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EFFECTS OF THEOPHYLLINE ON SALICYLATE TRANSPORT IN ISOLATED RAT JEJUNUM

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Summary

Salicyclic acid flux at pH 7.4 across rat jejunum was measured before and after perturbation with theophylline and/or an electric field. After the flux reached a steady state control condition theophylline was added to the system, which resulted in establishment of a new higher steady state flux. Dependence of flux on an externally applied potential difference was observed to have a strong exponential behavior. It appears that salicylate transport is primarily via the shunt pathway and the effect of theophylline is to increase transport by the low resistance pathway.

Introduction

Intestinal transport studies by in vitro methods have elucidated numerous aspects of the role of tissue structure and function in absorption and secretion [1,2]. The technique developed by Ussing and Zerham [3] to study transport of inorganic ions across high resistance tissue was later modified by Schultz and Zalusky [4] to study inorganic ion transport across low resistance tissue and has also been used to study the flux of weak electrolytes across rat jejunum [5]. The accumulated data strongly support the view that the tissue is structured such that multiple transport pathways exist: one a high resistance transcellular route, and another which is a parallel paracellular low-resistance shunt pathway. Barnett and Licko [6] proposed a model which includes the parallel pathways plus a serial compartment corresponding to nonepithelial portions of the tissue. We report a study on the transport of salicylate across rat jejunum using the in vitro technique. The flux of tracer quantities of the weak electrolyte

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is measured before and after theophylline is added to the system in order to observe its effect on the flux. We also measured the flux dependence on an externally applied electric field as we investigate the nature of the transport and the effect of theophylline tissue response on transport across the small intestine.

Methods

Salicylic acid fluxes for mucosal to serosal (M→S) and serosal to mucosal (S→M) transport were measured by the technique of Ussing and Zerham [3] as modified by Schultz and Zalusky [4]. The tissue chamber (E.W. Wright, Inc., New Haven, Conn.) consisted of two half chambers with a conical shape and 1.13 cm^2 cross-sectional area. Each half chamber was connected to a gas lift perfusion apparatus containing a 10-ml volume of buffer solution and was heated to 37°C by water jackets. Composition of the buffer in mM was: 143.5 Na/6 K/1.2 Mg/2.5 Ca/128.3 Cl/25 HCO_3/1.2 H_2PO_4/1.2 SO_4/25 glucose. The pH was adjusted to 7.4 with CO_2. Solution mixing was effected by the gas used, O_2 95%/CO_2 5%. Salt bridges consisting of 4% agar in buffer solution were connected to Ag|AgCl electrodes located approximately 22 mm from the mounted tissue while calomel electrodes were placed via salt bridges at about 2 mm from the tissue. Sprague Dawley male rats, weight range 300—400 g, were killed by decapitation, and four sections of tissue were excised from one animal for mounting on the cells by cutting along the mesenteric line from the segment of intestine 25 to 50 cm distal to the stomach. Radioactive salicylic acid, 2 μCi and 0.1 mM, was introduced to one side of the tissue at 25 ± 5 min after death of the animal, and the increasing concentration as a function of time was measured on the other side. The potential across the tissue was recorded as the spontaneous potential difference across the tissue. Current was applied periodically to determine the current needed to force the potential to zero. The theophylline solution was made with the buffer as solvent and gave a concentration of 2.8 mM for the 10 ml bathing solution otherwise specified. To calculate the time dependence of the tissue resistance, we used the graphical method of Clarkson and Toole [7].

For tissues from which the serosa and muscularis were stripped from the serosal surface of the intestine the flux values were about double the values found for the unstripped tissue [8]. This is the expected behavior for a membrane-limited diffusion process, as opposed to an unstirred-layer controlled process, since the tissue thickness is greater for the unstripped tissue while unstirred layers at the membrane surfaces should be approximately the same for two tissue types.

Flux studies

The results are summarized in Table I. The process M→S, T_S gives the flux for salicylic acid before and after theophylline is added to the serosal solution for 9 different tissues taken from 5 different animals. The average initial flux in units of nmol · cm^{-2} · h^{-1} is $J_{ms} = 5.36$ with a standard error of ± 0.52 After theophylline was added the average flux became $J^T_{ms} = 6.94 \pm 0.44$. The

TABLE I

FLUX OF SALICYCLIC ACID ACROSS RAT JEJUNUM BEFORE AND AFTER ADDITION OF THEOPHYLLINE

Flux values are given in units of nmol · cm^{-2} · h^{-1} ± standard error of the mean. For the process M → S the labeled salicylate was added to the mucosal bathing solution at 10 min intervals from 20 min after label was added until 110 min. For S → M the label was added to the serosal solution and measurements were taken from the mucosal solution. Theophylline was added at 60 min to either the serosal (T_S) or mucosal (T_M) sides of the tissues. Salicylate flux (*) was also studied in an additional number of animals. Average values presented here include the studies above before theophylline was added plus these additional studies.

Process	Salicylate flux		Ratio	Significance	Tissues, animals
	Before	After			
M → S, T_S	5.36 ± 0.52	6.94 ± 0.44	1.29	$P < 0.0005$	9, 5
S → M, T_M	3.35 ± 0.51	4.59 ± 0.61	1.37	$P < 0.0005$	9, 5
M → S *	5.81 ± 0.38				21, 10
S → M	3.09 ± 0.29				17, 9

flux ratio (J^T_{ms}/J_{ms}) = 1.29. For transport in the opposite direction the process S→M,T_M had a flux ratio (J^T_{sm}/J_{sm}) of 1.37 also showing the stimulatory effect of theophylline on salicylate flux. Since each tissue served as its own control, the stimulatory effect of theophylline on salicylic acid flux was evaluated using paired *t*-test statistics and is reported in Table I. The average salicylate flux M→S taken for the total of 21 tissues from 10 animals, before theophylline was added, is 5.81 ± 0.38, while the S→M flux, for 17 tissues from 9 animals, is 3.09 ± 0.29, thus giving a directional flux ratio (J_{ms}/J_{sm}) = 1.9 which compares well with the results of Jackson et al. [5] for a number of weak acids.

The tissue response to theophylline has been observed to vary with time for rabbit ileum intracellular cyclic AMP levels and for tissue conductance [9]. Therefore, we also ran several experiments where the tissue was pretreated with theophylline and found that the salicylate flux was constant within our experimental error for as long as two hours for both M→S,T_S and S→M,T_M processes.

The relation between salicylate flux and average tissue resistance, as reported in Fig. 1, is seen to be approximately linear. The circles refer to values of J_{ms}, related to the average tissue resistance before T_S, and the squares denote values of J^T_{ms}, with the average resistance determined after T_S. Statistical analysis showed no difference in the J_{ms} and J^T_{ms} dependence. While the regression coefficient of 0.77 for the data in Fig. 1 indicates considerable scatter, the results for salicylate anion flux are comparable to those reported for the behavior of Na cation transport S→M as a function of the tissue resistance of rabbit ileum [9].

Flux dependence on applied potential

To investigate some details of the transport mechanism for our system we have applied the technique of Schultz and Zalusky [4] to study flux behavior under an externally applied electric field. From the Ussing flux ratio treatment they developed the approximate expression which for the total M→S flux is:

$$J_{ms} = J_a + J_b \exp(-zFV_t/2RT) \quad (1)$$

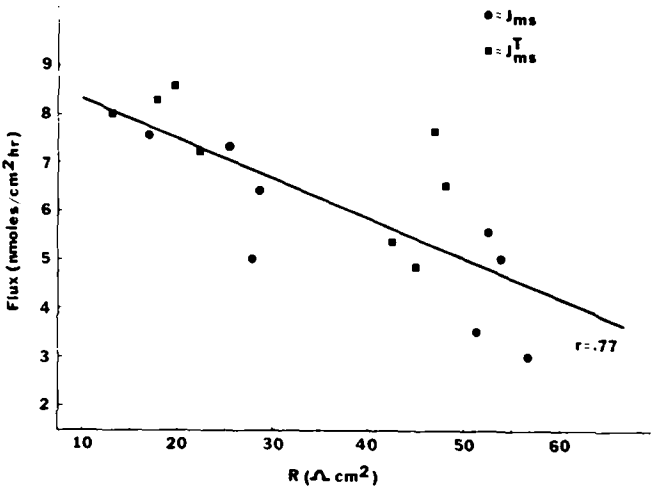

Fig. 1. The relationship between salicylate flux M→S, T_S and the average tissue resistance before (the J_{ms} points) and after (the J^T_{ms} squares) theophylline treatment of rat jejunum. The solid line is a linear least squares fit to all 16 data points.

which consists of a part that is insenstitive to the applied potential difference across the tissue, J_a, plus a portion that has an exponential dependence on the transmural tissue potential V_t. By measuring J_{ms} and J^T_{ms} as a function of $\exp(-zFV_t/2RT) = \xi$, we found the results presented in Fig. 2. First, the value of V_t was manually set, then the radioactive salicylate was added to the mucosal solution and at 50 min theophylline was added to the serosal solution. The circles in Fig. 2 refer to the flux before the biochemical perturbation, J_{ms}, and the squares refer to the flux afterwards. The data consists of 15 sets of paired data points representing 15 tissues taken from 5 animals. In all cases but one (i.e., at $\xi = 0.80$) we found $J^T_{ms} > J_{ms}$. Excluding the 2 sets of points in brackets, a least squares fit of the data for 13 tissues from 4 animals gave:

$$J_{ms} = (-0.29) + (5.86)\xi$$

$$J^T_{ms} = (-0.62) + (7.45)\xi \qquad (2)$$

Statistically, both intercepts are essentially zero, and the slopes are significantly different. A normalized residuals plot of these data shows that the scatter of included data is within 2 standard deviations and that the excluded points for J_{ms}, our control, are definite outliers (more than 5 standard deviations) thus justifying their exclusion from the linear fittings. A comparison with data from Table I shows the average J_{ms} and J^T_{ms} values for the M→S,T_S experiments with a natural tissue potential difference $\xi = 1.07 \pm 0.05$, lie slightly below the fitted lines but are both within the corresponding normal residuals scatter. To demonstrate the linearity of flux over a wide range of potentials for one animal, we have included labels in Fig. 2 for the four tissue samples of animals numbered 1 and 2. The other four pairs of points at $\xi = 1.57$ represent data from one animal with the same potential set for each tissue sample. This set of tissues yielded clustered flux measurements and one smaller value.

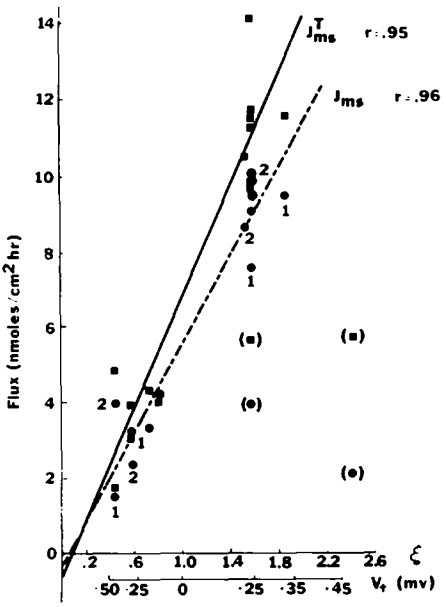

Fig. 2. The dependence of salicylate flux M→S,T_S on an applied potential difference V_t where $\xi = e^{-zFV_t/2RT}$. The 15 sets of data points are circles for flux before theophylline treatment, J_{ms}, and squares after, J^T_{ms}. The two sets of points in brackets were excluded from linear least squares fits of the data for 13 tissues from 4 animals. For J_{ms} the intercept is −0.29 ± 0.64 (the S.D.), and the slope is 5.86 ± 0.51. For J^T_{ms} the intercept is −0.62 ± 0.95, and the slope is 7.45 ± 0.76. The symbol 1 denotes four tissues taken from the same animal that had V_t values of −44 mV, −29 mV, 24 mV and 33 mV. The data points denoted by 2 are four tissues from the same animal with V_t values of −44 mV, −30 mV, 22 mV and 24 mV. The other 3 pairs of points at 24 mV are from the same animal.

Our results are similar to those of Munck and Schulz [10] for the flux of several inorganic ions into rat jejunum and the Na flux across rabbit ileum reported by Nellans, Frizzell and Schultz [9]. The latter workers have pointed out the need for a better method to account for the solvent resistance correction when determining the transmural tissue potential, especially at large values of V_t, as tissue displacement of fluid results in an overestimate of solvent resistance. In their effort to correct for this error they report a tissue thickness of 0.05—0.06 cm for rabbit ileum that has been stripped of its submucosal tissue while Fromm and Field [11] reported a thickness of 0.037 cm for the same type of tissue which has had only the serosa and muscularis removed. This demonstrates the difficulty of defining tissue thickness and thereby making a meaningful correction to the method of Clarkson and Toole [7]. Furthermore, since the in vitro tissue resistance is a decreasing function of time we found that we could only keep the applied potential constant to approximately ±5% of the reported V_t values. The result of these limitations on the data of Fig. 2 is to affect the points with negative V_t values very little, but it allows that the flux values for the positive V_t may have slightly larger values of ξ. While the effect is identical for J_{ms} and J^T_{ms}, it suggests that the slopes in Fig. 2 would be slightly decreased and the negative intercepts, which are the result of experimental limitations, would be increased. Other sources of error may include current leakage through damaged tissue edges, (this is our only sugges-

tion to account for the two sets of divergent data points in Fig. 2) and electro-osmosis which could become a noticeable second-order effect at large applied voltages. Clearly, the results in Fig. 2 demonstrate that the dependence of J_{ms} and J^{T}_{ms} on applied potential difference across the tissue are both reasonably well described by the model of Eqn. 1.

The usual interpretation of this model is to identify J_a, the intercept, with the flux across the cellular membranes while J_b, the slope, is assumed to describe transport via a parallel paracellular low-resistance shunt pathway under short circuit conditions ($V_t = 0$). From Eqn. 2 then, the flux of salicylate anion via the lipoid transcellular route is essentially zero while transport via the aqueous extracellular pathway accounts for the total flux across this low resistance tissue. The effect of theophylline is simply to increase the flux via this pathway. The conclusion drawn from Eqn. 2 is that salicylate transport is exclusively passive diffusion that occurs through the tight junction or shunt pathway of the epithelial tissue.

Flux studies of K, Rb, Na and Cl transport into in vitro rat jejunum by Munck and Schultz [10] concluded that the effective diameter of the solvent pathway is no larger than 8 Å, which easily accomodates the salicylate anion. A model proposed by Jackson [5,12] to describe weak electrolyte transport across rat jejunum requires two membranes barriers in series and includes as a basic assumption that one of the barriers is impermeable to anion transport. The experimental support for this assumption is that he observed no dependence of benzoate flux on applied potential difference across the tissue which is, of course, in direct contraindication to our findings.

Conclusions

Our results correlate well with other findings on rat jejunum. In vivo studies, which show a linear increase of salicylate flux with concentration [13], show the permeabilities of salicylate and benzoate to be approximately equal [14]. Defining the directional permeability as directional flux per unit concentration, we have salicylate permeabilities of $P^{S}_{ms} = 0.0581$ cm · h^{-1} and $P^{S}_{sm} = 0.0309$. For the same type of tissue the data of Jackson et al. [5] give benzoate permeabilities of $P^{B}_{ms} = 0.052$ and $P^{B}_{sm} = 0.021$. These organic anion values are considerably smaller than the inorganic cation permeabilities found for rat jejunum [10]. The latter data suggests the extracellular pathway has a negative electric field of intermediate intensity. Surprisingly, the chloride value, $P^{Cl} = 0.02$, is smaller than the organic anion result. This may be due to the larger charge/volume ratio for Cl ion allowing a greater solvent drag effect within the diffusion pathway. The increased salicylate permeability due to theophylline is an expected result if one assumes that the effect of theophylline on extracellular space for rat jejunum is similar to the observed action of ADH on other epithelia [1,15].

The strong dependence of salicylate flux on applied potential difference across the tissue allows us to suggest that transport is primarily via the low resistance pathway, in agreement with other findings on low resistance epithelia [4,6,9,16]. However, one must be cautious in drawing conclusions on the basis of simple modeling of in vitro biological systems. Further refinement of

the experimental procedure is still needed to include, e.g., time-dependent measurements of cellular and extracellular amounts of labeled solute in the tissue. Clearly, the model for ion transport across intestinal tissue is not an exact one.

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