FURTHER INVESTIGATIONS ON THE TRANSPORT MECHANISM OF CEPHALEXIN AND AMPICILLIN ACROSS RAT JEJUNUM

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The transport mechanisms of cephalixin (CEX) and ampicillin (AB-PC) across rat jejunum were examined using the electrophysiological technique in vitro. From the experiments under the short-circuit condition, it became evident that the specific transport system participates in the mucosal-to-serosal transport of CEX, while AB-PC is transported only by the simple diffusion. In addition, the transport routes of these drugs were examined using the voltage-clamp technique. Salicylate, a model drug of the weak electrolyte, was transported mainly via a paracellular route, while CEX, in the absence of Na, penetrated the membrane via two routes, i.e. the transcellular route and the paracellular route. Similar result was obtained as to AB-PC. So it seems to be likely that these two drugs diffuse through the intestinal epithelium via the similar pathways in the Na-free condition.

Keywords—electrophysiological technique; rat jejunum; cephalixin; ampicillin; passive diffusion; specific transport system; transcellular route; paracellular route

INTRODUCTION

The mechanism of drug absorption or transport across the intestinal barrier has been extensively investigated.1,2) Drug transport across the epithelial layer is not only by the transmembrane transcellular route, but also the parallel paracellular shunt pathway may exist. In order to examine the permeation pathways of weak electrolytes in the low resistance tissue, Schultz and Zalusky3) applied the electrophysiological technique, based on the method of Ussing and Zerhan.4) The electrophysiological experiments have indicated the existence of the paracellular pathway for low-molecular-weight compounds, such as salicylate5) and 3-O-methylglucose.6)

In this study, we investigated the transport mechanism of two β-lactam antibiotics, cephalixin (CEX) and ampicillin (AB-PC), across rat jejunum by using the electrophysiological technique in vitro.

MATERIALS AND METHODS

Materials — AB-PC (Takeda Chemical Industries, Osaka, Japan) and CEX (Shionogi Co., Osaka, Japan) were used as supplied. All other reagents used in these experiments were of reagent grade and were used without further purification.

Preparation and Incubation of Rat Jejunum — Male Wistar rats weighing 200–250 g were used. Under sodium pentobarbital anesthesia, lower jejunum, about 10 cm-length, was isolated and immediately mounted between two Lucite half chambers.7) The area of the opening was 1.0 cm². Both sides of the tissue were filled with 11 ml of Ringer's solution, bubbled with 95% O₂-5% CO₂ during the experimental period. The chambers were placed in the temperature-controlled box to maintain the solution temperature at 37°C. Ringer's solution was adjusted to pH 7.4 at 37°C prior to the experiment, and its composition in milimoles per liter
was as follows: NaCl, 125; KCl, 5; CaCl₂, 1.4; NaH₂PO₄, 1.2; NaHCO₃, 10; and 200 mg/dl of D-glucose. In Na-free experiments, NaCl, NaH₂PO₄, and NaHCO₃ were replaced with choline chloride, KH₂PO₄, and KHCO₃, respectively.

**Electrical Measurements** — The transmucosal electrical potential difference (PD) and the short-circuit current (Iₑ) were measured at 10 min intervals. The tissue electrical resistance (Rₑ) was calculated by Ohm's law. Details were described in our previous report. PD and Iₑ reached the steady-state conditions about 20 min after mounting the membrane, and then the experiment was started. At the subsequent experiments, we used the membranes whose PD was above 3mV and Rₑ was in the range of 30 to 60 ohm-cm².

**Measurement of Unidirectional Transmural Fluxes** — The drug containing Ringer's solution was introduced to one side of the tissue at 25 min after the mounting. One ml samples were taken every 10 min from the other side for 1 h. The volume of the bathing solution was kept constant by the addition of fresh Ringer's solution. Except the potential-applying experiments, the preparations were maintained in the short-circuit condition by means of an automatic voltage-clamp apparatus.

**Analytical Methods** — AB-PC and CEX were determined spectrophotometrically according to the method of Barbhaiya et al. Salicylate was determined by the high performance liquid chromatography (HPLC). HPLC (Shimadzu LC-3A, Kyoto, Japan) equipped with a variable wave-length UV detector (Shimadzu SPD-2) was used in a reversed phase mode with Cosmosil 5C₁₈ column (4.6 mm i.d.×150 mm, Nakarai Chemicals Co., Kyoto, Japan). The column temperature was kept at 30°C. A mixture of methanol/0.05% phosphoric acid (1/1 by volume) was used as the mobile phase at a flow rate of 0.8 ml/min. The effluent was monitored at 237 nm.

**RESULTS**

**Unidirectional Fluxes of CEX and AB-PC**

Fig. 1 shows a cumulative plot of the amount of CEX and AB-PC transferred versus time. In these experiments, drug concentration in the last sample could be negligible compared to the concentration of the initially drug added compartment. Therefore, it may be safely to assume that the backward flow of the drug is negligible during the time of experiment. Transmural unidirectional flux rates were calculated from a least-squares fit of the linear portion of the cumulative plots. Jₘs and Jₘₐₙ are used as symbols for mucosal-to-serosal and serosal-to-mucosal flux rate, respectively. Jₘs of CEX (0.319±0.012 nmol/cm²·min) was significantly faster than the Jₘₐₙ (0.181±0.009 nmol/cm²·min), while in the case of AB-PC, Jₘs (0.0827±0.0012 nmol/cm²·min) and Jₘₐₙ (0.0965±0.0047 nmol/cm²·min) were not significantly different and, in addition, Jₘs of CEX was four-times larger than that of AB-PC.

**Effect of Drug Concentration on Jₘs of CEX and AB-PC**

Fig. 2 shows Jₘs of CEX and AB-PC as a function of their mucosal concentrations. Jₘs of AB-PC was linearly increased as the mucosal concentration of AB-PC was increased. This linear relationship was unchanged when AB-PC concentration was increased up to 20 mM. Accordingly, the flux may be described by the first-order equation, i.e. Jₘs = (0.094)·C, where C is the mucosal concentration of AB-PC. On the other hand, Jₘs of CEX was found to be saturable. The flux may be described as the sum of a saturable term displaying Michaelis-Menten kinetics plus a second nonsaturable term, since the flux tended to become proportional to the concentration offered as the latter was increased. Thus the flux can be described by the following equation

\[
Jₘs = \frac{Jₘax·C}{Kₘ+C} + P·C
\]

where Jₘax is the maximum velocity of transepithelial carrier-mediated transport, Kₘ is the mucosal CEX concentration at which the carrier-mediated transport is half-maximal, and P is the permeability coefficient (proportionality constant
FIG. 1. Cumulative Mucosal-to-Serosal (○) or Serosal-to-Mucosal (●) Transfer of CEX (a) and AB-PC (b) after the Addition of Drugs to the Mucosal or Serosal Solution, Respectively. The initial drug concentration was 1 mM and results are expressed as the mean ± S.E.

FIG. 2. The Unidirectional Mucosal-to-Serosal Flux Rate ($J_{ms}$) of CEX (a) and AB-PC (b) as a Function of Mucosal Drug Concentration. Results are expressed as the mean ± S.E. The simulation curves (dotted lines in (a)) represent the saturable and nonsaturable components of $J_{ms}$ of CEX and correspond to the equations:

$$J_{ms} = \frac{(0.42) \cdot C}{(1.39) + C} \quad \text{and} \quad J_{ms} = (0.12) \cdot C.$$
for nonsaturable process). The calculation by approximating the parameters of eq. (1) to the experimental data provided the parameters $J_{\text{max}} = 0.42 \text{ nmol/cm}^2\text{-min}$, $K_m = 1.39 \text{ mM}$, and $P = 0.12 \mu\text{mol/cm}^2\text{-min}$ and the following equation was obtained.

$$J_{\text{ms}} = \frac{(0.42) \cdot C}{(1.39) + C} + (0.12) \cdot C$$  \hspace{1cm} (2)

The satisfactory fit of the calculated curve to the values measured is illustrated in Fig. 2(a). As is evident, from the simulation curve of each component of this equation (dotted lines), at the higher concentration range, contribution of the saturable component to $J_{\text{ms}}$ was small and the linear component became dominant.

**Effects of Sodium Replacement of $J_{\text{ms}}$**

In this series, experiments were carried out at drug concentrations of 2 mM, because the nonsaturable and the saturable components of $J_{\text{ms}}$ of CEX became equal around this concentration (Fig. 2(a)). As shown in Fig. 3, replacement of Na in the bathing solution with choline and K markedly inhibited $J_{\text{ms}}$ of CEX. $J_{\text{ms}}$ of CEX in the absence of sodium do not differ markedly from the nonsaturable component of the $J_{\text{ms}}$ calculated as in Fig. 2(a). On the other hand, Na-free medium was without significant effect on $J_{\text{ms}}$ of AB-PC.

**Flux Dependence on Applied Potential**

In order to investigate some details of the transport mechanism for CEX and AB-PC, the technique of Frizzell and Schultz\(^9\) which studied flux behavior under an externally applied electric field was utilized. From the Usings flux ratio treatment they developed the approximate expression in which the total mucosal-to-serosal flux is:

$$J_{\text{ms}} = J_m + J_d$$  \hspace{1cm} (3)

$$J_d = 0 \cdot J_d \cdot \exp(-zFV_t/2RT)$$  \hspace{1cm} (4)

$$J_{\text{ms}} = J_m + 0 \cdot J_d \cdot \xi$$  \hspace{1cm} (5)

Equation (3) consists of a part that is insensitive to the applied potential difference across the tissue, $J_m$, plus a portion that has an exponential dependence on the potential ($V_t$), $J_d$, which can be expressed as eq. (4). Where the subscript 0 refers to the short-circuit condition (i.e. when $V_t = 0$), and $z$, $F$, $R$, and $T$ have their usual meanings. Further, when exp ($-zFV_t/2RT$) is expressed as $\xi$, eq. (5) is given. In a tissue when the passive conductance of the extracellular route is many times greater than that of the transcellular route, it may not be unreasonable to assume that most if not all of the observed $J_d$ traverses the paracellular shunt pathway. A plot of $J_{\text{ms}}$ versus $\xi$ should yield a straight line whose intercept on the ordinate represents $J_m$ and whose slope represents $0 \cdot J_d$. By measuring $J_{\text{ms}}$ of salicylate (at 1 mM), an anion at pH 7.4 (i.e. $z = -1$), under various externally applied potential differences, we obtained the result presented in Fig. 4. From a least squares fit of the data, $J_{\text{ms}}$ of salicylate can be described as $J_{\text{ms}} = (-0.21) + (1.32) \cdot \xi$. The negative intercept is thought to be the result of experimental limitations and may be essentially zero. Therefore, a most part of the flux is dependent on $V_t$, suggesting the shunt pathway.

![FIG. 3. Effects of Na-Replacement on $J_{\text{ms}}$ of CEX (Open Column) and AB-PC (Dotted Column)](image-url) Mucosal drug concentration was 2 mM. Results are expressed as the mean ± S.E. The hatched column represents the calculated nonsaturable component of $J_{\text{ms}}$ of CEX in the presence of Na as shown in Fig. 2(a).
Similar experiments were carried out for CEX and AB-PC in the condition of Na-free, where only diffusional flux are operative. The two drugs are amphoteric compounds (CEX, pKₐ 2.67, pKₐ 6.96; AB-PC, pKₐ 2.67, pKₐ 6.95) and show complex dissociations. We used the anion ratios at pH 7.4, which were calculated by the method of Purich et al.,¹⁰ multiplied by −1 as the z values. They are −0.734 for CEX and −0.738 for AB-PC. The plots of Jₘs versus ξ are shown in Fig. 5 and their relationship could be described as

\[
J_{ms} = (0.094) + (0.185) \cdot \xi \quad \text{for CEX}
\]
\[
J_{ms} = (0.090) + (0.153) \cdot \xi \quad \text{for AB-PC}
\]

These results suggest that about one-third of Jₘs for both antibiotics is mediated by the transcellular pathway under the short-circuit condition when Na is absent.

**DISCUSSION**

It has been well known that some weak electrolytes such as salicylate are well absorbed from the alimentary tract though these drugs exist as ionized forms at the physiological pH of the small intestine (pH 6.5). As the permeability of the brush border membrane of the intestinal epithelial cell to the ionized species of these drugs is poor, it has been proposed that the transport of weak electrolytes across the intestinal epithelium includes a paracellular route which may be penetrated by small ionized molecules more easily.⁵³

According to Schultz and Zalusky,³ the total transmembrane flux of ionized molecules is represented as described in eqs. (3) − (5), and the usual interpretation of this model is to identify Jₘ, the intercept, with the flux across the transcellular high-resistance route, while Jₜ, the slope, is assumed to describe transport via a paracellular low resistance route. From the voltage-clamp studies based on this theory, it was concluded that the transport of salicylate across the intestinal membrane is exclusively passive diffusion that might occur through the tight-junction or shunt-pathway of the epithelial tissues. This result is compatible with the previous report⁵¹ and if the effective diameter of the paracellular channel is evaluated to be about 10−15 Å,¹¹ the low-molecular weight drug such as salicylate anion would be possible to penetrate through this route.

Among β-lactam antibiotics, amino-β-lactam antibiotics are amphoteric and exist as ionized forms over the whole range of pH, zwitterion being dominant ionic species at the physiological pH of the small intestine. However, these antibiotics can be administered orally and well absorbed from the alimentary tract.¹²−¹⁵ In this study, we examined the transport mechanisms of AB-PC and CEX across the intestinal membrane by electrophysiological technique and obtained following interesting results.

**FIG. 4. Effect of Transmural PD on Jₘs of Salicylate**

The explanation of ξ, the abscissa, is described in detail in the text.
2. The total flux of CEX was expressed as the sum of two different components, one is a saturable and Na-dependent component which followed the Michaelis-Menten equation and the other is a diffusional one. However, the contribution of the saturable component to the whole flux of CEX was very small as compared with amino acids or sugars which had been reported by Munck et al.\textsuperscript{[16–18]}. In some previous reports,\textsuperscript{[13–15]} especially in-situ studies, the absorption of CEX failed to show the saturation phenomena. These discrepancies might be due to such small contribution of the saturable component.

3. When Na was eliminated from the medium, only the carrier-mediated transport of CEX was abolished almost completely while the diffusional flux remained unchanged.

4. From the voltage-clamp experiments carried out in the absence of Na, it was proved that besides the transcellular route, the paracellular route exists to account for the total transport of the two drugs across the intestinal epithelium. In addition, the similar results for CEX and AB-PC indicate the possibility that these two drugs might be transported via the similar route in the Na-free condition.

Though one must be careful in drawing conclusions on the basis of this simple modeling of in vitro experimental systems, such investigations which employ the electrophysiological technique will be able to give new suggestions to clarify the mechanisms of drug absorption.

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