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RUBIDIUM AND CREATININE TRANSPORT ACROSS ISOLATED MESENTERY*

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Abstract—The movement of Rb^{86} cations across isolated rabbit mesentery displays the kinetic pattern associated with passive diffusion. The permeability constant can be increased by the application of several drugs. Histamine markedly stimulates the movement of rubidium ions, while epinephrine and acetylcholine are much less effective. By measuring the contemporaneous transfer of the Rb^{86} cation and of un-ionized creatinine, an attempt was made to expose the nature of the permeability barrier. A 10° rise in temperature produced a relatively greater elevation in creatinine permeability, while 5-HT was relatively more effective in enhancing the permeability to rubidium. The differential effects of these two environmental stimuli on the trans-mesenteric movement of two tracer substances indicate that these two stimuli must affect different parts of the transport process.

IN recent years considerable attention has been focused on the transfer of solutes across natural and artificial membranes composed of living cells. The naturally occurring barriers that have attracted the most attention have been the frog skin,[1, 2] toad bladder,[3] gastrointestinal mucosa,[4, 5] and capillary endothelium.[6] Artificial membranes include tumor cells that have been caught by filtration on Millipore filters.[7] In most of these systems the emphasis has been directed toward solutes that are transported across the membranes against electrochemical gradients, particularly sugars,[8] amino acids,[7] and electrolytes.[1–5] There have been no demonstrations, however, of active transport across the capillary wall or across the mesentery. Passive diffusion and facilitated diffusion,[8, 9] while commonly conceded to represent important modes of transport in biological systems, have received little attention in the recent past.

Isolated mesentery is a convenient example of a cellular barrier through which diverse solutes migrate apparently only in the direction of the chemical gradient. Such migration, however, is not a simple phenomenon, as is demonstrated in this report. The original observations[10] were limited to the passage of Rb^{86} cations across the isolated rabbit mesentery. It was shown that the rubidium flux can be modified by a variety of chemical factors, e.g. pH, calcium ion concentration, oxygenation, 2 : 4-dinitrophenol, and mercuric chloride. In many respects the permeability responses of isolated mesentery proved to be similar to the reactions of intact capillaries.[11]

The present report extends this study to include observations on the influence of

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several drugs on the Rb^{86} flux. To learn more about the nature of the permeability process, the simultaneous movements of Rb^{86} and of creatinine have also been determined.

METHODS

In the passage of a substance through a membrane by passive diffusion, the flux dQ/dt is a function of the membrane thickness d, the area of the membrane A, and the concentration gradient across the membrane $(C_1—C_2)$:

$$\frac{dQ}{dt} = \frac{DA(C_1—C_2)}{d} \tag{1}$$

D in this equation[12] is a diffusion constant (cm^2/min).

For any one membrane and any one substance, D and d can be combined to give a permeability coefficient K, which has the units of cm/min. In a situation in which the sink volume and the source concentration of the diffusing substance are held constant (as in our system), the diffusion process can be defined by a definite integral of equation (1):

$$(C_\infty—C_t) = (C_\infty—C_0)e^{-kt} \tag{2}$$

where C_∞ is equal to the sink concentration at equilibrium or to the constant source concentration; C_t is the sink concentration at any time t (min); C_0 is the sink concentration at zero time; and k is the first-order rate constant in reciprocal minutes. The rate constant k was evaluated by the same methods as described previously.[10] The value of the permeability coefficient K is related to this first-order rate constant as follows:

$$K = \frac{kv}{A} \tag{3}$$

where v is the sink volume in cm^3, and A is the area of the membrane in cm^2. In this paper we have presented all of the flux data in terms of the permeability coefficient K. P-values in the tables represent probability levels at which drug-induced changes in K were judged to be significant according to the Student's t.

The general technique employed in measuring the Rb^{86} flux in these experiments was like that used before.[10] The rabbit mesentery free of macroscopic blood vessels and fat was tied onto the end of a glass tube, which was suspended in such a way that the membrane did not touch the walls or bottom of a 10-ml beaker, the contents of which served as the diffusion sink. The sink fluid was pumped continuously in closed system through a small chamber contained in a scintillation well-counter and back to the sink. The pumping not only served to stir the fluid but also permitted continuous monitoring of the changing radioactivity in the sink. Throughout each experiment the volume of the diffusion sink was maintained constant.

Either Krebs–Ringer bicarbonate or a modified Krebs–Ringer phosphate (with half the usual phosphate concentration) was used as the base solution; both were buffered at a pH of 7·4[13]. The apparatus was immersed in a constant temperature bath. The superfusate (source solution) contained tracer amounts of $Rb^{86}Cl$ and in some experiments creatinine (about 8 m-moles/l). Otherwise, the source and sink fluids were identical and had essentially the same osmotic pressure. The apparatus

was assembled in such manner that no hydrostatic pressure difference existed across the membrane.

When creatinine was measured along with Rb^{86}, the experiment consisted in a series of consecutive periods of about 45 min each. During each period the Rb^{86} was monitored as described above. The creatinine flux was determined by analyzing (alkaline picrate method) the creatinine concentration in the sink fluid at the end of each period. On the assumption that creatinine movement can be described by equation (2) above, K was calculated. The sink fluid was then replaced with an equal volume of base solution containing no creatinine or Rb^{86}, at which time the next experimental period began.

This replacement was accomplished by inserting a large container completely filled with unlabeled base solution into the plastic tube which returned the fluid to the sink. The labeled fluid coming from the well counter was pumped into the bottom of this large reservoir, displacing from the top of it into the sink an equal volume of unlabeled solution. The pumping process was continued for about 5 min until the radioactivity of the sink fell to background. This method of exchanging the sink fluid allowed both the sink volume and the tension on the membrane to remain constant. The next experimental period began at the moment the exchange reservoir was removed from the system.

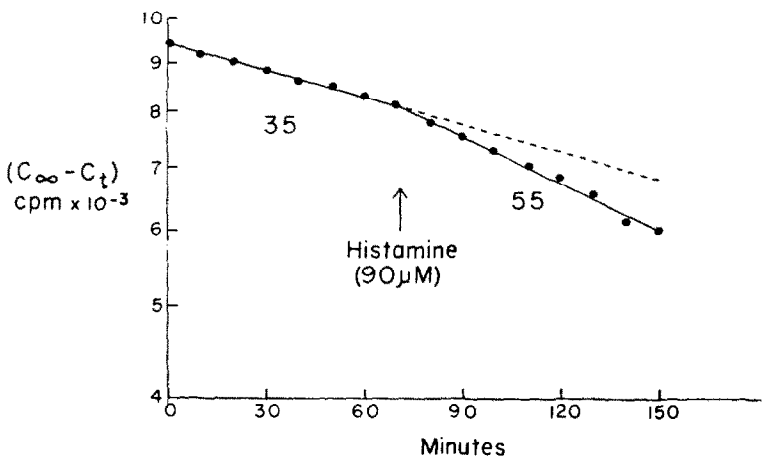

FIG. 1. The effect of histamine on Rb^{86} movement through isolated rabbit mesentery. The difference in radioactivity across the membrane (C_∞—C_t) is depicted on a logarithmic scale. Histamine (90 μmoles/l.) was added at the arrow and the concentration was maintained thereafter. The number under each straight line is the permeability coefficient K calculated for that period.

The drug solutions used in these experiments were always freshly prepared from the dry salts just before use and were introduced into the source reservoir after an appropriate control period or periods. The source solution bathing one surface of the membrane was replaced continuously in order to maintain constant the concentrations of drug and of the penetrating solute or solutes. The drugs used were epinephrine bitartrate, acetylcholine iodide, histamine dihydrochloride, and 5-hydroxytryptamine creatinine sulfate.

RESULTS

Histamine

Because histamine has been shown to be very effective in altering capillary permeability,[11] this drug was tested on the Rb^{86} flux across the isolated rabbit mesentery. As seen in Fig. 1, the result was a prompt and sustained increase in the permeability of the mesentery. The data from this and similar trials (no creatinine present) are summarized in Table 1. A different rabbit was used for each experiment. Although inactive in phosphate-buffered Ringers at 23 °C, histamine at 38 °C appeared to be almost as effective in phosphate as in bicarbonate. In all but one trial (omitted from Table 1), the rise in permeability was significant even at the 0·01 level of significance (Student's *t* test). In general, histamine was as effective in increasing the permeability of mesentery as was 5-hydroxytryptamine (as was reported in an earlier paper).[10]

TABLE 1. DRUG EFFECTS ON THE K FOR Rb^{86} THROUGH ISOLATED RABBIT MESENTERY AT 38 °C

Drug*	Buffer	Control K (cm $min^{-1} \times 10^3$)	Mean %Δ†	Significant at P = 0·05
Histamine	Phosphate	64	+21	2 out of 2 expts.
Histamine	Bicarbonate	41	+41	4 out of 4 expts.
Epinephrine	Phosphate	48	+11	1 out of 1 expts.
Epinephrine	Bicarbonate	45	+21	3 out of 5 expts.
Acetylcholine	Phosphate	63	0	0 out of 2 expts.
Acetylcholine	Bicarbonate	42	+26	2 out of 5 expts.

* The drug concentrations, in μmoles per liter, were as follows: histamine 90, epinephrine 55, and acetylcholine 66.

† In this and other tables, %Δ indicates the percentage change in K that attended exposure to the drug.

Epinephrine

Table 1 also shows that epinephrine increased the permeability of the mesentery in four out of six trials, but the magnitude of the rise was not large. The characteristic red color of adrenochrome (oxidation product of epinephrine) was always seen, sometimes as soon as 30 min after preparing the test solution. There appeared to be an inverse relationship between the magnitude of the permeability response and the formation of the red color as judged visually. Perhaps our difficulty in obtaining reproducible results was due to the destruction of epinephrine, or the ability of the oxidation product to interfere with the action of epinephrine in enhancing the permeability of this tissue, or both.

Acetylcholine

Since acetylcholine is widely believed to promote conduction and transmission in certain specialized tissues by altering their permeability to electrolytes, this drug was tested on the Rb^{86}-mesentery system. There is no evidence in the literature to indicate that acetylcholine alters capillary permeability; the edema formation it produces has

been ascribed to an increased capillary hydrostatic pressure secondary to arteriolar dilatation.[14, 15]

The data in Table 1 indicate that acetylcholine was not nearly as effective as histamine in stimulating Rb^{86} flux across rabbit mesentery. In fact, statistically significant effects were obtained in only two of the seven experiments. At the present time no physiological or pharmacological significance can be ascribed to these observations with acetylcholine.

5-Hydroxytryptamine (5HT)

The influence of 5HT on Rb^{86} movement alone has been reported before.[10] In the bicarbonate buffer at 38 °C, this drug, in a concentration of 55 μmoles/l. produced consistently a marked increase in the Rb^{86} permeability of the isolated mesentery. In order to investigate the mechanism of this action and to uncover more information about the transport process, we have measured the contemporaneous movement of Rb^{86} and creatinine. Appropriate control experiments demonstrated that creatinine did not modify the Rb^{86} flux.

In Fig. 2 are presented the flux data for a control experiment, together with the values of K calculated for each period. The creatinine data are shown as straight

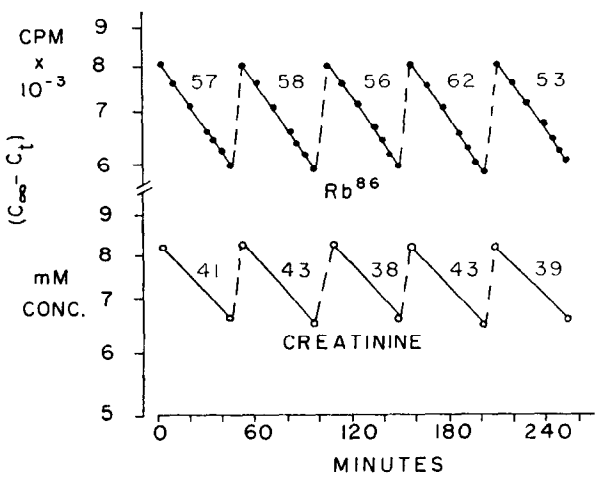

FIG. 2. Control flux data for the simultaneous movement of Rb^{86} and creatinine. See text for a complete explanation.

lines, even though there are only two points from which to construct each line. The assumption underlying this procedure has been discussed above. This analysis makes it possible to calculate values for K, in order that a comparison can be drawn between the permeability to Rb^{86} and to creatinine. In each case, the value for Rb was larger than that for creatinine. In this and other experiments, the mean difference was about 40 per cent of the creatinine value. Fig. 2 demonstrates also the stability of the Rb^{86} flux during each individual period, as well as the reproducibility of both the rubidium and creatinine data collected over several consecutive periods.

When the permeability was altered by the addition of 5HT, the fluxes of Rb^{86} and of creatinine were enhanced, as is seen in Table 2. Of particular interest is the observation

that in each experiment the K for Rb^{86} rose more markedly than the K for creatinine.

Changes in temperature

As was reported earlier,[10] an elevation in temperature from 28° to 38 °C caused an increase in the Rb^{86} flux. When the migrations of Rb^{86} and creatinine were measured simultaneously, the fluxes of both solutes were enhanced by a 10° rise in temperature (Table 3). In contrast to the results obtained with 5HT (Table 2), the temperature elevation led to a greater percentage increase in the permeability constant for creatinine than in the K for Rb^{86}.

TABLE 2. INFLUENCE OF 5-HYDROXYTRYPTAMINE ON THE SIMULTANEOUS MOVEMENTS OF Rb^{86} AND OF CREATININE IN TWO EXPERIMENTS

	K (cm $min^{-1} \times 10^3$)		
	Control*	5HT* (55 μmoles/l.)	%Δ
Rb^{86}	33	49	+48
Creatinine	25	32	+28
Rb^{86}	28	50	+76
Creatinine	23	33	+44

* Each datum is the average value of K obtained from two consecutive periods of observation.

TABLE 3. INFLUENCE OF TEMPERATURE ON THE SIMULTANEOUS MOVEMENTS OF Rb^{86} AND CREATININE IN TWO EXPERIMENTS

	K (cm $min^{-1} \times 10^3$)				
	28 °C		38 °C		
Period no.→	1	2	3	4	%Δ*
Rb^{86}	32	32	45	46	+41
Creatinine	18	17	27	28	+59
Rb^{86}	39	40	50	58	+25
Creatinine	15	16	26	28	+62

* Each percentage change was calculated from the values in periods 2 and 3.

DISCUSSION

Fig. 1 demonstrates that histamine produced a rise in the permeability of the isolated rabbit mesentery and that this rise occurred as soon as the drug was introduced. A new level of permeability was reached in from 5 to 10 min and was sustained thereafter. The other drugs tested in this system also elicited prompt and sustained responses, as did imposed changes in the environmental temperature. Thus equations

(2) and (3) describe the rubidium flux equally well during control and experimental periods, the only difference being in the magnitude of the permeability coefficient K. That the drug responses in terms of creatinine permeability were also prompt and sustained is not so well established, but is implicit in demonstrations that the creatinine permeability coefficient remained constant for at least two consecutive 45-min periods following exposure to 5HT (Table 2) and following a 10°-rise in temperature (Table 3).

Do these data suggest a mechanism or mechanisms for the transport process? Because neither a hydrostatic pressure gradient nor an osmotic pressure difference was permitted across the membrane, no movement of water was expected and none was observed. Whatever bioelectric potential fields exist between the inside and outside of mesothelial cells, there is no evidence for the existence of a net potential difference between the two outside surfaces of the mesentery, and none would be anticipated in view of its complete bilateral symmetry in terms of both structure and embryogenesis. These considerations restrict the number of possible modes of solute transport.

If either rubidium or creatinine, or both, diffuse passively through either the cells or pores of the mesentery, one presumes that the flux data should be consistent with Fick's diffusion equation for a one-dimensional diffusion process. The relationship between Fick's law and the common empirical equation for membrane permeability (equation 1) is not obvious. Of course the latter is readily inferred from the former if the membrane is infinitesimally thin or if the concentrations (activities) of solute are not allowed to change on the two sides of the barrier. Obviously neither of these conditions was realized in the present system. As long as a steady state is not maintained at the two outside surfaces, the activity gradient along which diffusion occurs cannot be strictly linear within even a homogeneous membrane.

However, Northrup and Anson[16] and McBain and Liu[17] found equation (1) to be satisfactory for describing diffusional fluxes through non-living membranes much thicker than mesentery (sintered glass and alundum), even when the source concentration was allowed to fall and the sink concentration to rise. On theoretical grounds, Barnes[18] has validated these approximations. Thus, if the membrane is thin enough, and the change in sink (or source) concentration is slow enough, equation (1) provides an adequate description of the changing flux. This is equivalent to saying that, as the sink concentration rises, the system promptly approaches a new steady state so that the spacial concentration gradient within the membrane remains sensibly linear, even while its slope gradually falls toward zero (quasi-stationary state). In a multiphase membrane consisting of several layers through which solutes travel in series, the gradient within each phase behaves as though it remains linear.In a membrane such as mesentery, measured values of the permeability coefficient K represent a compromise among the true values for K of the various phases. The form of the compromise for a two-phase system has been derived by Jacobs.[19] The linearity of data like those in Fig. 1 is evidence that the necessary conditions for these approximations of Fick's law have been realized in the present system. Thus, these flux data are consistent, at least qualitatively, with simple passive diffusion as the sole mode of transport.

The present results do not prove that penetration of mesentery by these solutes was due to passive diffusion, and they do not indicate the mechanism or mechanisms by

which drugs modified the permeability constants. As demonstrated here, the permeability to Rb^{86} cations was enhanced by a rise in temperature and by the addition of histamine or of 5-hydroxytryptamine (5HT). Sometimes acetylcholine produced a similar effect, but a response to this substance was not seen consistently. A rise in permeability was also noted in the presence of epinephrine, at least in those instances in which auto-oxidation of the drug did not occur rapidly.

Even more puzzling is the demonstration that environmental factors were able to influence differentially the membrane permeability to two different solutes. Like rubidium, creatinine moved faster under the influence of a 10°-rise in temperature and after the introduction of 5HT. The drug, however, was relatively more effective in enhancing the permeability to rubidium, while a temperature rise produced a relatively greater elevation in the permeability to creatinine. Consequently, one must conclude that 5HT and a rise in temperature operated by different mechanisms to enhance the permeability of mesentery. In other words, if one of these stimuli was active by altering the number, diameter, or length of pores through the mesentery, an entirely different mechanism must be postulated for the other.

An analysis of the environmental factors which influence differentially the permeability to two unlike solutes is expected to clarify the mechanism or mechanisms of membrane transport. At the present time it is premature to speculate about the role of the mesothelial cell in this process, except to note that these cells and perhaps the connective tissue cells must play a vital role, since none of these drug effects can be duplicated with non-living cellulose membranes. Although the specific procedures described in this report are somewhat awkward, these results serve at least to demonstrate the feasibility and potential usefulness of studying the simultaneous penetration of two solutes. With a more convenient double-isotope technique that has now been developed in this laboratory, more extensive studies of this nature will be reported later.

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