Universidad Nacional de San Luis, San Luis, Argentina. We used bats on the same day of capture in order to minimize stress associated with keeping bats in captivity. Average mass was 15.66 ± 0.26 g and nearly all bats had visible abdominal adipose stores. All bats were adults and the great majority was female (6M, 29F). All animal procedures adhered to institutional animal use regulations and approved animal use protocols by the Animal Care and Use Committee of the Universidad Nacional de San Luis.

To examine tissue-level absorption, we used in situ intestinal luminal perfusions. In vitro methods such as everted sleeves, which may be ideal for measuring mediated uptake into enterocytes, are not suitable for measuring paracellular absorption because paracellular convective fluid flow is negligible (Pappenheimer, 1998) and blood flow is absent. Paracellular absorption is thought to rely on fluid absorption powered by osmotic gradients across tight junctions generated by Na^+-coupled concentrative transport of sugar and/or amino acids (Pappenheimer, 2001), and villus blood flow is probably essential for washing away absorbed solute and maintaining a high gradient for movement into capillaries (Pappenheimer and Michel, 2003).

For a given bat, we used one of three perfusates, which were designed to be isosmotic but vary in nutrient (proline and glucose) concentration, with the variation in nutrient concentration offset primarily with sodium chloride (Table 1). Both nutrients were set at 75 mM, 10 mM or 1 mM. For each perfusion, buffers were labeled with a tracer amount of [1-^{14}C]-L-arabinose and a tracer amount of [2,3-^{3}H]-L-proline, [methyl-^{3}H]-3-*O*-methyl-D-glucose (3OMD-glucose), or [galactose 6-^{3}H]-lactulose. We used radiolabeled 3OMD-glucose (a nonmetabolizable D-glucose analogue) rather than D-glucose to avoid complications associated with metabolism had we instead used radiolabeled D-glucose. We will address the implications of this methodological point in the discussion.

Table 1
Perfusion buffer components.

	Low nutrient (mM)	Mid nutrient (mM)	High nutrient (mM)
D-Glucose	1	10	75
L-Proline	1	10	75
L-Arabinose	1	1	1
Lactulose	0	1	0
$NaHPO_4$	1.2	1.2	1.2
NaCl	116	110	65
KCl	5	5	2.5
$MgSO_4$	1	1	1
$CaCl_2$	2	2	1
$NaHCO_3$	20	20	5

All buffers were pH 7.4.

Anesthesia was used throughout the surgery and perfusion (0.8 L/min oxygen flow, 3.5–4% isoflurane during surgical preparation, 1.5–2% isoflurane during perfusion). Anesthetized bats were taped to a heating pad (Deltaphase Isothermal Pad, Braintree Scientific Inc., Braintree, MA, USA) that maintained a constant 37 °C. Once on a surgical plane, the abdominal cavity was opened and the intestine was cannulated ~1 cm from the stomach using a rat gavage needle as the cannula, which was secured with suture. The mesenteric vasculature was maintained intact throughout all procedures. An exit cannula was placed distally, with an attempt to perfuse as much of the intestine as possible. The intestine was then flushed with prewarmed saline for 15 min to remove its contents, using a perfusion pump (1 mL min^{-1}). The saline was removed from the system and the experimental perfusion was started with a flow rate of 1 mL min^{-1}. Upon exiting the intestine, the perfusate returned to a reservoir and was continuously recirculated. The reservoir was kept in a water bath at 37 °C. The perfusion continued for approximately 2 h (117 ± 1.65 min) and then the perfusate was collected.

The perfusate was carefully weighed before and after the perfusion. Subsamples (50 μL) of the perfusate collected before and after the perfusion were counted using 5 mL Ultima Gold TM scintillation cocktail (Perkin Elmer) in 8 mL glass scintillation vials with a scintillation counter (Wallac 1409 DSA, Perkin Elmer). Immediately following the perfusion and euthanasia, the perfused section of intestine was removed from the abdomen and the length was measured using calipers. The intestine was then cut longitudinally and laid flat to measure circumference (we used the average of 3 measurements taken along the length of the perfused section). We calculated a 'nominal surface area perfused' (smooth bore tube) as the product of the length × circumference.

Absorption of each probe was calculated from the decrease in total radioactivity during the experiment, and was normalized by dividing by the duration (min) of the perfusion and either the length (cm) or nominal surface area (cm^2) of the perfused section of intestine. For arabinose and lactulose, we also calculated clearance, which accounts for slight changes in probe concentration over the course of the experiment. Clearance (μL min^{-1} cm^{-1} or μL min^{-1} cm^{-2}) was calculated by dividing absorption (calculated as above) by $[(C_{initial} - C_{final})/(C_{initial}/C_{final})]$, where C is the concentration (Sadowski and Meddings, 1993). Clearance values for glucose and proline were not calculated because they are absorbed by both carrier-mediated and non-mediated mechanisms.

To estimate the proportion of nutrient (glucose or proline) absorption that was paracellular, we used arabinose. Because its absorption is not carrier-mediated (Lavin et al., 2007), arabinose absorption rate is directly proportional to its luminal concentration. Thus, to calculate arabinose absorption at 10 mM, we multiplied arabinose absorption (which was measured at 1 mM) by 10. To then estimate the percent proline absorption that was paracellular, for example, we then divided this calculated arabinose absorption at 10 mM by the proline absorption measured at 10 mM and multiplied this fraction by 100%. We recognize that arabinose is not a perfect comparison molecule for proline because it has larger MW and is neutral rather than slightly nonpolar/hydrophobic like proline, but its use allows comparison to absorption measurements in intact animals of this species (Fasulo et al., 2012). In the Discussion we consider how differences between arabinose and the nutrients affect this estimate.

2.1. Statistics

We tested for differences between initial and final probe concentrations using paired t-tests. We used student's t-tests to detect significant differences between the absorption of glucose and proline. We used a paired t-test to detect differences in absorption and