Research Note

Intestinal D-Glucose and L-Alanine Transport in Japanese Quail (Coturnix coturnix)

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ABSTRACT The mechanisms involved in D-glucose and amino acid transport in the intestine of birds are still not clear. In chickens, D-glucose and amino acid absorption occurs via carrier-mediated transport, but in wild birds a passive paracellular mechanism seems to be the predominant pathway. The purpose of this work was to determine the existence of carrier-mediated sodium cotransport of D-glucose and L-alanine in the small intestine of Japanese quail (Coturnix coturnix), a granivorous bird. Intestinal transport was determined by changes in the short-circuit current (Isc), proportional to ion transmembrane flux, in the middle segment of the intestine of Japanese quail with a Ussing chamber. D-Glucose produced an increase of the Isc, and this effect was reverted by phloridzin, indicating the presence of a D-glucose transport mediated by the sodium/glucose cotransporter 1. Addition of L-alanine also produced an increase of the Isc. We concluded that there is carrier-mediated cotransport of D-glucose and L-alanine with sodium in the small intestine of the Japanese quail.

(Key words: Japanese quail, L-alanine, D-glucose, intestinal transport, short-circuit current)

INTRODUCTION

The major functions of the intestine are processing, transporting, and absorbing nutrients. An optimized function of the avian intestine depends on various parameters, such as efficiency of digestion, morphology of the intestine, transit time, and nutrient absorptive function. Limitations in any of these parameters might constrain the intestinal function and consequently limit the energy supply, affecting health and growth (Starck, 1996). Therefore, the measurement of nutrient absorption is an initial step toward a better understanding of intestine function in birds.

In the intestine, D-glucose and amino acids are absorbed by 2 types of mechanisms: paracellular diffusion and transcellular transport. In paracellular diffusion, the substrates are passively absorbed through intercellular spaces by a process known as solvent drag. The passive absorption does not require input of energy and adjusts the rate of absorption to match substrate concentration, thereby eliminating the need for modulation of nutrient transporters (Pappenheimer, 1993). The disadvantage is that the passive absorption is nonspecific, which increases vulnerability to absorption of hydrophilic toxins (Afik et al., 1997b; Levey and Martínez del Río, 2001). In transcellular transport, there is an active, energetically costly absorption of D-glucose (mediated by the sodium/glucose cotransporter-1, SGLT1) and amino acids via carrier proteins in the apical and basolateral membranes of the epithelial cells (Pappenheimer, 1993).

Paracellular and transcellular mechanisms of absorption may be present in different birds. The chicken (Gallus gallus) has transcellular transport of D-glucose and amino acids in the small intestine and colon (Dyer et al., 1997; Soriano and Planas, 1998; Garriga et al., 1999a,b; Soriano-Garcia et al., 1999; Laverty et al., 2001). Active transport is also present in the small intestine of doves (Columba livia; Patil et al., 1986; Obst and Diamond, 1989). However, there is evidence that paracellular absorption of D-glucose predominates in the small intestine of wild birds (Karasov and Cork, 1994; Caviedes-Vidal and Karasov, 1996; Karasov, 1996; Karasov et al., 1996; Levey and Cipollini, 1996; Afik et al., 1997b, Chediack et al., 2001; Chang and Karasov, 2004). The Japanese quail (Coturnix coturnix) is a granivorous bird and is reported to have transcellular transport in the colon.

Abbreviation Key: Isc = short-circuit current; PD = transepithelial potential difference; R = resistance; SGLT1 = sodium/glucose cotransporter-1.
The purpose of this work was to determine the presence of carrier-mediated sodium cotransport of D-glucose and L-alanine in the small intestine of Japanese quail (Coturnix coturnix).

### MATERIALS AND METHODS

#### Birds

Eight adult male Japanese quails were obtained from Inversiones Agro Avícolas Pigarin (Carrizal, Venezuela) and were killed by cervical dislocation. Transport of L-alanine was determined in 4 birds (group 1, mean body weight of 134.5 ± 2.6 g) and transport of D-glucose was determined in the other 4 birds (group 2, mean body weight 132.8 ± 8.7 g).

#### Tissue Preparation

The small intestine was removed from the pancreatic loop to the ileocecal junction and placed in cool (4°C) Ringer buffer (161 mM NaCl, 4.7 mM KCl, 20 mM NaHCO₃, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, and 1.2 mM MgSO₄; Afik et al., 1997a). The intestinal contents were washed with cool Ringer buffer and were divided in 3 equal segments [proximal (closest to the pancreatic loop), medial, and distal (nearest to the ileocecal junction)]. Each medial section was opened along the mesenteric border and mounted intact in an Ussing chamber with an exposure area of 0.38 cm². During the experiments, the serosal and mucosal surfaces of the tissues were bathed in 3 mL of Ringer buffer at room temperature (20 to 24°C) and were continuously gassed with a mixture of 95% O₂ and 5% CO₂.

#### Electrical Measurements

Transepithelial potential difference (PD; mV), short-circuit current (Isc; µA/cm²), and electrical resistance (R; Ω cm²) were measured with a voltage-clamp apparatus² as previously described (Ussing and Zerahn, 1951). Spontaneous PD was measured between calomel electrodes connected to the chamber by KCl agar bridges. Any PD existing between the calomel cells before the tissue was in place was automatically compensated for at the beginning of the experiment. Except for short periods, when the open-circuit PD was measured, the tissue was short-circuited by passing the appropriate current through Ag/AgCl electrodes. The electrical parameters (PD, Isc, and R) were expressed relative to the mucosal side, and a positive Isc indicated mucosal to serosal flow of current. The tissue R was determined by creating a potential difference of 10 mV for 1 s across the tissue every 10 min and dividing the applied potential difference by the recorded change in the Isc according to Ohm’s law. The basal measures of PD, Isc, and R were taken after a stabilization period of 30 min.

For D-glucose transport, a concentrated solution of the substrate was added on the luminal side, reaching a final concentration of 10 mM. In all cases, sorbitol was added to the serosal side at 10 mM in order to maintain osmolarity. Electrical response to D-glucose was measured each 5 min until the Isc was stable. The effect of D-glucose was also studied adding phloridzin, a specific inhibitor of the transporter SGLT1 (Wright et al., 1994), to the mucosal side (final concentration: 0.1 mM), once the electrical effect induced by D-glucose had been reached.

For L-alanine transport, the amino acid was added on the luminal side (final concentration: 10 mM), whereas sorbitol was added to the serosal side. Electrical response to L-alanine was measured every 5 min until the Isc was stable. An experiment with Na+-free Ringer (161 mM N-methyl-D-glucamine chloride, 4.7 mM KCl, 20 mM KHCO₃, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, and 1.2 mM MgSO₄) was run to confirm that L-alanine transport was sodium dependent.

#### Statistical Analysis

Values are expressed as means (±SD). Values of PD, Isc, and R with glucose or phloridzin conditions were compared using a Kruskal-Wallis analysis, and L-alanine conditions were compared using a Mann-Whitney U test (Siegel and Castellan, 1988). In all cases, a probability level of 5% (P < 0.05) was considered significant.

### RESULTS AND DISCUSSION

The small and large intestines of domestic birds have high absorptive rates for water and electrolytes. The movement of ions responsible for the current across the epithelium includes mainly the absorption of Na⁺ and the secretion of Cl⁻ (Grubb, 1991). A number of nutrients transported with sodium are added to the luminal side of intestinal tissue, the carrier-mediated transport is stimulated, and a concomitant rise in sodium uptake occurs. The brush border membrane depolarization and the rise in sodium cytosolic concentration stimulate Na⁺/K⁺-ATPase, which, in turn, increases the net flux of sodium from the luminal to the serosal side. All of these processes modify the electrical variables of the tissue as PD and Isc (Wright et al., 1994; Amat et al., 1999).

Results of Isc, PD, and R for Japanese quail small intestine are shown in Table 1. Addition of L-alanine in a sodium ringer buffer produced a significant increase of PD and Isc values, whereas no changes occurred in the Na⁺-free ringer buffer. These electrical changes indicate the presence of a cotransport of L-alanine and sodium in the small intestine of Japanese quail. The constant transepithelial R indicated that the paracellular resistance remained unaffected during the mucosal ad-

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²VCC600 Physiologic Instruments, San Diego, CA.
tion of L-alanine, provided the junctional pathway had no ion selectivity. This report is the first on sodium L-alanine cotransport in small intestine of Japanese quail, whereas amino acid cotransport has been reported in the colon of quail with L-leucine and L-lysine (Laverty, 1997).

D-Glucose cotransport was also demonstrated. Increased Isc by addition of substrate reflected a higher net flux of sodium from the mucosal to the serosal side (Table 1). Phloridzin added after the maximal Isc reached decreased Isc to the basal values, indicating involvement of SGLT1 transporter (Wright et al., 1994). The D-glucose produced a significant increase of Isc, but the PD and R values remained constant. Similar responses have been observed in the colon of Japanese quail (Laverty, 1997), chicken small intestine and colon (Amat et al., 1999), and hen colon (Bindslev et al., 1997).

Basal values of Isc and R in the small intestine of the Japanese quail were lower than values reported for the colon (Laverty, 1997), although the PD was higher. Basal PD, Isc and R values for the small intestine were lower than those reported for chicken jejunum (Grubb et al., 1987; Amat et al., 1999) and hen colon (Laverty et al., 2001).

There are few studies with the Ussing chambers to study intestinal transport in birds, and they have mostly focused on the colon and coprodeum (Bindslev et al., 1997; Laverty, 1997; Laverty et al., 2001). Our study is, to our knowledge, the first to describe the electrical properties of the small intestine of Japanese quail.

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REFERENCES


