Simultaneous *in Vitro* Measurement of Intestinal Tissue Permeability and Transepithelial Electrical Resistance (TEER) Using Sweetana–Grass Diffusion Cells

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A simple modification of the commercially available Sweetana–Grass (S-G) side-by-side diffusion cells, allowing the simultaneous measurement of tissue permeability and transepithelial electrical resistance (TEER), has been described and validated for rat excised, muscle-free intestinal tissue. The TEER-lowering effects of a series of acylcarnitines were shown to be correlated with previously reported *in vitro* (i.e., membrane perturbation) and *in vivo* (i.e., absorption enhancement) activity. The TEER-lowering effect of palmitoyl carnitine chloride (PCC) was also shown to be reversible. The effects of PCC on TEER and the permeability of poorly absorbed compounds (cefoxitin and lucifer yellow) were simultaneously determined. Compared to controls (mannitol-treated), PCC immediately produced a rapid drop in colon TEER. By 5 min post-PCC addition, colon TEER was 50% of control; by 10 min post-PCC addition, colon TEER was 17% of control. After a lag of about 5–10 min post-PCC addition, the cefoxitin or lucifer yellow permeability coefficient increased more than 20-fold. The modified S-G cells provide a simple and reproducible method whereby flux and TEER can be simultaneously determined, providing a valuable link between the effect of absorption enhancers on TEER measurements and the increased permeability of poorly absorbed compounds.

KEY WORDS: acylcarnitines; cefoxitin; lucifer yellow; rat colon; tissue permability; transepithelia electrical resistance (TEER); Sweetana–Grass diffusion cells.

INTRODUCTION

Electrophysiologic [Ussing chamber-type (1)] measurements have been routinely performed during the past 40 years. Although several apparatuses with minor improvements have been made, the Ussing chamber apparatus remained deficient in several categories (see below). Recently, Grass and Sweetana (2) have introduced a new S-G diffusion cell with several advantages over the classical Ussing chamber apparatus, including reduced leakage (i.e., there are no connections between the tissue compartment and the drug reservoir), better and more easily controlled mixing and heating (i.e., simultaneous heating of six cells), an increased working tissue surface area (compare to the typical 10-ml Ussing chamber), and easier cleaning (see Ref. 2 for details).

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However, the commercially available S-G cells are not equipped for electrophysiologic measurements. Since the advantages found in the S-G cells make them better suited than Ussing chambers for flux measurements of drugs, the capability to obtain electrophysiologic measurements may provide useful additional information in the study of absorption enhancers. For example, the transepithelial electrical resistance (TEER) can be a useful index of tissue integrity and viability and would be useful data to obtain for the study of absorption enhancer effects (3,4).

The acylcarnitines are a series of medium- to long-chain fatty acid esters of carnitine that have been extensively studied as absorption-promoting adjuvants (5). The 16-carbon acylcarnitine, palmitoyl carnitine chloride (PCC), appears to be the most potent in this regard, as shown both *in vivo* (5) and *in vitro* (8).

We report here a simple modification of the S-G cells that allows them to be utilized in the Ussing configuration for electrophysiologic measurements. This method may help elucidate the mechanism of action whereby absorption enhancers modify membrane permeability. We also report the results of experiments where TEER and the PCC-enhanced flux of poorly absorbed compounds were simultaneously measured in rat intestinal tissue.

EXPERIMENTAL

Chemicals. Sodium cefoxitin (Mefoxin) was obtained from Merck Sharp & Dohme Research Laboratories (Westpoint, PA); lucifer yellow, from Molecular Probes (Eugene, OR); and Krebs–Henseleit buffer and all acylcarnitines (mixed isomer chloride salt), from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade.

Sweetana–Grass Apparatus Modifications. The S-G diffusion cells and heating block were purchased from Precision Instrument Design (Los Altos, CA). The exposed tissue surface area was 1.8 cm^2, and the volume of each half-cell was 7.5 ml.

Diffusion Cell Cap. The acrylic cell cap (Rohm-Haas U.V. stabilized plexiglass grade G.M. sheet) was fabricated as a two-piece construction, bonded (No. 3 methylene chloride, Cope Plastics Inc., Topeka, KS) together to form a round plug (Fig. 1). The rectangular top was (H × W × L) 0.142 × 1.082 × 1.094 in., and the round plug was machined to a sliding fit matching the inside diameter (≈0.760 in.) of the diffusion chamber. A tapered hole was machined in the cap at a 10° angle to the vertical axis of the cell chamber. The depth of this hole depended on the type of pipette tip employed as the reversible electrode. A second hole was machined to accept the hair-pin current injecting electrode.

Electrodes. The Ag/AgCl reversible electrodes (No. 5415, A-M Systems, Inc., Everett, WA) were mounted in agar-filled pipette tips (No. 53503-167, VWR Scientific, Inc., Chicago, IL), which were compression-fitted into the diffusion cell cap. The matching 7.5° taper of the pipette tips held the tip rigidly in place and maintained the fixed distance of the tip relative to the membrane surface of the tissue mounted in S-G cells.

The current-injecting electrodes were hand-fabricated

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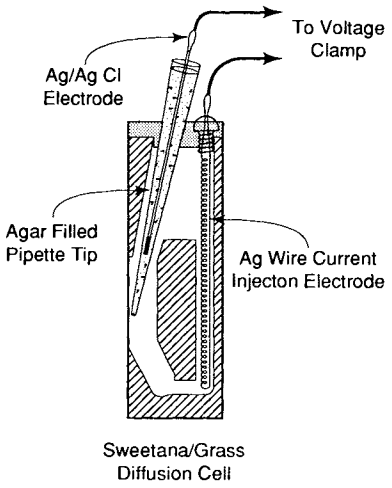

Fig. 1. Schematic of the modified S-G diffusion cell, showing the cap and electrodes in proper orientation for TEER measurements.

from high-purity (99.9%) 0.015-in.-diameter silver wire (A-M Systems, Inc.). All solder connections were made using No. 5 stainless-steel solder (Welco, Kings Mountain, NC). Miniature gold pin connectors (No. 5201, 5202, 5100, A-M Systems, Inc.), wire, plugs and insulated solderless tip plugs (Newark Electronics, Chicago, IL) completed the electrical connections to the voltage-clamping equipment (No. TR100-F, TR100-FC, JWT Engineering, Overland Park, KS).

Transparent Front Heating Block. To aid in visually confirming the location of the electrodes, a plexiglass front heating block was used instead of the aluminum heating block provided with the heated water circulator. This block was fabricated from two 1 × 13-in. square plexiglass tubes (Thermoplastics Process, Inc., Sterling, N.J.), and two 1 × 2-in. square solid plexiglass blocks (Cope Plastics Inc.).

In Vitro *Experiments: Excised Intestinal Tissue.* Unfasted Sprague–Dawley rats (250–350 g) were anesthetized with intraperitoneal pentobarbital (50 mg/kg). A 2.5- to 3.0-cm strip of intestinal tissue was excised, then stretched onto a glass rod, the serosal layers of muscle were dissected away, and the intestinal tissue was mounted onto the S-G cells (3). The entire procedure typically took 2–3 min. Krebs–Henseleit buffer was used in all experiments and circulation of the buffer was maintained by a gas-lift using 95%/5% O_2/CO_2. Temperature was maintained at 37°C and monitored (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) with a probe in contact with the serosal chamber buffer. A preincubation of 25–30 min was normally required to reach temperature and resistance (TEER) equilibrium. TEER was calculated by briefly clamping the tissue at 0.0 and 2.0 mV and measuring the current flow at each setting. The ratio of the change in current to the clamping voltage is resistance (Ohm's law). Experiments with absorption enhancers were initiated only after temperature and TEER equilibrium were established. In reversibility experiments, the buffer on the mucosal side was replaced with warm, fresh buffer, and TEER measurements were taken every 2–5 min until no further changes were observed. Experiments typically lasted no longer than an hour. Figure 2 includes micrographs of freshly excised tissue and tissue removed at the end of a typical experiment. As can be seen, the colon mucosa remained intact during the course of the experiment.

Acylcarnitine effects were examined by adding aliquots of the chloride salt of octanoyl carnitine (OOC, C8), decanoyl carnitine (DCC, C10), lauroyl carnitine (LCC, C12), or palmitoyl carnitine (PCC, C16) in stock solutions to a final mucosal chamber concentration of 2.5 or 5.0 m*M*.

The control and enhanced fluxes of the poorly absorbed compounds cefoxitin and lucifer yellow were also determined. Aliquots of cefoxitin or lucifer yellow stock solutions were added to the mucosal chamber to a final concentration of 10 or 0.05 mg/ml, respectively. Mannitol (controls) or PCC was then added to the mucosal chamber, 100-μl samples were taken from the serosal chamber for assay, and the volume was replaced with fresh buffer.

Analytical. Sodium cefoxitin was assayed according to published methods (6). Lucifer yellow concentration was measured by fluorescence spectroscopy (Hitachi F-3010 flu-

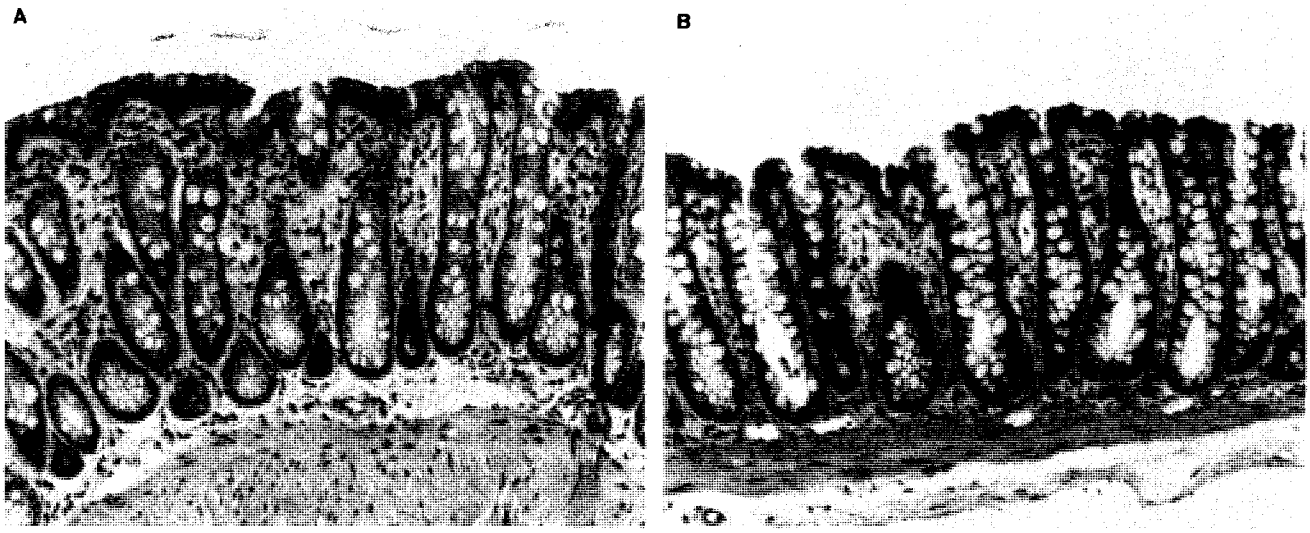

Fig. 2. Rat colonic tissue before stripping and mounting onto S-G cells (A) and 60 min after mounting (B). H&E staining. 200×; reduced 0% for reproduction.

orometer, Grover, MO) at 428-nm excitation and 540-nm emission.

Kinetics and Statistics. In vitro permeability coefficients were calculated (2) from the following equation:

$$P = \frac{V \cdot dC}{A \cdot C_0 \cdot dt}$$

where dC/dt is the change in concentration as micrograms per milliliter per unit time and V is the volume of the receptor phase as cubic centimeters. A is the surface area of the intestinal segment and C_0 is the initial concentration of the diffusing drug.

Mean ± SD are provided throughout, and differences (ANOVA or t test) were considered significant at the $P < 0.05$ level (7).

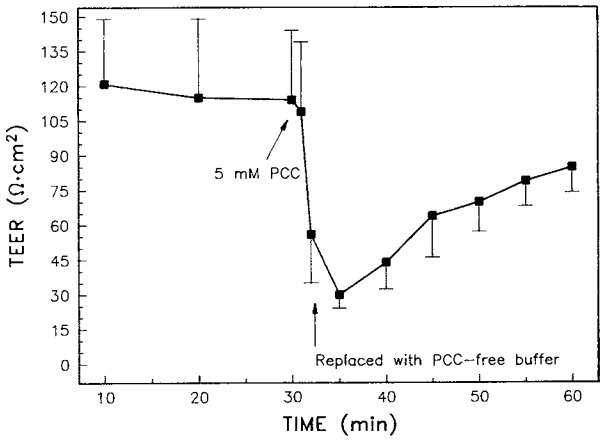

Fig. 3. Reversibility of the PCC-induced TEER-lowering effects. Representative colon TEER following the addition and subsequent removal of 5.0 m*M* PCC.

RESULTS AND DISCUSSION

Small and Large Intestine TEER. Experiments with mucosal tissue from the small intestine (duodenum, jejunum) determined that the electrical resistance of this tissue was too low (20–50 $\Omega \cdot cm^2$) to permit quantitation of the resistance-lowering effects of absorption enhancers. Stripped large intestine (ascending, transverse and descending) mucosa TEER, however, was much larger (range, 100–275 $\Omega \cdot cm^2$). All subsequent experiments were performed using stripped mucosa from the large intestine only.

Effects of Acylcarnitines on TEER. Following exposure to effective acylcarnitines (see below), a rapid drop in TEER was usually observed within a few minutes. As shown in Table I, OCC (octanoyl carnitine, C8) did not alter TEER, and DCC (decanoyl carnitine, C10) had only a slight effect on TEER. In contrast, both LCC (lauroyl carnitine, C12) and PCC (palmitoyl carnitine, C16) produced an 80% decrease in the TEER value relative to control. This apparent chain-length dependency was similar to that reported for *in vitro* membrane perturbing effects (8) and *in vivo* drug absorption-enhancing activity (5).

When PCC was removed from the mucosal chamber and replaced with fresh, warm buffer, TEER rapidly recovered to 75% of control levels (Fig. 3). These results indicate that the effects on colonic mucosa TEER are reversible, as has been shown *in vivo* for the absorption-enhancing activity of PCC (5).

Table I. Acylcarnitine Effects on Transepithelial Electrical Resistance (TEER) of Rat Colonic Mucosa Mounted in S-G Cells

Acylcarnitine	Change in TEER[a]
OCC (C8)[b]	0.0 ± 0.0[c]
DCC (C10)	11.4 ± 6.4
LCC (C12)	81.1 ± 1.8
PCC (C16)	80.1 ± 5.2

[a] Calculated as the maximum decrease in TEER relative to normalized initial values.

[b] Refer to octanoyl, decanoyl, lauroyl, and palmitoyl carnitine chloride, with corresponding acyl chain length indicated.

[c] Mean ± SD of three to six preparations.

Simultaneous Measurement of TEER and Permeability Coefficient. The poorly absorbed cephalosporin antibiotic cefoxitin was added to the mucosal side of the S-G cells for the simultaneous measurement of TEER and flux. In controls, where mannitol was added instead of PCC, TEER was maintained between 90 and 126% of the initial value during the 60-min experiment with no change in cefoxitin permeation. Following the addition of PCC, TEER began to decrease almost immediately, and within 20 min, the cefoxitin permeability coefficient (P) increased 20-fold ($P < 0.05$), from 12.9 ± 6.5 × 10^{-6} to 266 ± 79.8 × 10^{-6} cm/min (Fig. 4).

A similar measurement of TEER and lucifer yellow P, in response to PCC, was also determined. The decrease in TEER again immediately preceded increased tissue permeability. Following 5 m*M* PCC, lucifer yellow P increased 22-fold ($P < 0.05$), from 2.58 ± 3.58 × 10^{-5} cm/min to 5.76 ± 1.65 × 10^{-4} cm/min. When PCC was removed from the medium, lucifer yellow P decreased toward preexposure values (data not shown).

These preliminary results suggest that PCC initially decreases the electrical resistance of the intestinal mucosal tissue before increasing the tissue permeability to these compounds. Although these experiments were not designed to determine cause and effect, and these two effects could be unrelated, reversible changes in TEER are believed to involve paracellular effects (3,4). Since the effects were reversible, tissue integrity/viability was probably not compromised by the enhancer, and enhancement of drug permeability may be the result of improved paracellular flux as evidenced by decreased TEER.

In conclusion, the improved side-by-side diffusion cell introduced by Sweetana and Grass has been modified to accept electrodes for electrophysiologic measurements. Through the use of this modification, the simultaneous study of TEER and tissue permeability effects of absorption enhancers is now possible. This represents an advantage over conventional Ussing apparatus and provides a tool for the further study of absorption enhancer mechanism of action.

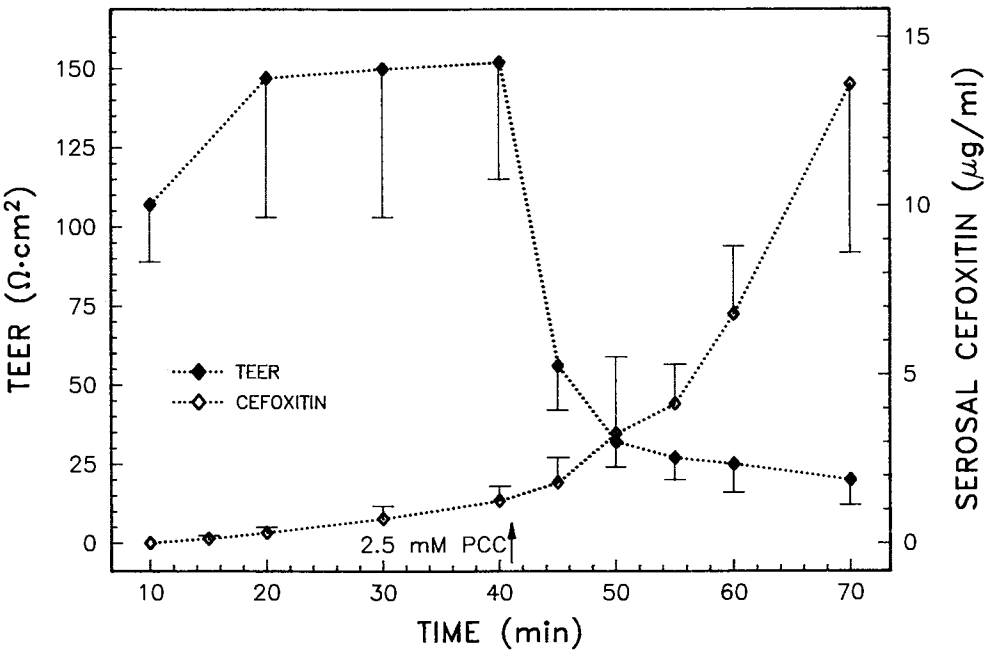

Fig. 4. Simultaneous measurement of TEER and cefoxitin transport following the addition (↑) of 2.5 m*M* PCC.

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