BBA 75388

DRUG TRANSPORT

II. THE EFFECT OF VARIOUS CATIONS ON THE PASSIVE TRANSFER OF DRUGS ACROSS THE EVERTED RAT INTESTINE*

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(Received August 25th, 1969)

SUMMARY

1. It has been shown in a previous report that replacement of Na^+ by K^+ in a Krebs bicarbonate buffer significantly reduced the passive transfer of several water-soluble drugs across the everted rat intestine. As an extension of that study the influence of other cations (*viz.* NH_4^+, Li^+, $Tris^+$, and $guanidine^+$) and the dependence of the drug-transfer rate on K^+ concentration, have been examined.

2. It is shown that ouabain (1 mM) in the Na^+ control buffer has no effect on the transfer process of riboflavin, salicylate and sulfanilamide. NH_4^+ and $guanidine^+$, as well as K^+ significantly reduced the transfer of riboflavin while $Tris^+$ had no effect; NH_4^+ and K^+ decreased salicylate transfer and Li^+ and K^+ inhibited sulfanilamide transfer. Increasing concentrations of K^+ caused a progressive decrease in riboflavin transfer.

3. The effect of these various cations on the tissue fluid uptake was examined. Good agreement with previously published results has been obtained. The fluid uptake by rat intestinal segments is strongly influenced by the major cation in the buffer solution and decreases in the following order; $K^+ > NH_4^+ > Li^+ > Na^+$ control $> guanidine^+ > Tris^+$.

4. The inhibition of riboflavin transfer by various cations appears to correlate well with the degree of tissue fluid uptake by intestinal tissue.

INTRODUCTION

The active processes responsible for the transport of several classes of nutrients, particularly amino acids and sugars, have been shown to be specifically dependent upon the presence of Na^+ (refs. 1–4). Changes in the ionic environment have a profound effect upon the transfer of these compounds. Replacement of Na^+ by other monovalent cations such as K^+ or Li^+, causes a significant decrease in the membrane uptake of the actively transported compound as well as a decrease in the total membrane transfer[5].

* A preliminary report of this study: see ref. 7.

Interestingly, little research has been conducted to determine what effect various cations may have upon the transfer of compounds that penetrate biological membranes by passive diffusion. This process depends upon the concentration gradient across the membrane for the compound being transferred as well as on several other physical factors embodied in Fick's law.

To the authors' knowledge there has been only one preliminary indication that various cations may effect the diffusion across the intestine of a passively transferred compound. NOGAMI *et al.*[6], have shown that when K^+ replaces Na^+ as the principal cation in the drug solution, there is a decreased transfer rate of several sulfa drugs across the everted rat intestine. The authors did not present an explanation for their findings. More recently, MAYERSOHN AND GIBALDI[7], reported similar findings with a more diverse group of compounds. These investigators found a decrease in the rate of passive transfer of all compounds studied when K^+ replaced Na^+ in a conventional physiologic Krebs bicarbonate buffer. It was proposed that K^+ inhibits the transfer of these compounds rather than there being a specific Na^+ requirement. This suggestion was based upon the observation that ouabain, a glycoside known to specifically inhibit Na^+ transport[8], in concentrations of 1 mM had no effect on the transfer of the compounds studied.

The purpose of the present investigation was to examine further the inhibitory effects of certain cations on the apparently passive transfer of several water-soluble compounds across the everted rat intestine, and to explore a possible mechanism for this phenomenon.

METHODS AND MATERIALS

Preparation of rat intestine

Male, Sprague–Dawley strain rats (Blue Spruce Farms, Altamont, N.Y.) weighing approx. 250 g were fasted 20–24 h prior to the experiment. Water was allowed *ad libitum*. The animals were anesthetized with diethyl ether and a midline abdominal incision made. The intestine was isolated and severed at the ileo-cecal junction. The entire length of the intestine was removed and freed by cutting the intestine at the pyloric juncture. The intestine was placed immediately into normal saline at room temperature, and the lumen was washed with normal saline to remove any solid matter. An initial 15-cm portion of the proximal intestine was discarded to insure use of the jejunal region of the intestine. The intestine was then everted and two 10-cm segments were attached to individual glass cannulas, according to the method of CRANE AND WILSON[9]. The 10-cm segments were measured after stretching the entire intestine with an 11-g weight. The initial, proximal segment was designated Segment 1 and the distal portion as Segment 2. A weight (7 g) was attached to each segment and maintained during the course of the experiment. Both segments were then placed into test tubes containing 100 ml of the mucosal drug solution, previously equilibrated at 37°. Due to the large solution volume the mucosal concentration of drug remained essentially constant throughout the experiment. 2 ml of buffer solution (devoid of drug) were then placed into the serosal compartment. A gas mixture of O_2–CO_2 (95:5, v/v) was constantly bubbled through the mucosal solution.

The serosal compartment is sampled every 10 min during the entire course of the 2-h experiment. The entire serosal volume is removed at the sampling time. 2 ml

of buffer solution are then introduced into the serosal compartment as a rinse, immediately removed, and added to the previous sample. Finally, another 2-ml portion of buffer is placed into the serosal compartment and withdrawn at the next sampling interval.

Although results have been presented in terms of the cumulative amount of drug transferred in 2 h, essentially identical results were obtained when 0.5- or 1-h intervals were chosen for comparison of cation effects.

Buffer solutions

The buffer solution was a modified Krebs bicarbonate buffer (pH 7.4). In all cases the total cation concentration was 154 mM. The pH of the mucosal solution never varied by more than ± 0.2 pH unit before and after each experiment. Table I lists the composition of all buffer solutions used. The anion composition of all the solutions was identical. In addition to the buffers listed in Table I, three other solutions were prepared that were identical in composition to the Na^+ control buffer but contained varying concentrations of Na^+ and K^+. This was accomplished by varying the concentrations of KCl and NaCl, but without changing the individual anion concentrations.

In addition, solutions of 1 mM ouabain (Nutritional Biochemicals Corp., Cleveland, Ohio, lot No. 5481) in the Na^+ control buffer were used with each drug. In these studies the serosal compartment also contained 1 mM ouabain in the control buffer.

Drug solutions and assay procedures

The drugs examined were riboflavin (General Biochemicals, Chagrin Falls, Ohio, lot No. 84134) (20.0 μg/ml), salicylate (sodium salicylate: Fisher Scientific Co., Fair Lawn, N.J., lot No. 762784; salicylic acid: Eastman Organic Chemicals, Rochester, N.Y.) (2.0 mg/ml), and sulfanilamide (Eastman Organic Chemicals, Rochester, N.Y.) (0.1 mg/ml). At pH 7.4, salicylate is completely ionized and sulfanilamide and ribo-

TABLE I

IONIC COMPOSITION OF VARIOUS BUFFER SOLUTIONS

All cation concentrations are given in mM. The final total cation concentration is 154 mM. All solutions were gassed with O_2–CO_2 (95:5, v/v) to give pH 7.4.

Salt	*Buffer solutions*					
	Na^+ (control)	*K^+*	*Tris$^+$*	*Li^+*	*NH_4^+*	*Guanidine$^+$*
KCl	5	127				
KH_2PO_4	1	1	1	1	1	1
NaCl	122					
$NaHCO_3$	26			26	26	26
$KHCO_3$		26				
Tris			26			
Tris·HCl			127			
LiCl				127		
NH_4Cl					127	
Guanidine·HCl						127

flavin essentially nonionized. These compounds were chosen for investigation since they have a wide divergence in structure, physical-chemical properties and presumably permeability characteristics. In addition, these materials are also presumed to be passively transferred across biological membranes. The passive nature of the transfer process for the compounds investigated here has been inferred previously (sulfanilamide[6], riboflavin[10-12], and salicylate[13]). The cumulative amount of drug transferred in 2 h was determined and an analysis of variance in a 2 × 2 factorial layout fashion was used to evaluate the results of the influence of various cations on transfer. This statistical method is useful since it generates F-ratio values for comparison of cations, segments and interaction.

Riboflavin was assayed fluorometrically using the Turner fluorometer, Model 110 (G. K. Turner Associates, Palo Alto, Calif.), based upon the procedure reported by LEVY AND JUSKO[14]. Salicylate was assayed spectrophotometrically with the Hitachi–Perkin–Elmer Model 139 spectrophotometer (Perkin–Elmer Corp., Palo Alto, Calif.), by the method of TRINDER[15]. Sulfanilamide was assayed spectrophotometrically using the method of BRATTON AND MARSHALL[16].

Determination of tissue fluid uptake

The intestine was everted and the first 15 cm discarded. The next 30 cm of intestine was used to prepare six 5-cm segments. Six segments from one rat were used per buffer solution. A ligature was attached to each segment and the segment was briefly and carefully blotted on Whatman No. 40 filter paper to remove any excess adhering fluid and then weighed. The weight of the ligature was subtracted from this initial weight of tissue. Each segment was then placed into 50 ml of buffer solution in individual Erlenmeyer flasks maintained at 37°. A gas mixture of O_2–CO_2 (95:5, v/v) was then constantly bubbled through this solution. At the end of 20 min the segments were removed, drained and placed into weighing cups. It was found that blotting the tissue at this point caused a significant loss of mucosal material. For this reason blotting was considered inappropriate. Once the segment was placed onto the weighing dish there was formation of a small amount of fluid (*i.e.*, fluid adhering to the membrane). This fluid was wiped up, and the final tissue weight determined. Fluid uptake was expressed in mg fluid per g initial wet tissue, and was calculated by subtracting the initial tissue weight from the final tissue weight after 20 min incubation in buffer solution, and dividing by the initial tissue weight. These data were analyzed statistically by Student's t test.

RESULTS

Effect of various cations on drug transfer

As can be seen in Table II, there is a significant decrease ($P<0.01$) in the total amount of each drug transferred when Na^+ is replaced by K^+. It should be noted, that, with the exception of salicylate, there is a tendency for Segment 2 to transfer a smaller amount of drug (and therefore at a lower rate) during the 2-h experimental period. This difference in apparent permeability was found to be significant ($P<0.05$). The F-ratio value for the interaction of the two levels of comparison was not significant in any case.

It has been shown previously[7] that the K^+ inhibition of riboflavin transfer is

TABLE II

CUMULATIVE MUCOSAL-TO-SEROSAL TRANSFER OF VARIOUS DRUGS ACROSS THE EVERTED RAT INTESTINE AT pH 7.4 FROM Na^+ AND K^+ BUFFERS

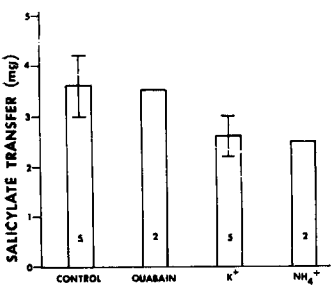
For experimental details see METHODS AND MATERIALS. Number of experiments is given in parentheses. N.S. = not significant.

Drug	*Amount transferred in 2 h ± S.D.*				*Level of significance*	
	Segment 1		*Segment 2*		Na^+ *vs.* K^+	*Segment 1 vs. Segment 2*
	Na^+	K^+	Na^+	K^+		
Riboflavin (μg)	14.2 ± 2.5 (5)	6.3 ± 2.0 (5)	11.8 ± 0.8 (5)	4.6 ± 2.0 (5)	$P<0.01$	$P<0.05$
Salicylate (mg)	3.6 ± 0.7 (5)	2.6 ± 0.4 (5)	3.7 ± 0.5 (5)	1.7 ± 0.3 (5)	$P<0.01$	N.S.
Sulfanilamide (μg)	131.2 ± 6.2 (4)	86.2 ± 23.5 (4)	126.4 ± 23.7 (4)	41.5 ± 2.0 (4)	$P<0.01$	$P<0.05$

readily reversible. In the same report it was noted that 1 mM ouabain, a glycoside known to specifically inhibit Na^+ transport, had no effect on the drug transfer process. This is shown in Figs. 1 and 2 for salicylate and sulfanilamide, respectively and in Table III for riboflavin.

Fig. 3 illustrates what appears to be a linear relationship between riboflavin transfer (expressed as percent of control) and the concentration of K^+ in the buffer solution. The open circles are data from Segment 1 and the closed circles are from Segment 2. The line was fitted by least squares linear regression analysis through all points (*i.e.* using data from both segments). It is appropriate to use all points since there was no significant interaction between the two main variables (*i.e.* cation *vs.* segment). The correlation coefficient of this line is 0.946.

Table III summarizes the results of the effect of various cations on riboflavin transfer. It is apparent that not only K^+, but also NH_4^+ and guanidine$^+$ cause a



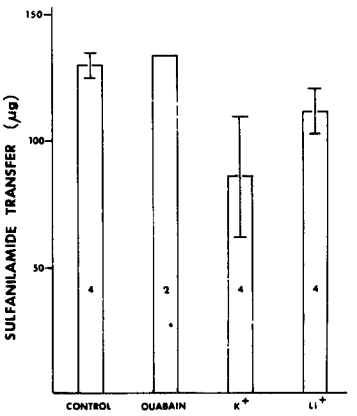


Fig. 1. The cumulative amount of salicylate transferred in 2 h (mg). The vertical bars denote the standard deviation of the mean. The number of experiments is given in each bar. These results represent data from Segment 1. Essentially identical results were obtained with Segment 2.

Fig. 2. The cumulative amount of sulfanilamide transferred in 2 h (μg). The vertical bars denote the standard deviation of the mean. The number of experiments is given in each bar. These results represent data from Segment 1. Essentially identical results were obtained with Segment 2.

significant decrease ($P<0.01$) in the transfer rate of the drug, whereas $Tris^+$ and Li^+ have no effect. The F-ratio value calculated for segmental differences was significant ($P<0.05$) in all cases (*i.e.* when any treatment was compared to the Na^+ control), but values for interaction were not significant. Salicylate transfer in the NH_4^+ buffer

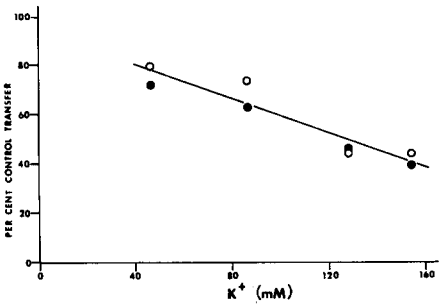


Fig. 3. Relationship between K^+ concentration (mM) in the mucosal drug-buffer solution and the percent of control transfer for riboflavin. ○, Segment 1; ●, Segment 2. Average of five experiments. Correlation coefficient 0.946.

TABLE III

CUMULATIVE MUCOSAL-TO-SEROSAL TRANSFER OF RIBOFLAVIN ACROSS THE EVERTED RAT INTESTINE AT pH 7.4, FROM VARIOUS BUFFER SOLUTIONS

For experimental details see METHODS AND MATERIALS. Numbers of experiments are given in parentheses. N.S. = not significant.

Major cation in buffer	*Amount transferred in 2 h ± S.D.*		*Amount transferred in 2 h ± S.D.*		*Level of significance Na^+ vs. cation*
	Segment 1 (μg)	*Control* (%)	*Segment 2* (μg)	*Control* (%)	
Na^+–control	14.2 ± 2.5 (5)		11.8 ± 0.8 (5)		—
Ouabain (1 mM)	15.0 (2)	106	14.7 (2)	125	—
K^+	6.3 ± 2.0 (5)	44	4.6 ± 2.0 (5)	39	$P<0.01$
$Tris^+$	13.9 ± 1.5 (5)	98	12.7 ± 1.5 (5)	108	N.S.
Li^+	12.7 ± 1.5 (5)	89	11.3 ± 1.3 (5)	94	N.S.
NH_4^+	6.8 ± 1.3 (5)	48	6.0 ± 1.5 (5)	51	$P<0.01$
$Guanidine^+$	11.2 ± 1.7 (5)	79	8.8 ± 1.5 (5)	75	$P<0.01$

was also evaluated and the results support the riboflavin–NH_4^+ data, as shown in Fig. 1. There is a significant decrease in the transfer rate of salicylate in the presence of NH_4^+ as compared to the rate observed in the Na^+ buffer. The effect of Li^+ on the transfer of sulfanilamide is shown in Fig. 2. There is a small but significant decrease ($P<0.01$) in the transfer of sulfanilamide in the presence of Li^+ compared to the Na^+ control.

Determination of tissue fluid uptake

Table IV shows the amount of fluid taken up by the intestinal tissue in the presence of the various cations. It is apparent that both K^+ and NH_4^+ cause a large increase in tissue fluid uptake compared to the control Na^+ buffer. $Tris^+$ seems to decrease tissue fluid uptake relative to the control, as does $guanidine^+$, whereas Li^+

TABLE IV

FLUID UPTAKE BY A 5-cm SEGMENT OF EVERTED RAT INTESTINE IN THE PRESENCE OF VARIOUS CATIONS AT pH 7.4 AND 37°

For experimental details see METHODS AND MATERIALS. Average of six experiments.

Cation	*Fluid uptake (mg/g initial wet tissue ± S.D.)*
Na^+-control	75 ± 34
K^+	176 ± 57
NH_4^+	126 ± 45
Li^+	80 ± 33
Guanidine$^+$	70 ± 34
Tris$^+$	43 ± 34

produces a slight increase above the control value. The fluid uptake in the presence of K^+ was significantly ($P<0.01$) greater than the control. The variability seen in this series of experiments was also observed when a longer (1 h) incubation period was used, but both methods produced the same rank-order with respect to fluid uptake for the various cations.

DISCUSSION

The striking generality of the reduced drug transfer rate in the presence of K^+ is apparent in Table II for a diverse group of compounds. In addition to this K^+-reduced drug transfer, NH_4^+ and guanidine also decrease riboflavin transfer. Salicylate transfer was decreased in the presence of NH_4^+, and Li^+ decreases sulfanilamide transfer. It appears that these effects are due to inhibition by the various cations rather than being due to a specific requirement for Na^+. This is evidenced by the fact that ouabain in the Na^+ control buffer has no effect on the transfer process and by the observation that in the Tris$^+$ buffer, with no Na^+ present, transfer is essentially the same as in the Na^+ control.

In order to understand the nature of this reduced drug transfer rate, consideration must be given to the properties of the various cations investigated. With respect to the effect of K^+ on the permeability of the membrane, there is apparently no irreversible inhibition of drug transfer in the absence of Na^+. This is evidenced by data presented previously[7], where it has been shown that although K^+ has a profound inhibitory effect on drug transfer, the effect is readily and rapidly reversible once the tissue is exposed to the Na^+-containing buffer. A similar observation has been made by NOGAMI *et al.*[6]. In addition, CSAKY AND THALE[17] found that there was a complete reversal of the inhibition of sugar transport after 2 h incubation in a Li^+ buffer. These authors concluded that there was no permanent damage to the transport mechanism in the absence of Na^+.

The cations examined in this study fall into several categories based upon penetrating ability. K^+, Li^+ and NH_4^+ are considered to be penetrating ions, whereas Tris$^+$ is non-penetrating[5]. The exact classification of guanidine$^+$ is not certain, although it most probably resembles urea which is a penetrating compound, based upon similarity in structure and molecular volume. There seems to be a reasonably good

relationship between penetrability and inhibition of drug transfer. Thus, in the case of riboflavin, K^+, NH_4^+, and guanidine$^+$ decrease the transfer rate and these ions are penetrating. Tris$^+$, a non-penetrating ion, has no effect on the transfer of riboflavin. NH_4^+ decreases salicylate transfer rate and Li^+ has a similar effect on sulfanilamide.

Since these ions have different penetrating ability it is expected that replacement of Na^+ by them would lead to redistribution of the fluid within the tissue. This appears to be the case, based upon the results of the tissue fluid uptake study. The results obtained for fluid uptake in the presence of various cations resemble those obtained previously[5], and differences may be due to different species used.

The relationship between gut fluid uptake and extent of inhibition of drug transfer is illustrated in Fig. 4. Apparently, a good linear relationship exists between these two parameters. A least squares regression line has been drawn through all points. The open circles represent data from Segment 1, and the closed circles data from Segment 2. The correlation coefficient of this line is 0.931 and is highly significant

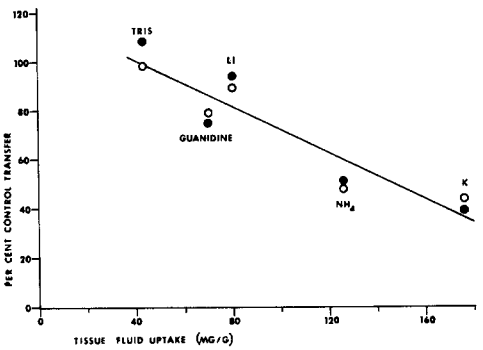


Fig. 4. Relationship between riboflavin transfer rate (expressed as percent of control value) and the fluid uptake by intestinal tissue (mg per g wet tissue). ○, Segment 1; ●, Segment 2. Correlation coefficient 0.931, $P<0.01$.

($P < 0.001$). It would appear from this relationship that the greater the fluid uptake the greater the inhibition of transfer. Since it has been illustrated previously[5] that the greater the K^+ concentration in the buffer solution the greater the tissue fluid uptake, one would expect, that as the K^+ concentration in the drug-buffer solution increases so would the extent of inhibition of drug transfer. In fact, this is exactly what is seen in Fig. 3. Thus, one may conclude that as the K^+ concentration increases, so does the fluid uptake and one observes a resulting decrease in drug transfer.

From recent work[13], it appears that tissue fluid uptake may be accounted for, at least in part, by an increase in the volume of the epithelial cell. Thus an increase in epithelial cellular volume may result in smaller effective drug concentrations in the tissue with a resulting decrease in the transfer rate.

ACKNOWLEDGEMENTS

This investigation was supported in part by Grant AM-11498 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service. Michael Mayersohn is a fellow of the American Foundation for Pharmaceutical Education, 1969–1970.

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