In Vitro Nasal Transport Across Ovine Mucosa: Effects of Ammonium Glycyrrhizinate on Electrical Properties and Permeability of Growth Hormone Releasing Peptide, Mannitol, and Lucifer Yellow

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Transport of growth hormone releasing peptide across ovine nasal mucosa in the absence or presence of ammonium glycyrrhizinate (AMGZ) was studied *in vitro*. Ovine nasal mucosa was stripped from underlying cartilage and mounted in Ussing chambers. Transepithelial conductance (G_t) and short-circuit current (I_{sc}) were monitored during experiments to assess tissue viability and integrity. Radiolabeled mannitol (Man; MW 182) and growth hormone releasing peptide (GHRP, SK&F 110679; MW 873) were employed to measure transport rates across the epithelium, and fluorescence spectroscopy was employed to measure rates of lucifer yellow (LY; MW 521) transport. Effects of AMGZ on ovine nasal mucosal viability and transport were determined from changes in electrical properties or fluxes of [^{3}H]GHRP, [^{3}H]Man, and LY. Results demonstrate that electrical properties of ovine nasal mucosa are stable over the time course of the experiments (G_t = 8.3 ± 0.5 mS/cm^2 and I_{sc} = 3.7 ± 0.2 μEq/hr · cm^2; n = 21). Man fluxes were comparable in the mucosal (m)-to-serosal (s) and s-to-m directions [0.10 ± 0.01 (n = 17) and 0.10 ± 0.01 (n = 4) %/hr · cm^2, respectively]. Transport of GHRP and LY in the m-s direction was similar to that of Man [0.08 ± 0.01 (n = 11) and 0.09 ± 0.01 (n = 3) %/hr · cm^2, respectively]. GHRP flux was equivalent in the m-s and s-m directions. GHRP did not significantly alter ion transport processes as indicated by the lack of any change in G_t or I_{sc}. Luminal addition of AMGZ (2%, 24 m*M*) increases G_t and transport of both LY and [^{3}H]Man approximately fourfold without altering transport of [^{3}H]GHRP. No changes in transport or G_t were seen with luminal addition of AMGZ (1%, 12 m*M*). These studies suggest that transport of the hexapeptide GHRP occurs by a passive process and that AMGZ selectively increases the permeability of the mucosa to the low molecular weight molecules, Man and LY, but not to GHRP *in vitro*.

KEY WORDS: nasal; peptide; transport; growth hormone releasing peptide; ammonium glycyrrhizinate.

INTRODUCTION

Although there have been numerous attempts to identify effective routes for peptide delivery, including oral, buccal, pulmonary, transdermal, and rectal, none of these has proved to be efficient or generally acceptable. The nasal route, however, is effective and acceptable for several peptides, including desmopressin (1), calcitonin (2), and buserelin (3). Nasal administration offers several advantages, including rich vascularity, avoidance of first-pass metabolism, and absence of an unfavorable luminal environment like that found in the intestine.

Peptides cited above are hormones or hormone mimetics and thus dose requirements for systemic availability are lower than may be required for drugs currently being developed, such as peptide antagonists or enzyme inhibitors. In an attempt to increase the bioavailability of peptides administered by the nasal route, a number of absorption promoters have been investigated. Among these are bile salts, which increase permeability but also are reported to alter nasal morphology (4–7) and thus may not be suitable for chronic use. Other strategies proposed to increase nasal absorption include inhibition of protease activity (8), increases in formulation viscosity or a decrease in ciliary activity, thereby increasing residence time in the nasal cavity (9–11), and alterations in physical characteristics of the peptide [e.g., reduced aggregation (12)]. Ammonium glycyrrhizinate has been reported to enhance the nasal absorption of calcitonin (13) and glycyrrhetinic acid derivatives have also been reported to promote nasal absorption of insulin (14).

Objectives of this study were (i) to establish techniques for studying transport across nasal mucosa *in vitro*, (ii) to determine routes and mechanisms of transport of growth hormone releasing peptide (GHRP; SK&F 110679) across ovine nasal mucosa *in vitro*, and (iii) to determine effects of ammonium glycyrrhizinate (AMGZ) on transport of the passive permeability markers, mannitol (Man) and lucifer yellow (LY), and the hexapeptide, GHRP. An *in vitro* model utilizing ovine nasal mucosa mounted on Ussing chambers was used to study intranasal drug transport. The rationale for selecting this model is (i) a demonstrated correlation between previous results with ovine nasal studies *in vivo* and human nasal studies (15) and (ii) previous data suggesting that this *in vitro* model provides a rapid method for evaluating transport of drugs and effects of absorption promoters (7). Use of Ussing chambers is advantageous since tissue viability and integrity can be continuously monitored by measuring transepithelial conductance (G_t) and short-circuit current (I_{sc}). Ussing chambers can also provide information about routes and mechanisms of drug transport, metabolic stability, and morphologic correlations (7,16–20).

MATERIALS AND METHODS

Materials

GHRP and [^{3}H]GHRP (sp act, 31.9 Ci/mmol) as the acetate salt were obtained from SB. [^{3}H]GHRP was stored at −80°C in ethanol (40 μCi/mL). [^{14}C]Mannitol (mannitol, D-[1-^{14}C], 55.0 mCi/mmol) and [^{3}H]mannitol (mannitol, D-[1-^{3}H(N)], 30.0 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Lucifer yellow (potassium salt) was purchased from Molecular Probes (Eugene, OR). Mannitol

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and 2-[*N*-morpholino]ethanesulfonic acid (MES) were obtained from Sigma Chemical Co. (St. Louis, MO). Except where indicated, all other reagents were from Sigma Chemical Co.

A Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) including SCL-6A system controller, LC-6A pumps, SIL-6A auto injector, SPD-6AV UV-VIS spectrophotometric detector, and C-R3A chromatopac together with an LKB 2211 SuperRac fraction collector (LKB-Produkter AB, Bromma, Sweden) was employed for analysis of GHRP. Zorbax RX-C8 columns (4.6 mm × 15 cm) were purchased from Rockland Technologies, Inc. (Chadds Ford, PA). The luminescence spectrometer LS 50, FL Data Manager software package, and IBM Personal System/2 Model 50 Z personal computer were purchased from Perkin Elmer (Buckinghamshire, England).

Tissue Preparation for Ussing Chambers

Ovine tissue obtained locally was mounted in Ussing chambers (1.13-cm^2 exposed area) within 1 hr of collection as described previously (7). Tissues were equilibrated for 60–120 min in 10 mL bicarbonate-buffered Ringer solution (BBRS) containing 10 m*M* glucose in the serosal bath and 10 m*M* mannitol in the mucosal bath. Throughout the equilibration period, transepithelial potential difference (PD) with reference to the mucosal bathing solution and short-circuit current (I_{sc}) were measured using a current/voltage clamp (VCC600, Custom Control, Houston, TX). Tissues were continuously short-circuited via electrodes at the distal ends of each half-chamber except for brief intervals (<10 sec) during which time *PD* was measured. Transepithelial conductance (G_t) was calculated as described previously (7).

Transepithelial Unidirectional Flux Measurements

Subsequent to the equilibration period, reservoirs and chambers were clamped, drained, and refilled with 10 mL of BBRS containing 10 mM glucose in the serosal bath and 10 m*M* mannitol in the mucosal bath. GHRP (100 μ*M*) was then added to both bathing solutions and 5 μCi of [^{3}H]GHRP and 5 μCi of [^{14}C]mannitol were added to either the serosal bathing solution for serosal (s)-to mucosal (m) fluxes or the mucosal bathing solution for m-to-s fluxes. The rational for including unlabeled GHRP in both the donor and the receiver compartments is to avoid nonspecific adhesion which could complicate interpretation of results. Inclusion of unlabeled GHRP in both bathing solutions will not alter interpretation of the transport results since radiolabeled tracer is being measured. *PD* and I_{sc} were measured throughout the experiment as indicated above. One-milliliter samples were taken from the receiver reservoir at 30-min intervals for 3 hr. After each sample, the volume removed was replaced with the appropriate buffer to maintain constant volume. Samples of 100 μL were taken from the donor reservoir at 0, 60, and 180 min. These volumes were not replaced. Each sample was mixed with 10 mL of scintillation fluid (Ready Safe, Beckman Instruments, Inc., Fullerton, CA), and radioactivity determined with a Packard Tri-Carb 4640 scintillation counter using the external channel's ratio to correct for quenching. Results are presented as percentage of the administered dose or as a flux of substance per hour per square centimeter, taking into account dilution effects of the replacement buffer.

Enhancer Studies

Effects of luminal AMGZ on m-s fluxes of [^{14}C]- or [^{3}H]mannitol, lucifer yellow (LY), and [^{3}H]GHRP were determined. Flux studies in the presence of luminal AMGZ (2%) were performed with all the above compounds, and flux studies in the presence of luminal AMGZ (1%) were performed with [^{3}H]mannitol and LY. Initially a 1-hr radiolabeled mannitol flux was determined in each tissue to assess tissue permeability under control conditions. Chambers were drained and refilled as indicated above except that the mucosal reservoir was replaced with BBRS without or with AMGZ. A subsequent 3-hr flux in the same tissue was then conducted. During the initial 1-hr flux period, 1-mL samples were taken from the serosal reservoir at 0, 15, 30, and 60 min and 100-μL samples were taken from the mucosal reservoir at 0 and 60 min. For LY flux studies, 1 mg of LY was added to the mucosal bathing solution. One-milliliter samples were taken at 0, 15, 30, and 60 min from the serosal reservoir and 100-μL samples were taken from the mucosal reservoir at 0 and 60 min for LY analysis. Fluorescence of LY samples was measured at excitation 428 nm and emission 540 nm. Transport of LY across nasal tissue was quantitated from a standard curve. Volume was maintained constant by addition of the appropriate buffer throughout the experiment. *PD* and I_{sc} were measured throughout the experiment as indicated above. At the end of the experiment, amiloride was added to the mucosal bathing solution to determine its effect on electrical properties (7).

AMGZ Reversibility Studies

Ovine nasal tissue was mounted in Ussing chambers as indicated above. After the equilibration period, tissues were incubated for 20–60 min in the absence or presence of luminal AMGZ (2%). Tissues were subsequently washed and reincubated for 1 hr with BBRS. *PD* and I_{sc} were measured throughout the study as indicated above. At the end of the experiment, amiloride was added to the mucosal bathing solution to determine its effect on electrical properties.

GHRP Stability

Ovine nasal tissue was stripped from the underlying cartilage as stated above. Tissues were mounted in diffusion chambers (1.13-cm^2 exposed area) and equilibrated for 60–120 min in 5 mL of BBRS with 10 m*M* glucose in the serosal bath and 10 m*M* mannitol in the mucosal bath. At the end of the equilibration period, chambers were drained and refilled with 5 mL of BBRS with 10 m*M* glucose in the serosal bath and 10 m*M* mannitol in the mucosal bath. GHRP (100 μ*M*) and 15 μCi of [^{3}H]GHRP were then added to the mucosal bathing solution. One-milliliter samples were taken from both the mucosal and the serosal reservoirs at 0, 60, and 180 min. These volumes were not replaced. Acetonitrile was added to each mucosal sample to obtain a 20% acetonitrile concentration. Mucosal samples were analyzed by HPLC for degradation of GHRP. The column was eluted with 20%

acetonitrile/ammonium phosphate buffer (5 m*M* hexanesulfonic acid, 0.1 *M* ammonium dihydrogen phosphate, and 20 m*M* triethylamine, pH 4.0). Flow rate was 1.5 mL min^{-1}, and run time was 20 min. GHRP was detected at 280 nm. One-minute fractions were collected throughout each run and counted to determine the elution profile of radioactivity. These elution profiles were compared with the UV absorbance profiles of authentic GHRP.

Statistics

Results are presented as means ± SE. Statistical analysis was performed with Student's paired or unpaired *t* test as appropriate. A value of $P < 0.05$ was considered statistically significant (21).

RESULTS

Transepithelial Unidirectional Flux Measurements

From the time course for transport of radiolabeled mannitol across ovine nasal mucosa, it was found that the m-s and s-m fluxes did not significantly differ as expected for a diffusional marker [m-s = 0.10 ± 0.01%/hr · cm^2 (n = 17) and s-m = 0.10 ± 0.01%/hr · cm^2 (n = 4)]. Both fluxes are linear with time as indicated by correlation coefficients of 0.987 and 0.996 (m-s and s-m, respectively). Values for ^{14}C-mannitol and ^{3}H-mannitol transport rates were not different. Figures 1 and 2 illustrate G_t and I_{sc} values for mannitol flux studies. Electrical properties were similar between tissues employed for determination of m-s and s-m fluxes. During the initial 60- to 120-min equilibration period, G_t decreased and I_{sc} increased in all tissues, consistent with reestablishment of tight junctional integrity and ion transport processes. Similar changes in electrical properties have been reported previously for nasal (7), tracheal (20), and intestinal epithelium (17). Throughout the 3-hr flux period G_t and I_{sc} averaged 8.3 ± 0.5 mS/cm^2 and 3.7 ± 0.2 μEq/hr · cm^2 (n = 21), respectively. No apparent changes in G_t and I_{sc} upon addition of mannitol or GHRP were observed.

The time course of [^{3}H]GHRP transport across ovine

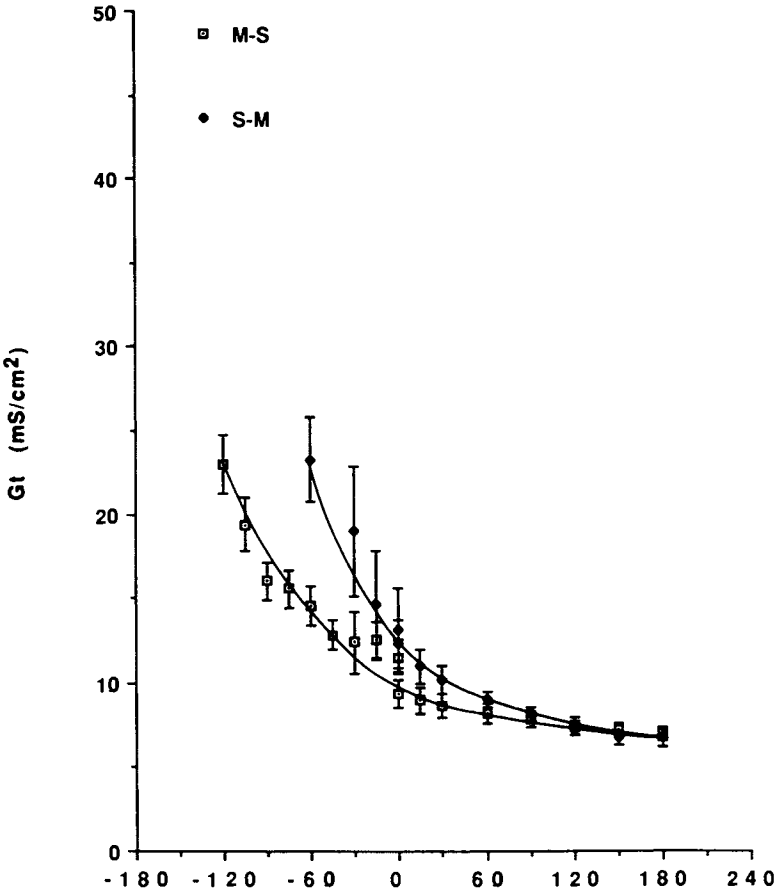

Fig. 1. G_t of ovine nasal mucosa during mannitol flux studies. The G_t was measured over the time course of each flux experiment. Negative time points indicate the equilibration period. Results are mean G_t (±SE) for 17 tissues with m-s and 4 tissues with s-m fluxes.

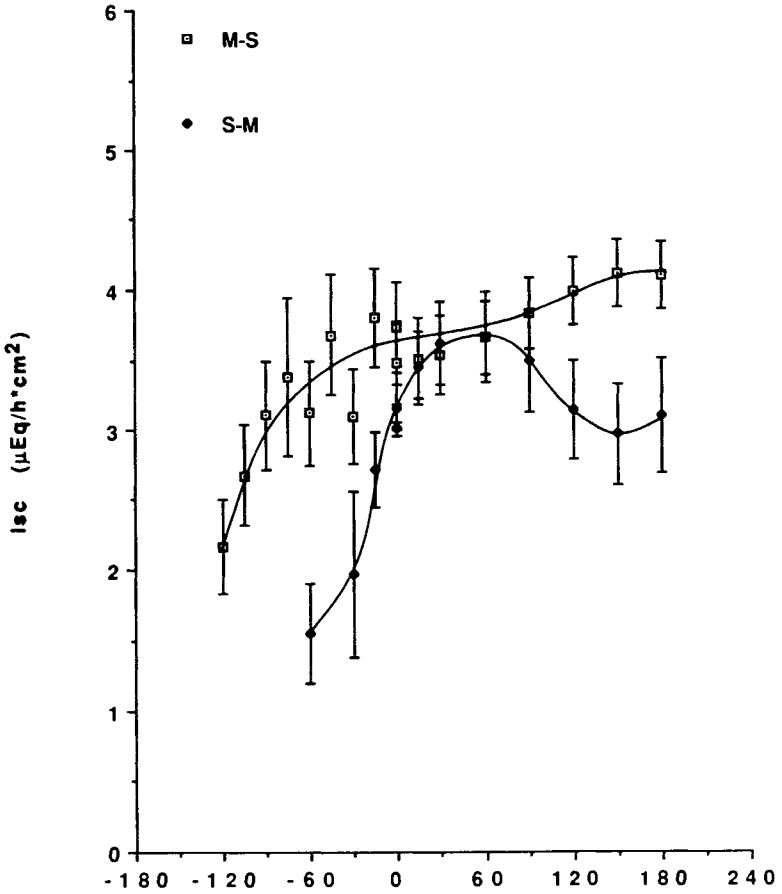

Fig. 2. Time course of I_{sc} across ovine nasal mucosa during mannitol flux studies. The I_{sc} was measured from each flux experiment. Negative time points indicate the equilibration period. Results are mean I_{sc} (±SE) for 17 tissues with m-s and 4 tissues with s-m fluxes.

nasal tissue demonstrated that [^{3}H]GHRP fluxes in the m-s and s-m direction were not significantly different from each other [0.08 ± 0.01 (n = 11) and 0.09 ± 0.02 (n = 4) %/hr · cm^2, respectively] or from mannitol fluxes. Addition of GHRP (100 μM) to the luminal or serosal bathing solutions did not significantly alter ion transport across ovine nasal mucosa as evidenced by the absence of any change in I_{sc} or G_t (Table I).

Effects of AMGZ

Mucosal-to-serosal transport of radiolabeled mannitol across ovine nasal tissue in the absence or presence of AMGZ is shown in Fig. 3. For untreated tissue, radiolabeled mannitol transport values were taken from basal studies described above (0.10 ± 0.01%/hr · cm^2). In tissues treated with 1% AMGZ, radiolabeled mannitol transport was not significantly altered (0.07 ± 0.02%/hr · cm^2). In the presence of 2% AMGZ, a four- to fivefold increase in mannitol transport was observed (0.48 ± 0.06%/hr · cm^2).

Mucosal-to-serosal transport of LY across ovine nasal tissue was qualitatively similar in untreated tissues (0.09 ± 0.01%/hr · cm^2) and tissues treated with 1% AMGZ (0.07 ± 0.03%/hr · cm^2). In the presence of 2% AMGZ, a four- to fivefold increase in LY transport is observed (0.44 ± 0.11%/

Table I. Effect of GHRP on the conductance (G_t) and the Short-Circuit Current (I_{sc}) of Sheep Nasal Mucosa[a]

Condition	ΔG_t	ΔI_{sc}
− GHRP (control)	1.8 ± 0.4	0.0 ± 0.1
+ GHRP		
Mucosal	1.8 ± 1.3	−0.3 ± 0.2
Serosal	2.0 ± 1.0	−0.6 ± 0.3

[a] Results are means ± SE for three experiments in which all conditions were examined. The change in G_t (ΔG_t) and the change in I_{sc} (ΔI_{sc}) were calculated by subtracting the final G_t and I_{sc} values of the equilibration period from the G_t and I_{sc} values 15 min after addition of GHRP.

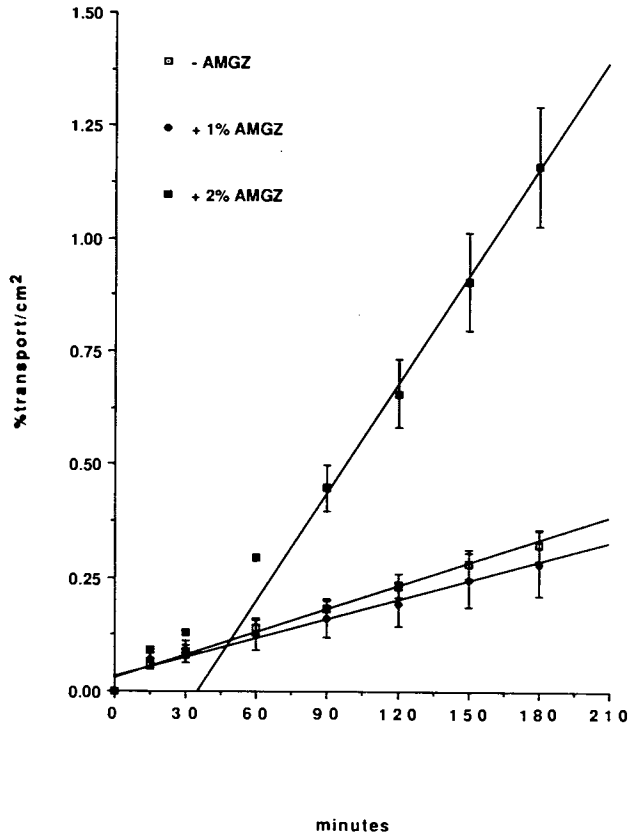

Fig. 3. Mannitol transport across ovine nasal mucosa in the presence of AMGZ. The m-s fluxes in the absence or presence of 1% AMGZ and 2% AMGZ were determined as detailed under Materials and Methods. Results are mean percentage cumulative transport (±SE) as a function of time. Correlation coefficients (R^2) determined by linear regression analysis are 0.987 (n = 17 tissues), 0.972 (n = 9 tissues), and 0.998 (n = 18 tissues) for 0% AMGZ, 1% AMGZ, and 2% AMGZ, respectively.

hr · cm²). However, 2% AMGZ does not alter the m-s flux of [³H]GHRP across ovine nasal tissue (0.08 ± 0.01%/hr · cm²).

Figure 4 presents the effects of AMGZ on G_t. In untreated tissues and tissues treated with 1% AMGZ, G_t remained constant. Addition of 2% AMGZ to the luminal bathing solution produced a four- to fivefold increase in G_t (8.3 ± 0.5 to 36.2 ± 5.3 mS/cm²). In tissues treated with luminal AMGZ (2%), I_{sc} decreased from 3.8 ± 0.3 to 1.7 ± 0.3 μEq/hr · cm².

In studies with 2% AMGZ, the pH of the luminal bathing solution was reduced from 7.4 to 5.5 and the osmolarity was increased from 280 to 327 mOsm. To determine whether effects on I_{sc}, G_t, and passive permeability as assessed by changes in LY fluxes were due to changes in luminal pH or osmolarity, electrical properties and LY fluxes were determined with BBRS, which was adjusted to pH 5.5 with MES or with BBRS in which the osmolarity had been increased to 370 mOsm by the addition of mannitol. Results from these studies demonstrate that lowering the pH to 5.5 did not significantly alter I_{sc}, G_t, or LY transport. Increasing the osmolarity to 370 mOsm resulted in a decrease in I_{sc} [I_{sc} at 280 and 370 mOsm = 3.0 ± 0.5 and 1.7 ± 0.3 μEq/hr · cm², respectively (n = 4)], G_t [G_t at 280 and 370 = 6.6 ± 1.0 and 5.0 ± 1.3 mS/cm², respectively (n = 4)], and LY transport [LY fluxes at 280 and 370 mOsm = 0.04 ± 0.03 and 0.010 ± 0.008%/hr · cm², respectively (n = 4)]. Thus, changes in pH or osmolarity alone do not appear to account for the changes in transport observed with 2% AMGZ.

Previously, it was shown that amiloride, a Na^+ channel blocker, reduces I_{sc} across ovine nasal mucosa, consistent with inhibition of Na^+ absorption (7). Since Na^+ absorption is dependent on the basolaterally located Na/K-ATPase (7), the presence of an amiloride-inhibitable I_{sc} provides a measure of the ability of the tissue to generate ATP and, thus, tissue viability. In control tissues, luminal addition of amiloride reduced I_{sc} from 3.7 ± 0.2 to 1.4 ± 0.1 μEq/hr · cm². In the presence of luminal AMGZ, amiloride decreased I_{sc} by only 0.2 μEq/hr · cm² (2% AMGZ). These results indicate that 2% (but not 1%) AMGZ abolishes the amiloride-inhibitable portion of I_{sc}, which may be due either to an indirect effect on energy metabolism or to a direct effect on the Na^+ conductance pathway. The observation that I_{sc} was not zero in the presence of 2% AMGZ or after addition of 10 μ*M* amiloride suggests that some other ion transport process is responsible for this portion of the current and that AMGZ does not have a nonspecific effect on the tissue.

Figure 5 shows the reversibility of effects of 2% AMGZ on G_t of nasal mucosa. The G_t increased from 10.7 ± 0.8 to 15.0 ± 1.0 mS/cm² after a 60-min incubation with luminal AMGZ (2%). The G_t returned to a value of 10.3 ± 0.9 mS/cm² after replacing both bathing solutions with fresh BBRS and incubating for one hour.

The correlation of mannitol flux with G_t is illustrated in Fig. 6. A linear relationship, in which G_t increased as mannitol flux increased, was observed with a correlation coefficient of 0.779. A qualitatively similar correlation of LY flux with G_t was observed with a correlation coefficient of 0.968. No correlation between mannitol or LY flux and I_{sc} was observed (data not shown).

GHRP Stability Study

The ability of sheep nasal mucosa to metabolize GHRP was investigated employing HPLC analysis of samples taken from the luminal bathing solution after 60 and 180 min. As shown in Table II, after 60 and 180 min, greater than 90% of the radioactivity was still present as the parent molecule, GHRP. Analysis of low levels of radioactivity appearing in the serosal bathing solution were attempted but could not be quantitated.

DISCUSSION

In this study, transport rates for mannitol, lucifer yellow, and GHRP were determined in ovine nasal mucosa employing the Ussing technique. Transport rates for GHRP are equal in the m-s and s-m directions and are comparable to those of lucifer yellow and mannitol. These results suggest that transport of GHRP across ovine nasal mucosa occurs by a diffusional mechanism. In addition, luminal exposure to 2% AMGZ produces parallel increases in G_t and transport

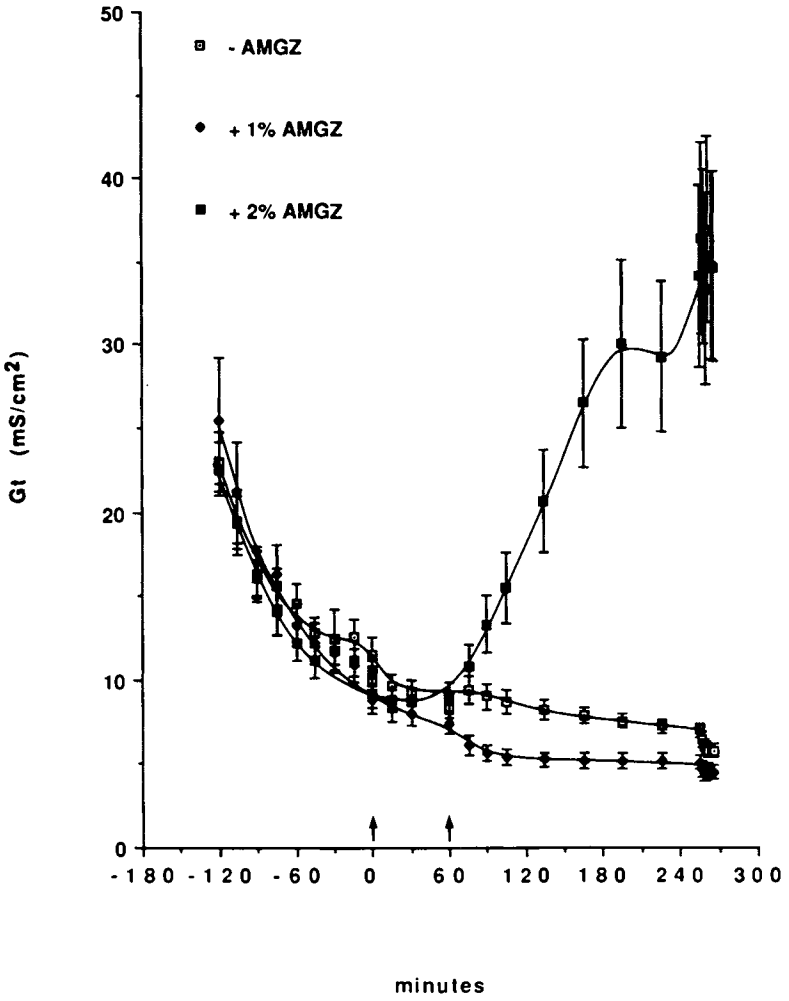

Fig. 4. G_t of ovine nasal mucosa in the absence or presence of AMGZ. G_t was measured over the time course of each flux experiment. Negative time points indicate the equilibration period, the first arrow indicates the initiation of a 1-hr m-s control mannitol flux, and the second arrow indicates the start of a 3-hr experimental m-s flux with the appropriate concentration of AMGZ. Results are mean G_t (±SE) for 17 tissues with 0% AMGZ, 9 tissues with 1% AMGZ, and 18 tissues with 2% AMGZ.

rates of the low molecular weight markers, mannitol and lucifer yellow, but does not alter the transport rates of GHRP. The increase in G_t elicited by AMGZ can be reversed by washing the epithelium with fresh buffer. These results are most consistent with a selective increase in the paracellular permeability of the nasal mucosa.

The Ussing technique provides a method for studying transepithelial transport, assessing tissue viability and integrity, and determining metabolic capabilities of epithelial membranes (16–20). In addition, this technique allows mechanistic studies to be conducted which would not be possible *in vivo*. Using the Ussing technique, it was found that the basal electrical parameters, I_{sc} and G_t, achieved stable values after approximately 3 hr *in vitro*. The I_{sc} is the current required to nullify the spontaneous potential difference and thus provides a measure of net active ion transport, an indirect measure of tissue viability. In ovine nasal mucosa, I_{sc} at steady state is approximately 3.5 μEq/hr · cm^2. The ionic basis for this I_{sc} appears to result predominantly from the active transport of Na^+ via an amiloride-sensitive uptake mechanism located at the apical cell membrane and exit of Na^+ across the basolateral membrane by the Na^+/K^+-ATPase (7).

The G_t across epithelia provides a measure of ionic permeability and has been employed to evaluate tissue integrity. A G_t value of 8.3 ± 0.5 mS/cm^2 for ovine nasal mucosal compares to a G_t value of 30 mS/cm^2 for rabbit small intestine (17), 5–10 mS/cm^2 for rabbit colonic mucosa (22), 2.0 mS/cm^2 for canine tracheal mucosa (20), and 0.03–0.5 mS/cm^2 for amphibian urinary bladder (23). Thus, ovine nasal mucosa can be classified as a moderately "leaky" epithelia. Comparison of the G_t and I_{sc} values for ovine nasal mucosa obtained in this study are in good agreement with those reported previously (7).

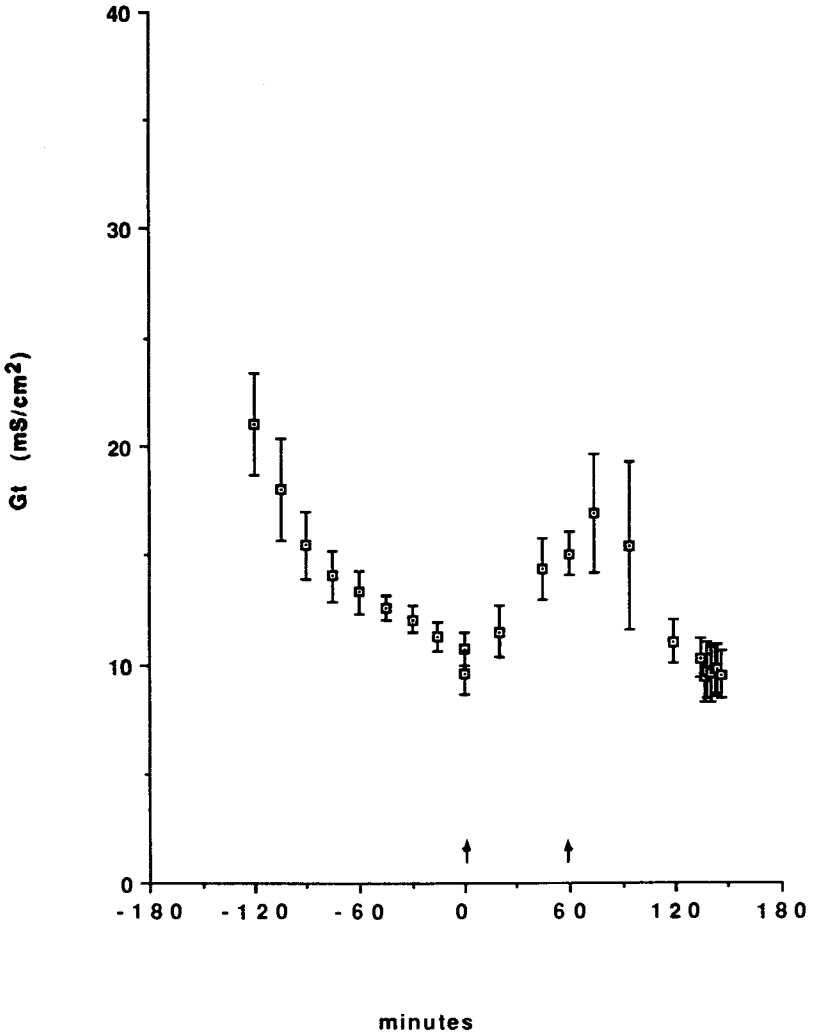

Fig. 5. Reversibility of the effects of AMGZ (2%) on G_t of ovine nasal mucosa. Negative time points indicate the equilibration period, the first arrow indicates the addition of luminal AMGZ (2%), and the second arrow indicates washing and incubation with fresh BBRS. Results are mean G_t (±SE) for four tissues.

A second method employed for assessing epithelial integrity is measurement of transport rates for low molecular weight solutes, such as mannitol (24,25). Mannitol (MW 182) does not affect tight junctional integrity or ion transport processes, as assessed by the lack of effect on G_t and I_{sc}. In this study, both mannitol, a noncharged, low molecular weight marker, and lucifer yellow (MW 521), a charged, low molecular weight marker, were employed to assess epithelial integrity. Transport rates for these molecules are similar (0.10 ± 0.01%/hr · cm^2 for mannitol and 0.09 ± 0.01%/hr · cm^2 for lucifer yellow). By comparison, transport rates for mannitol in rabbit jejunum and ileum are less than 0.1%/hr · cm^2 (22). It can be seen from Fig. 6 that there is a good correlation between G_t and percentage mannitol transport per hour per square centimeter or percentage LY transport per hour per square centimeter. This correlation further supports the proposal that mannitol and LY transport occur by a passive mechanism.

Transport of the hexapeptide, GHRP, also appears to occur by a diffusive process since both the m-s and the s-m fluxes are equal. GHRP does not affect tight junctional integrity or ion transport processes since G_t and I_{sc} values did not differ in the presence of 100 μ*M* GHRP. Experiments to investigate the concentration dependence or temperature dependence of transport were not conducted in this study since there was no indication that transport occurred by a facilitated mechanism, as has been proposed for amino acids and polypeptides in prior studies (26,27).

From these results, it can be seen that 2% AMGZ (24 m*M*) increases G_t and transport of the low molecular weight markers, mannitol and lucifer yellow, without altering the transport of GHRP. However, 1% AMGZ (12 m*M*) represents a no-effect concentration, as demonstrated by the lack of increase in G_t or transport of mannitol and lucifer yellow. This suggests that AMGZ enhances nasal transport in a concentration-dependent manner via an alteration of tight junc-

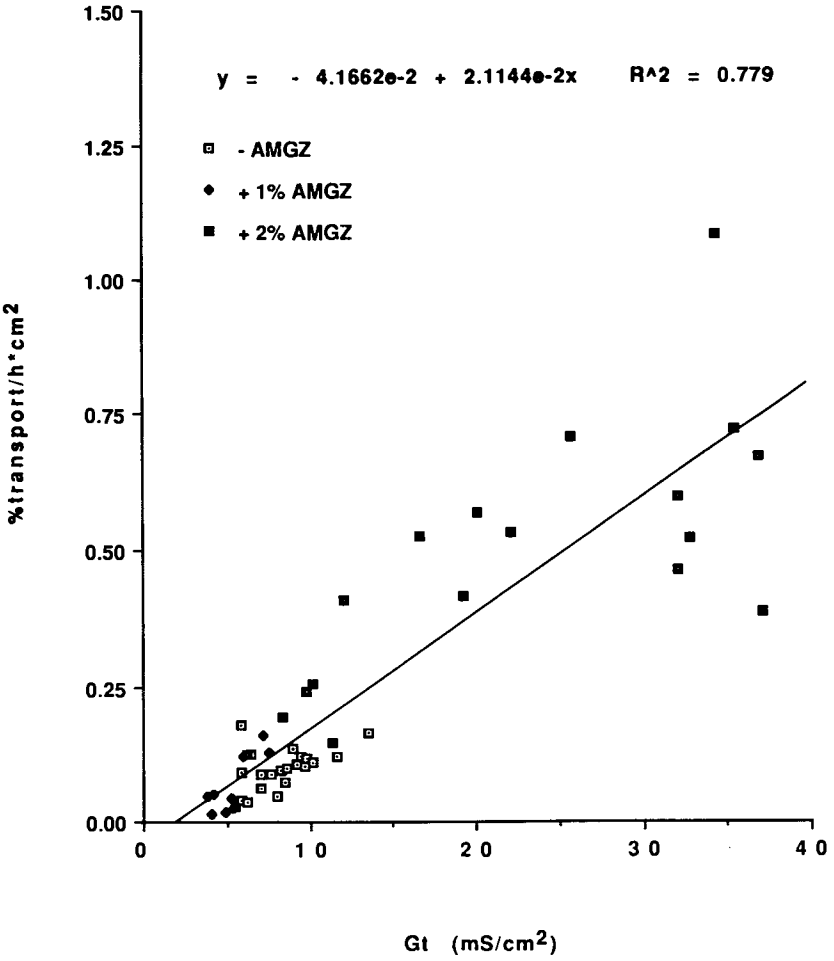

Fig. 6. Correlation between mannitol flux and G_t (mean for the appropriate flux period).

tional integrity. With the limited data available, it appears that this change in junctional permeability is limited by size. Additional studies will be required to confirm this proposal. In addition, the increase in G_t elicited by AMGZ is reversible. This finding together with the findings that AMGZ does not alter morphology or function suggests that AMGZ, at the concentrations employed, does not produce irreversible toxic effects (28,29).

These studies provide information regarding the mechanisms by which peptides traverse the nasal epithelium. In addition, these results support the use of ovine nasal mucosa as an *in vitro* model for evaluating formulation components.

Table II. Susceptibility of GHRP to Degradation by Ovine Nasal Mucosa[a]

Time (min)	% GHRP remaining
0	100.0
60	97.6
180	91.2

[a] GHRP added to the luminal bathing solution of ovine nasal mucosa was incubated for 0, 60, and 180 min, at which time 125-μL samples were removed and analyzed by HPLC as detailed under Materials and Methods. The percentage GHRP remaining at each time was calculated from the normalized dpm values recovered.

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