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# Poly(A)<sup>+</sup> RNA from Sheep Omasal Epithelium Induces Expression of a Peptide Transport Protein(s) in *Xenopus laevis* Oocytes<sup>1</sup>

Y.-X. Pan\*, E. A. Wong\*, J. R. Bloomquist†, and K. E. Webb, Jr.\*<sup>2</sup>

Departments of \*Animal and Poultry Sciences and †Entomology,  
Virginia Polytechnic Institute and State University, Blacksburg 24061-0306

**ABSTRACT:** To verify research from this laboratory indicating that sheep omasal epithelium contains mRNA encoding for a peptide transporter(s) and to determine di- to octapeptide transport capability, we injected poly(A)<sup>+</sup> RNA isolated from sheep omasal epithelium into *Xenopus laevis* oocytes. Poly(A)<sup>+</sup> RNA was functionally expressed in *Xenopus* oocytes 4 to 7 d after injection. Peptide (5 di-, 10 tri-, 6 tetra-, 2 penta-, 1 hexa-, 1 hepta-, and 1 octapeptide) transport capability was measured by impaling oocytes with a microelectrode to monitor membrane potential ( $V_m$ ). Oocytes were maintained in pH 5.5 buffer. Peptide transport was identified as being expressed when, in the presence of a buffered peptide substrate (1 mM), the oocyte membrane showed persistent depolarization (a more positive  $V_m$ ). In the absence of peptide

transport, the membrane became depolarized with the addition of buffered substrate, but it rapidly repolarized to the resting potential. Peptide transport was expressed for some di-, tri-, and tetrapeptides. Measured depolarization ranged from 9.6 mV to 42.1 mV. Larger peptides were not transported by the oocytes. When transport expression was measured with the substrates in a pH 7.5 buffer, no transport occurred, indicating that transport was dependent on a proton gradient. Thus, sheep omasal epithelium contains mRNA that codes for a protein(s) capable of proton-dependent di-, tri-, and tetrapeptide transport. Results from the present study provide further evidence that absorption of peptides from the ruminant stomach is possible.

Key Words: Sheep, Omasum, Peptides, Transport, Electrophysiology

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## Introduction

The omasum of ruminant animals has the ability to absorb VFA (McSweeney, 1988), ammonia and electrolytes (Oyaert and Bouckaert, 1961), and water (Holtenius and Bjornhag, 1989). However, the ability of the omasum to absorb peptides as a source of amino acid N has not been clearly defined.

Recent research has increased the understanding of the potential for peptide absorption to exist in the forestomach epithelium of ruminants. Matthews and Webb (1995) showed that carnosine and methionylglycine were transferred intact across ruminal and omasal epithelia when these tissues were mounted in parabiotic chambers. Using an expression system, poly(A)<sup>+</sup> RNA isolated from omasal epithelium was

injected into *Xenopus laevis* oocytes (Matthews et al., 1996a). Specific poly(A)<sup>+</sup> RNA fractions induced an increased rate of glycylsarcosine (Gly-Sar) absorption in mRNA-injected oocytes compared with water-injected oocytes. Thus, mRNA encoding for a protein(s) that is capable of dipeptide transport exists in sheep omasal epithelium. To elucidate the transport mechanisms of a larger number of small peptides, *Xenopus* oocytes injected with poly(A)<sup>+</sup> RNA from sheep omasal epithelium were used as experimental models using electrophysiological techniques to determine di- to octapeptide transport capability.

## Materials and Methods

**Total RNA Extraction from Animal Tissue.** We used a modification of the method of Puissant and Houdebine (1990) to extract total RNA from the omasal epithelium of crossbred sheep (average BW 60 kg). In the initial experiment, a composite of total RNA obtained from 16 sheep was used. In subsequent experiments, total RNA from another 19 sheep was extracted and composited. Briefly, omasal mucosal

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<sup>2</sup>To whom correspondence should be addressed.

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scrapings were homogenized in 4 M guanidine thiocyanate, 25 mM sodium citrate, pH 7.0, .1 M  $\beta$ -mercaptoethanol, and .5% N-laurylsarcosinate. Homogenates were then pooled into centrifuge bottles that contained 2 M acetic acid (pH 4.1), water-saturated phenol, and chloroform. The suspensions were centrifuged at  $12,000 \times g$  at 4°C for 20 min. The resulting supernatants were precipitated with isopropanol at -20°C for 12 h and centrifuged for 20 min at  $4,000 \times g$  at 4°C. The pellets were resuspended in 4 M LiCl and centrifuged at  $3,600 \times g$  at room temperature (-21°C) for 20 min. The RNA pellets were pooled again in 10 mM Tris-HCl (pH 7.6), 1 mM EDTA, .5% SDS, and chloroform. The solutions were then centrifuged at  $12,000 \times g$  at 4°C for 20 min. The supernatants containing total RNA were then stored at -80°C in the presence of .2 M sodium acetate (pH 5.0) and isopropanol. When needed, RNA was collected by centrifugation, washed in 70% ethanol, and suspended in water.

**Poly(A)<sup>+</sup> RNA Isolation and Fractionation.** Poly(A)<sup>+</sup> RNA was purified from total RNA on oligo(dT) cellulose following established procedures (Sambrook et al., 1989). Briefly, total RNA was recovered by centrifugation at  $12,000 \times g$  at 20°C for 25 min and then dissolved in diethyl pyrocarbonate (DEPC)-treated water. Typically, approximately 10 mg of total RNA was loaded on a column that contained approximately 200 mg of oligo(dT) cellulose. A second round of chromatography was performed on a column that contained 80 mg of oligo(dT) cellulose to further purify the poly(A)<sup>+</sup> RNA. For both rounds, the final RNA eluate was precipitated with an equal volume of isopropanol at -80°C.

Poly(A)<sup>+</sup> RNA was fractionated on a linear sucrose density gradient (8 to 20% wt/vol) prepared according to the method of Luthe (1983). Briefly, poly(A)<sup>+</sup> RNA was recovered by centrifugation at  $80,000 \times g$  at 2°C for 30 min. A 100- $\mu$ g (1  $\mu$ g/ $\mu$ L) sample of Poly(A)<sup>+</sup> RNA was loaded onto a 13-mL 8 to 20% (wt/vol) linear sucrose gradient containing 10 mM Tris-HCl (pH 7.6), 1 mM EDTA (pH 8.0), and 10 mM methyl mercuric hydroxide, and then it was centrifuged in a Beckman SW-41 ultracentrifuge rotor at  $80,000 \times g$  at 4°C for 15.5 h. Four fractions of 3 mL each were then collected by piercing the bottom of the centrifuge tube. An equal volume of 5 mM  $\beta$ -mercaptoethanol was then added to each fraction. The fractional RNA was precipitated with ethanol and 3 M sodium acetate and stored at -80°C until use.

**Oocyte Preparation, Storage, and Microinjection.** Female *Xenopus laevis* (Xenopus One, Ann Arbor, MI) were kept in water tanks at 15 to 16°C in dechlorinated water on an 8 h light and 16 h dark cycle and fed at least three times a week with a complete diet (Frog Brittle, Nasco, Fort Atkinson, WI). Following methods described by Goldin (1992) to obtain the oocytes, mature toads were anesthetized by immersion in ice-water supplemented with .15% ethyl-m-

aminobenzoate (MS222 from Sigma). A small incision was made in the lower abdominal quadrant and a lobule of ovarian tissue, containing up to 200 oocytes, was removed and placed in Ca<sup>2+</sup>-free medium (in mM: 96 NaCl, 2 KCl, 1 MgCl<sub>2</sub>, 5 HEPES titrated with NaOH to pH 7.5). The wound was closed in two layers, the toad was allowed to recover from the anesthesia in a separate tank containing .01% penicillin-G in water for several hours, and then the animal was returned to the colony.

The ovarian material was separated into pieces and treated at room temperature (-21°C) with collagenase A (EC 3.4.24.3, Boehringer Mannheim, Indianapolis, IN) at a concentration of .5 units/mL in Ca<sup>2+</sup>-free medium for approximately 40 min. Oocytes at stages V and VI were collected after the surrounding tissue layers were peeled off with watchmaker's forceps (size 5, George Tiemann and Company, Plainfield, NY). These defolliculated oocytes were stored again for at least 2 h in Ca<sup>2+</sup>-free medium and the adhering follicle cells were removed by gentle shaking. After washing, oocytes were incubated (model BK6160, Heraeus Instruments, South Plainfield, NJ) at 18°C in a culture solution (in mM: 96 NaCl, 2 KCl, 1 MgCl<sub>2</sub>, 1.8 CaCl<sub>2</sub>, 5 HEPES titrated with NaOH to pH 7.5) supplemented with 2.5 mM sodium pyruvate, 100 units/mL penicillin-G, and 100  $\mu$ g/mL streptomycin.

The poly(A)<sup>+</sup> RNA or size-fractionated poly(A)<sup>+</sup> RNA was dissolved in DEPC-treated water to a final concentration of 1.5 ng/nL. The oocytes were placed in injection buffer (in mM: 96 NaCl, 2 KCl, 1 MgCl<sub>2</sub>, 1.8 CaCl<sub>2</sub>, 5 HEPES titrated with NaOH to pH 7.5) in a tissue culture dish under a stereomicroscope. Using a microinjection system (Matthews et al., 1996b), 100 nL of either RNA solution or DEPC-treated water was injected into each oocyte in the vegetal pole, near the polar interface. The injected oocytes were returned to the culture solution and then incubated at 18°C for 1 to 7 d. The culture solution was changed daily, and damaged oocytes, as indicated by misshapen or ruptured oocytes, were discarded. Generally, 50% to 75% of injected oocytes were usable.

**Electrophysiological Recording from Injected Oocytes.** Conventional intracellular glass microelectrode recordings were used to monitor peptide transport via changes in the oocyte membrane potential. Potentials were monitored with an amplifier and analyzed with a MacLab (AD Instruments, Milford, MA), which is an analog-digital converter and software system that uses an Apple Macintosh computer for performing data acquisition (Soderlund et al., 1989).

Microelectrodes were pulled from borosilicate glass filament tubing of 1.5 mm outer diameter and .87 mm inner diameter using a vertical pipette puller. The electrode was filled with 3 M KCl, giving a tip resistance of 2 to 5 M $\Omega$  and a tip potential of no more than 5 mV. As long as the electrode retained its low resistance, it could be reused for several oocytes.

Normally, 4 to 7 d after injection with poly(A)<sup>+</sup> RNA or DEPC-treated water, a single oocyte was placed in a recording chamber (500  $\mu$ L) in the presence of 125  $\mu$ L of pH 5.5 measurement buffer (in mM: 96 NaCl, 2 KCl, 1 MgCl<sub>2</sub>, 1.8 CaCl<sub>2</sub>, 5 HEPES titrated with NaOH to pH 5.5). Oocytes were maintained in this pH 5.5 buffer for at least 20 min before impalement. Oocytes were always impaled to enable measurement of the resting membrane potential ( $V_m$ ) and then allowed to stabilize before the start of the experiment. In most cases (approximately 70% of successful impalements), the electrode was inserted into the dark, animal hemisphere for better visualization of the electrode. No difference was observed between potential responses,  $\Delta V_m$  from the animal or vegetal pole. Only oocytes at stage V or VI with a resting  $V_m$  more negative than -30 mV and that responded to the addition of buffer by becoming depolarized followed by rapid repolarization were used.

Screening for peptide transporters was performed by adding 125  $\mu$ L of appropriate 2 mM, pH 5.5 peptide solution to the recording chamber that contained 125  $\mu$ L pH 5.5 measurement buffer. Peptide solutions were prepared by dissolving the peptides in the appropriate measurement buffer. Changes in pH of the solutions resulting from dissolving peptides were compensated with NaOH or HCl. Peptide solutions flowed into the recording chamber through a plastic tube (1 mm). For treatment, the plastic tube was manually moved near the oocyte. Care was taken to minimize mechanical stimulation. To determine pH-dependency of peptide transport, the oocyte was placed either in pH 5.5 or pH 7.5 measurement buffer before impalement. Then appropriate peptide solutions were added to the recording chamber. All experiments were performed at room temperature (~21°C).

The uptake of peptide substrates (1 mM) from measurement buffer by oocytes was determined by the changes of oocyte  $V_m$ . Peptide transport was identified as being expressed when, in the presence of a buffered peptide substrate, the oocyte  $V_m$  showed persistent depolarization (a more positive  $V_m$ ). In the absence of peptide transport, the membrane became depolarized with the addition of buffered substrate but rapidly repolarized to the resting  $V_m$ . Similar responses were observed as early as 1982 in intestinal epithelial cells and showed that transport of intact peptides caused a depolarization of the brush-border membrane (Body and Ward, 1982).

**Peptides Examined.** Twenty six peptides (di- to octapeptides), which were all constituted with L- $\alpha$ -amino acid residues and purchased from Sigma Chemical (St. Louis, MO), were evaluated in this study.

**Statistical Analysis.** Data were analyzed by regression analysis to investigate the form of the relationship between transport and peptide characteristics. Positive responses were regressed against the molecu-

lar weight, hydrophobicity, and electrical charge of the peptides, respectively. The REG procedure of SAS (1989) was used in this study for regression analysis.

## Results and Discussion

**Identification of Peptide Transport Capability in Poly(A)<sup>+</sup> RNA-Injected Oocytes.** As an initial experiment, oocytes were injected with total poly(A)<sup>+</sup> RNA to determine whether mRNA encoding for proteins capable of peptide transport could be identified by electrophysiological measurements. Among the 13 peptides examined, peptide transport was shown to be expressed for one dipeptide (Gly-Sar), a mixture of three tripeptides (Lys-Tyr-Lys, Gly-Leu-Tyr, and Lys-Trp-Lys), and two tetrapeptides (Met-Gly-Met-Met and Val-Gly-Asp-Glu) in oocytes injected with total poly(A)<sup>+</sup> RNA in this initial experiment (Figure 1). Measured depolarization ranged from 9.6 mV to 42.1 mV. Two tetrapeptides (Val-Ala-Ala-Phe and Pro-Phe-Gly-Lys), two pentapeptides (Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu), one hexapeptide (Lys-Arg-Gln-His-Pro-Gly), one septapeptide (Arg-Val-Tyr-Val-His-Pro-Phe), and one octapeptide (Val-His-Leu-Thr-Pro-Val-Glu-Lys) were not transported by the injected oocytes in this study. Water-injected oocytes (as opposed to poly(A)<sup>+</sup> RNA injected oocytes) did not show any persistent depolarization with any of the tested peptides; this indicated that the endogenous ability of oocytes to transport peptides was low.

Using this electrophysiological measurement, the ability to transport peptides by injected oocytes could be detected on d 4 after injection of poly(A)<sup>+</sup> RNA. Most of the injected oocytes responded to the peptide treatments on d 5 to 7, which indicated that the expression of poly(A)<sup>+</sup> RNA in injected oocytes increased by day. In contrast, there was no clear change in  $V_m$  in water-injected oocytes by day. These data from this initial study were consistent with previous research from this laboratory (Matthews et al., 1996a). In that study, a time course trial was conducted to determine the optimal day after injection for measuring the absorption of [<sup>14</sup>C]Gly-Sar by the oocytes. Oocytes were measured for their ability to transport [<sup>14</sup>C]Gly-Sar on d 1 to 4 after injection of sheep omasal RNA or water. As compared with water-injected oocytes, the absorption of Gly-Sar by oocytes injected with RNA on d 3 and 4 was 1.5 and 2.6 times greater, respectively.

**Effects of pH Changes on Peptide Transport Capability in Poly(A)<sup>+</sup> RNA-Injected Oocytes.** The pH dependency of the peptide transport process was investigated by testing the effects of measurement buffer and peptide solution pH on  $V_m$  during the recording. The  $V_m$  of poly(A)<sup>+</sup> RNA-injected oocyte showed persistent depolarization when peptide solutions at pH 5.5 were added to measurement buffer also at pH 5.5 (Figure 2a). This was observed when Gly-Sar,

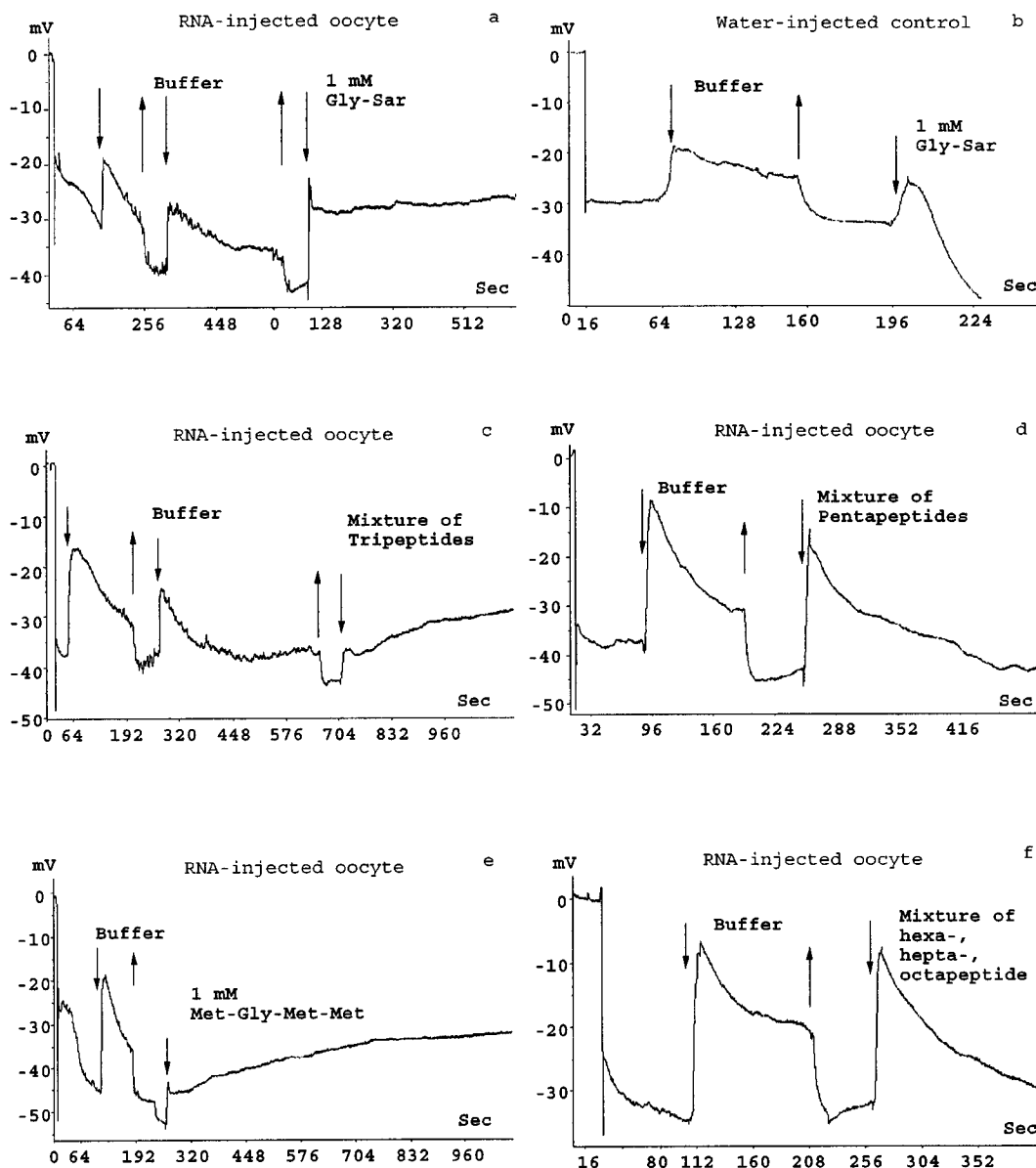


Figure 1. Membrane potential responses to peptide treatments in *Xenopus* oocytes 5 d after injection with (a, c–f) sheep omasal epithelial total poly(A)<sup>+</sup> RNA or (b) water. Oocytes were measured in pH 5.5 buffer. For all figures, downward arrows (↓) indicate time of treatment addition, and upward arrows (↑) indicate time of buffer removal.

Lys-Tyr-Lys, Leu-Leu-Tyr, Lys-Trp-Lys, Met-Gly-Met-Met, or Val-Gly-Asp-Glu were used as substrates. With the measurement buffer at a pH of 7.5, the membrane became depolarized with the addition of pH 7.5 peptide substrate, but it rapidly repolarized to the resting potential. In water-injected oocytes, neither at pH 5.5 nor at pH 7.5 did  $V_m$  show persistent depolarization indicative of peptide uptake (Figure 2b).

To determine whether these results were due to the pH dependency of the transport process or just the responses of sequential treatments, additional experiments were conducted. One trial was performed with a completely opposite sequence, where the  $V_m$  of poly(A)<sup>+</sup> RNA- or water-injected oocyte was first

recorded at pH 7.5 of measurement buffer when the peptide solution was at pH 7.5. The pH was then decreased to pH 5.5, and then a peptide solution at pH 5.5 was added (Figure 2c). Another trial was performed only at pH 7.5 of measurement buffer when peptide solution at pH 7.5 was added (Figure 2d). In both trials, only at a pH of 5.5 did  $V_m$  of poly(A)<sup>+</sup> RNA-injected oocytes show persistent depolarization. These data indicate that increasing pH or decreasing the proton gradient reduces the peptide transport ability of poly(A)<sup>+</sup> RNA-injected oocytes. These results were consistent with previous research that indicated that the quantity of induced [<sup>14</sup>C]Gly-Sar uptake by RNA-injected oocytes at pH 5.5 ( $91.9 \pm 32.2$  fmols·oocyte<sup>-1</sup>·40 min<sup>-1</sup>) was greater than that at pH

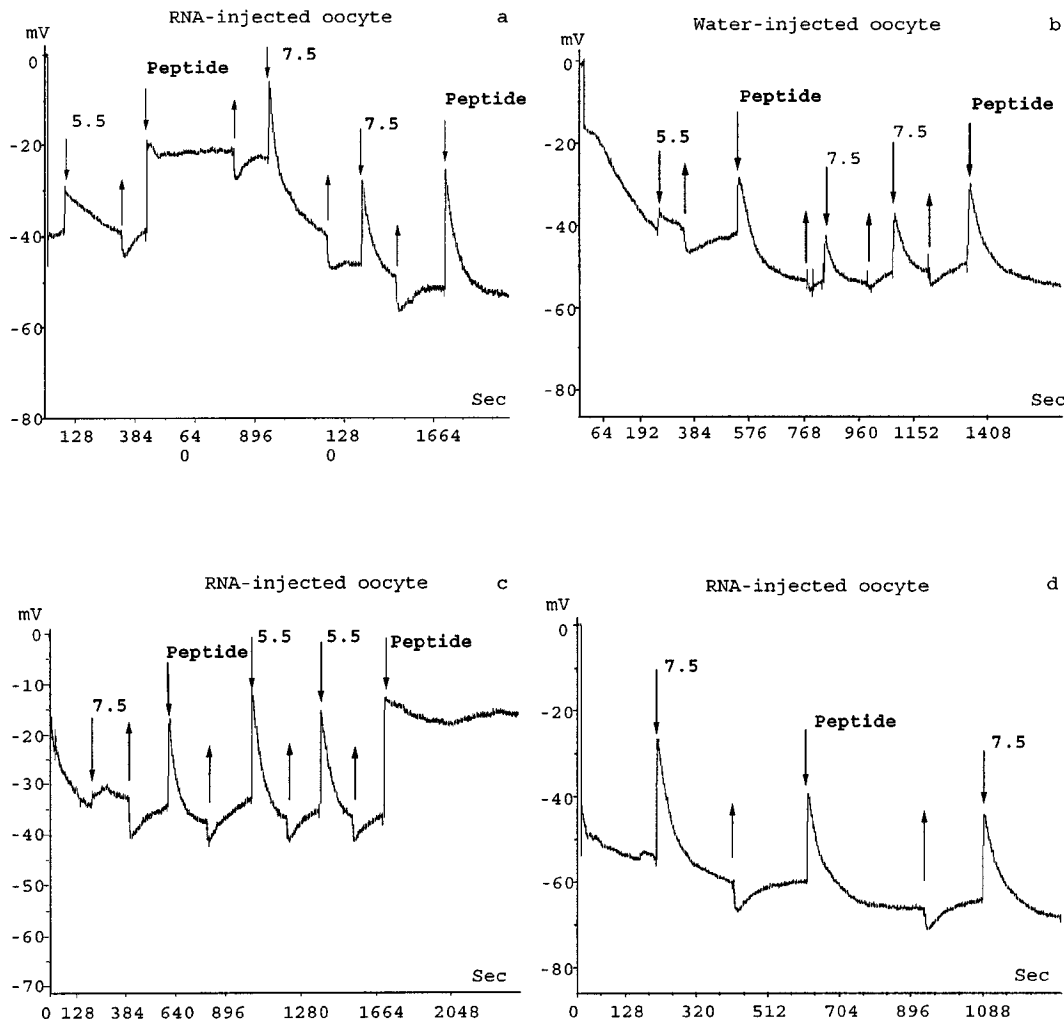


Figure 2. Responses to peptide treatments at various proton gradients in *Xenopus* oocytes 5 to 7 d after injection with sheep omasal epithelial poly(A)<sup>+</sup> RNA or water. The change of pH from 5.5 to 7.5 in poly(A)<sup>+</sup> RNA-injected oocytes (a) or water-injected oocytes (b) and pH change from 7.5 to 5.5 (c) or pH at 7.5 only (d) in poly(A)<sup>+</sup> RNA-injected oocytes are shown. For all figures, downward arrows (↓) indicate time of treatment addition, and upward arrows (↑) indicate time of buffer removal.

7.5 ( $-1.30 \pm 22.2$  fmols-oocyte<sup>-1</sup>·40 min<sup>-1</sup>; Matthews et al., 1996a). In two other studies on human small intestinal tissue, the current evoked by 1 mM Gly-Sar at pH 7.5 in RNA-injected oocytes was approximately 30% of pH 5.5 (Mackenzie et al., 1996), and [<sup>14</sup>C]Gly-Sar (30 μM Gly-Sar) uptake by RNA-injected oocytes at pH 7.5 was 54% that at pH 5.5 (Liang et al., 1995). Liang et al. (1995) attributed the discrepancy to an appreciable H<sup>+</sup>-uncoupled flux of Gly-Sar at pH 7.5.

**Demonstration of Peptide Transport Ability in Size-Fractionated RNA-Injected Oocytes.** The capacity of the oocytes to translate poly(A)<sup>+</sup> RNA is limited. Therefore, to obtain the maximal expression of sheep omasal epithelial RNA in oocytes and to enhance the recording of V<sub>m</sub> signals, poly(A)<sup>+</sup> RNA was fractionated on a linear sucrose density gradient. This allowed more accurate sizing and better enrichment of biologically active RNA than did just poly(A)<sup>+</sup> RNA on oligo(dT) cellulose. After centrifugation, four fractions of po-

ly(A)<sup>+</sup> RNA were collected (from fraction I to IV, the size of poly(A)<sup>+</sup> RNA decreased) and injected into *Xenopus* oocytes. The Gly-Sar, Leu-Ser-Phe, and Met-Gly-Met-Met peptides were used to determine which fraction had the ability to induce peptide transport in oocytes. Neither fraction I nor II showed any ability to elicit a change in V<sub>m</sub> as compared with that of water-injected oocytes (Table 1). Fractions III and IV showed the ability to induce peptide transport in oocytes. Fraction III and IV in the present study included the fractions giving positive responses in earlier studies (Matthews et al., 1996a).

**Dependency on Na<sup>+</sup> of Peptide Transport Ability in Size-Fractionated RNA-Injected Oocytes.** The Na<sup>+</sup> dependence of the V<sub>m</sub> change was investigated in size-fractionated RNA-injected oocytes. The Na<sup>+</sup>-free solutions were prepared by replacing NaCl with choline chloride in the measurement buffer and peptide

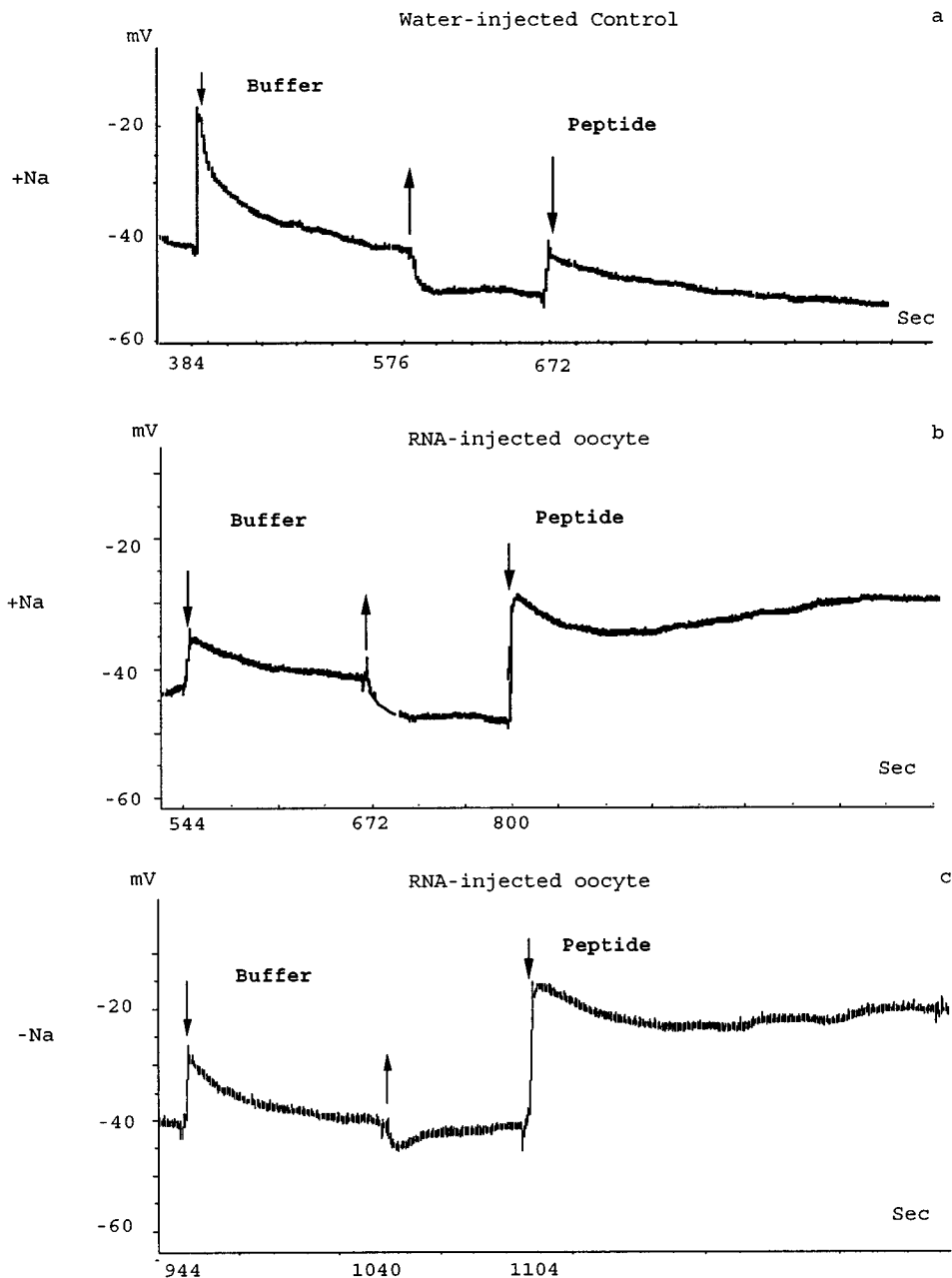


Figure 3. The  $\text{Na}^+$  dependence of responses to various peptides at pH 5.5 in *Xenopus* oocytes 5 to 7 d after injection with sheep omasal epithelial poly(A)<sup>+</sup> RNA or water. Examples of membrane potential changes in oocytes injected with water (a) or poly(A)<sup>+</sup> RNA (b, c) are shown. The +Na and -Na indicate the recorded responses in  $\text{Na}^+$ -containing and  $\text{Na}^+$ -free solutions, respectively. For all figures, downward arrows ( $\downarrow$ ) indicate time of treatment addition, and upward arrows ( $\uparrow$ ) indicate time of buffer removal.

solutions. A total of 10 peptides were used as the substrates, and the change in  $V_m$  of oocytes injected with either fraction III or IV was virtually identical in  $\text{Na}^+$ -free solutions and  $\text{Na}^+$ -containing solutions. Examples are shown in Figure 3. This finding indicates that peptide transport in size-fractionated RNA-injected oocytes is independent of  $\text{Na}^+$ . There have been conflicting reports with regard to the dependence of peptide transport on  $\text{Na}^+$ . However, recent evidence indicates that the absorption of peptides in the

mammalian epithelia of intestine, kidney, lung, placenta, and the blood-brain barrier is mediated by one or more  $\text{H}^+$ -coupled transporters (Meredith and Boyd, 1995). The sequential actions of the  $\text{Na}^+/\text{H}^+$  exchanger (Knickelbein et al., 1983) and  $\text{Na}^+/\text{K}^+$ -ATPase generate an inward  $\text{H}^+$  electrochemical gradient sufficient to drive the tertiary transport of peptides. The present study also showed that the inhibition of peptide transport by the total replacement of  $\text{Na}^+$  by choline was unable to be detected by

Table 1. Response of size-fractionated RNA-injected oocytes to peptides

Fraction <sup>a</sup>	Peptide		
	Gly-Sar	Leu-Ser-Phe	Met-Gly-Met-Met
I	0 (6) <sup>b</sup>	0 (6)	0 (6)
II	0 (5)	0 (5)	0 (5)
III	6 (8)	4 (8)	4 (8)
IV	4 (6)	3 (6)	4 (6)

<sup>a</sup>Fraction I is the 20% sucrose side and Fraction IV is the 8% sucrose side.

<sup>b</sup>Numerals are numbers of oocytes for which membrane potential was depolarized after addition of peptide. Numerals in parentheses are the number of oocytes tested.

single electrode membrane potential measurement. Thus, Na<sup>+</sup> might not have a role in the primary translocation step of peptide transport, or the single electrode membrane potential measurement did not detect this difference.

*Determination of Substrate Specificity in Size-Fractionated RNA-Injected Oocytes.* To determine the structural features of peptides that might influence their affinity for the peptide transporter, fractions III and IV were injected into oocytes and tested with 17 peptides. In oocytes injected with fractions III and IV, oocytes showed transport ability for the Gly-Sar, Gly-Leu, Gly-Pro, Phe-Leu, and Leu-Leu peptides (Table 2). Oocytes showed transport ability for all tripeptides tested, including Leu-Ser-Phe, Leu-Gly-Phe, Lys-Tyr-

Lys, Ala-Pro-Gly, Met-Leu-Phe, and Leu-Leu-Tyr. For tetrapeptides, oocytes showed transport ability for Met-Gly-Met-Met, Val-Gly-Asp-Glu, Ala-Gly-Ser-Glu, and Val-Gly-Ser-Glu, but no transport of Pro-Phe-Gly-Lys and Val-Ala-Ala-Phe. These peptides constitute a variety of substrates varying in their molecular size, hydrophobicity, and electrical charge under the experimental conditions used. Regression analysis of the percentage of transport induced by peptides showed that the relationship between transport and molecular weight was  $r = .02$  ( $P = .94$ ), and the relationship between transport and hydrophobicity values, which are calculated from their corresponding amino acid residues according to different scales, were  $r = .34$  ( $P = .21$ ; Kyte and Doolittle, 1982),  $r = .42$  ( $P = .11$ ; Fauchere and Pliska, 1983), and  $r = .44$  ( $P = .10$ ; Parker et al., 1986). Regression analysis performed with net charge data revealed that the relationship between transport and net charge was  $r = .1$  ( $P = .72$ ). Thus, the transporter(s) is capable of translocating substrates regardless of their molecular weight, hydrophobicity, or charge. The fact that the percentage of oocytes showing positive responses varied among peptides probably indicates that the affinities of the transporter(s) for the tested peptides may vary. Recent studies from other laboratories on the mammalian proton-coupled peptide transporter, PepT1, also demonstrate that peptide substrates are transported by PepT1 by electrogenic H<sup>+</sup> coupled cotransport that is independent of their physicochemical characteristics (i.e., size and charge; Daniel et al., 1996; Wenzel et al., 1996).

Table 2. Peptide substrate specificity in oocytes injected with fraction III or IV

Peptide	Transport, % <sup>a</sup>	MW <sup>b</sup>	Hydrophobicity <sup>c</sup>			Net charge <sup>d</sup>
			Fauchere	Kyte	Parker	
Gly-Leu	70 (10) <sup>e</sup>	206	.92	3.4	-3.5	0
Gly-Pro	77 (9)	190	1.59	-2.0	7.8	0
Gly-Sar	80 (10)	164	1.87	-8	7.6	0
Leu-Leu	33 (9)	262	.13	7.7	-18.4	0
Phe-Leu	75 (8)	296	.09	6.6	-12.4	0
Ala-Pro-Gly	55 (9)	279	1.56	-2	9.9	0
Leu-Gly-Phe	77 (9)	371	.67	6.2	-12.7	0
Leu-Leu-Tyr	38 (8)	443	.38	6.3	-20.3	0
Leu-Ser-Phe	77 (9)	401	.68	5.8	-11.9	0
Lys-Tyr-Lys	67 (9)	473	2.17	9.1	9.5	2
Met-Leu-Phe	55 (9)	445	.27	8.5	-22.6	0
Ala-Gly-Ser-Glu	63 (8)	416	1.92	-2.9	22.1	-1
Met-Gly-Met-Met	77 (9)	522	.91	5.3	-6.9	0
Pro-Phe-Gly-Lys	0 (8)	501	1.51	-3.1	4.3	0
Val-Ala-Ala-Phe	0 (7)	460	.92	10.6	-8.7	0
Val-Gly-Asp-Glu	77 (9)	472	1.88	-3.2	19.8	-2
Val-Gly-Ser-Glu	67 (9)	444	1.70	-5	16.3	-1

<sup>a</sup>Percentage of transport is the ratio of positive responses to the total oocytes tested.

<sup>b</sup>The relationship between transport ratio and molecular weight (MW) was  $r = .02$  ( $P = .94$ ).

<sup>c</sup>Hydrophobicity values of peptides are calculated as the average of the value of the constituent amino acids according to different scales. Relationships between transport ratios and hydrophobicity values were  $r = .34$  ( $P = .21$ ; Kyte and Doolittle, 1982),  $r = .42$  ( $P = .11$ ; Fauchere and Pliska, 1983), and  $r = .44$  ( $P = .10$ ; Parker et al., 1986).

<sup>d</sup>Net charge of peptides was calculated at pH 5.5. The relationship between transport ratio and net charge was  $r = .10$  ( $P = .72$ ).

<sup>e</sup>Numerals in parentheses are the number of oocytes tested.

It has been suggested that hydrophobic peptides and peptides resistant to mucosal-hydrolysis are absorbed faster than hydrophilic and hydrolysis-susceptible peptides (Gardner and Wood, 1989; Daniel et al., 1992; Pan et al., 1996). Expression of cloned rabbit intestinal H<sup>+</sup>/oligopeptide cotransporter rPepT1 in *Xenopus* oocytes displayed high apparent affinity for the anionic dipeptide alanyl-aspartate (Boll et al., 1996), whereas in rabbit intestinal brush-border membrane vesicles, neutral dipeptides, or those bearing a single positive charge, were generally favored (Wootton and Hazelwood, 1989). In the present study, the oocytes injected with fractions III or IV had a similar transport ability for di- to tetrapeptides, which indicates that more specific fractions should be prepared to separate transporters with different peptide affinities.

In summary, results of the present study indicate that there are mRNA present in sheep omasal epithelial cells that encode for a peptide transporter(s) and that peptide transport activity in RNA-injected oocytes can be detected by electrophysiological techniques. Substrate specificity of the transporter(s) indicates that many, but not all, di-, tri-, and tetrapeptides can be transported.

### Implications

The results of this study verify that mRNA encoding for a peptide transporter(s) is present in omasal epithelium. Studying the kinetic characteristics of the peptide transporter(s) should provide insight into structure-function relationships for the transport protein(s). If further research verifies this peptide transport capability in animal tissue, an important mechanism for nitrogen supplementation may be revealed.

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