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UNSTIRRED LAYER, SOURCE OF BIASED MICHAELIS CONSTANT IN MEMBRANE TRANSPORT

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SUMMARY

The apparent Michaelis constant K_m of a carrier-mediated transport system in a membrane is determined too high when an unstirred layer is present. The bias, for which an equation is derived, depends on the thickness of the unstirred layer, the maximal transport rate, and the diffusion constant. An additional error is introduced by the graphical determination of K_m using the Lineweaver–Burk plot, since after transformation the relation is not linear if an unstirred layer is present. A numerical example shows that differences between Michaelis constants obtained from *in vitro* and *in vivo* absorption experiments can be explained partly by a greater bias of the K_m determination caused by a larger unstirred layer *in vivo*.

INTRODUCTION

Recently Dietschy *et al.*[1] pointed out that the presence of an unstirred layer may distort the apparent kinetics of a carrier-mediated transport system. The apparent Michaelis constant will be determined too high. In the following the quantitative relation between the thickness of an unstirred layer and the bias introduced into the Michaelis constant is derived.

THEORY

The transport rate of a substance through an unstirred layer neighbouring a membrane can be described by the following equation:

$$\phi = \frac{D}{\delta}(C - C_s) \tag{1}$$

ϕ, transport rate per unit area (mole·min^{-1}·cm^{-2}); D, diffusion constant of the substance in the unstirred layer (cm^2·s^{-1}); δ, thickness of unstirred layer (cm), C, concentration of substance in well-mixed bulk phase (M), C_s, concentration at the surface of the membrane (M). There is no sharp boundary between the bulk phase and the unstirred layer. Therefore δ denotes an "equivalent" thickness. Water net flux is assumed to be zero so that solvent drag phenomena are excluded. The next equation represents in a simple form the carrier-mediated transport in the membrane:

$$\phi = \frac{VC_s}{K_m + C_s} \tag{2}$$

V=apparent maximal transport rate per unit area (mole·min^{-1}·cm^{-2}), K_m=apparent Michaelis constant (M). The concentration of the substance on the other side of the membrane is assumed to be low, so that the flux in the reversed direction can be neglected. The substance can pass the membrane only by the mentioned carrier-mediated transport system.

In the steady state the transport rate through the unstirred layer and the membrane is equal. The concentration of the substance at the membrane surface C_s is unknown. Only the concentration in the bulk phase can be measured or is given by the experimental conditions. After introducing Eqn 2 into Eqn 1 we get for C_s the following equation of the second order:

$$C_s^2 + C_s\left(K_m - C + \frac{V\delta}{D}\right) - CK_m = 0 \tag{3}$$

Introducing the solution into Eqn 1 a rather complicated expression for the transport rate is obtained:

$$\phi = \frac{D}{\delta}\left[0.5\left(K_m + C + \frac{V\delta}{D}\right) \pm \sqrt{0.25\left(K_m - C + \frac{V\delta}{D}\right)^2 + CK_m}\right] \tag{4}$$

For two special cases simplier equations can be derived:

First case: $C_s \ll K_m$, that is the region where ϕ is related in a nearly linear fashion to the concentration at the membrane surface. With $K_m + C_s \sim K_m$ we get from Eqns 1 and 2:

$$C_s = \frac{C}{1 + \frac{V\delta}{K_m D}} \tag{5}$$

and

$$\phi = \frac{C}{\frac{\delta}{D} + \frac{K_m}{V}} \tag{6}$$

The apparent transport rate is proportional to the bulk phase concentration, but the rate is less than if the unstirred layer were not present because of the additional term δ/D. The denominator of Eqn 6 can be interpreted as transport resistance. The first term of the denominator is the resistance of the unstirred layer and the second one the "resistance" of the carrier-mediated transport system. The whole resistance is the sum of both partial resistances.

Second case: $C_s \gg K_m$, that is the region of saturation for the carrier-mediated transport system. With $K_m + C_s \sim C_s$ we obtain from Eqns 1 and 2:

$$C_s = C - \frac{V\delta}{D} \tag{7}$$

and

$$\phi = V \tag{8}$$

The apparent transport rate coincides with the maximal transport rate of the carrier-mediated transport.

The apparent Michaelis constant of the carrier-mediated transport system is equivalent to the substance concentration at half the maximal transport rate. Setting $\phi = 0.5\ V$ in Eqn. 4 we get:

$$C(\text{for } \phi = 0.5V) = K_m + \frac{0.5V\delta}{D} \tag{9}$$

Using the bulk phase concentration instead of the concentration at the membrane surface a too high value is obtained for K_m as indicated by the "bias term" in Eqn 9. The error increases with the thickness of the unstirred layer δ and the maximal transport rate V.

Fig. 1 demonstrates graphically the influence of unstirred layer on the apparent kinetics of a carrier-mediated transport system. From Curve 1 (unstirred layer absent) K_m is determined correctly. From Curves 2 and 3 (unstirred layer present) a double and 6-fold higher K_m is obtained. Assuming $V = 1$ nmole$\cdot$s$^{-1}\cdot$cm^{-2}, $K_m = 1$ mM, and $D = 0.5\cdot 10^{-5}$ cm$^2\cdot$s^{-1}, Curve 2 corresponds to an unstirred layer of thickness $\delta =$ 0.1 mm and Curve 3 to $\delta = 0.5$ mm. The curves demonstrate that an unstirred layer reduces the transport rate at low concentrations. Approaching the region of saturation the reduction of transport rate decreases.

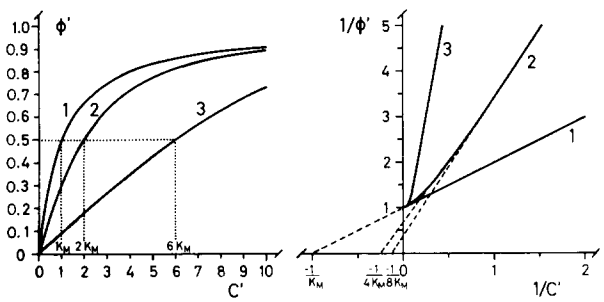

Fig. 1. Influence of unstirred layer on the apparent kinetics of a carrier-mediated transport system. Abscissa: concentration of bulk phase relative to Michaelis constant ($C' = C/K_m$). Ordinate: transport rate relative to maximal transport rate ($\phi' = \phi/V$). Curve 1, no unstirred layer ($\delta = 0$); Curve 2, 0.5 $V\delta/D = K_m$; Curve 3, 0.5 $V\delta/D = 5\ K_m$. Assuming $V = 1$ nmole$\cdot$s$^{-1}\cdot$cm^{-2}, $K_m = 1$ mM, and $D = 0.5\cdot 10^{-5}$ cm$^2\cdot$s^{-1}, Curve 2 corresponds to an unstirred layer of thickness $\delta = 0.1$ mm and Curve 3, $\delta = 0.5$ mm.

Fig. 2. Influence of unstirred layer on the Lineweaver–Burk plot of a carrier-mediated transport system. Abscissa: reciprocal of relative concentration, ordinate: reciprocal of relative transport rate. Further details, see Fig. 1.

Fig. 2 shows that an additional error is introduced if the apparent Michaelis constant is determined graphically by means of the Lineweaver–Burk plot. The double reciprocal plot does not yield a linear relation if an unstirred layer is present (Curves 2 and 3). Using data around half the maximal transport rate the graphical determination of K_m from Curve 2 results in a 4-fold too high value. Data from below half the maximal transport rate lead to a K_m 8-fold too high.

EXAMPLE

A numerical example shall demonstrate that, depending on the experimental conditions, the bias term of Eqn 9 cannot be neglected, especially in intestinal absorption experiments. Csáky and Ho[2] measured a maximal transport rate for glucose in the rat small intestine of 2.55 mmole·g^{-1}·h^{-1}. From the graph of the data an apparent Michaelis constant of about 30 mM can be obtained. By means of the specific mucosal surface area of 100 $cm^2 \cdot g^{-1}$ and the specific serosal surface area of 30 $cm^2 \cdot g^{-1}$ for a rat jejunum[3,4] the maximal transport rate can be related to the area of the villi (0.0255 mmole·cm^{-2}·h^{-1}) or the area of the unstirred layer (0.085 mmole·cm^{-2}·h^{-1}) assuming that the latter is approximated by the serosal surface area. The "effective absorption area" lies between these limits. With a diffusion constant of $0.68 \cdot 10^{-5} \cdot cm^2 \cdot s^{-1}$ for glucose[5] and assuming an unstirred layer of thickness 0.1 mm we get for the bias term 5 and 17 mM, respectively. An unstirred layer of 0.1 mm has been measured in a well-stirred *in vitro* preparation of a rabbit gall bladder[6]. Therefore, this value represents the lower limit for the conditions in the gut. The height of the villi[7] in the rat jejunum amounts to 0.4 mm. Moreover, a layer of mucus has to be considered, though its influence on the unstirred layer is not known. Therefore, an average thickness of the unstirred layer in the rat intestine of about 0.5 mm is not abnormal and a thickness of 1 mm in larger animals and in humans cannot be excluded[1]. The bias term increases to 25 and 50 mM (85 and 170 mM). It is possible that the apparent Michaelis constant obtained from the data of Csáky and Ho[2] at least partly consists of the bias term.

DISCUSSION

The influence of an unstirred layer on permeability parameters is well known. The apparent permeability coefficient is reduced[8–10], especially for easily penetrating substances. When the permeability coefficient exceeds the value $5 \cdot 10^{-4}$ $cm \cdot s^{-1}$ the unstirred layer (0.1 mm) becomes the rate limiting step[11]. In the presence of an unstirred layer the reflection coefficient is measured too low[10,12]. The influence on the hydraulic permeability coefficient is small[8].

In vigorously stirred cell suspensions the unstirred layer is very small: 5–10 μm (refs 13 and 14). Larger values of 20–170 μm were measured in well-stirred *in vitro* preparations: millipore filter[15], lipid bilayer[16–18], frog skin[19], gall bladder[6,11] and plant cells[20,21]. Because of the subepithelial tissue the unstirred layer on the serosal side of the gall bladder reaches the value 800 μm depending on the functional state[11,22]. Hitherto, in the intestine the unstirred layer has not been measured. An exact estimation is not possible, since the influence of the villus height and eventually the mucus is unknown. As outlined in the example a value of 100 μm represents the lower limit. Higher values up to 1000 μm may be expected[1], especially under certain experimental conditions, *e.g.* single administration of a solution into an isolated intestinal loop *in vivo*.

In cell suspensions the unstirred layer is so small that Miller[13] could rule out its influence on the K_m determination for the glucose transport in red cells. Under other experimental conditions agitation reduces the K_m for the acceleration of labelled sugar efflux from erythrocytes. This observation was interpreted as an unstirred layer

effect[23]. The example calculated above shows that in intestinal absorption experiments the bias term may reach or exceed the K_m of the carrier system. Experimental evidence is lacking. But differences between the Michaelis constants obtained from *in vivo* and *in vitro* absorption experiments may be explained at least partly by a larger unstirred layer *in vivo* which causes a greater bias in the determination of the apparent Michaelis constant. For instance, a K_m of 1.4 mM for phenylalanine was calculated from everted sacs experiments[24]. *In vivo* no saturation for the phenylalanine disappearance rate was observed in the range 0.05 to 100 mM (ref. 25). There are many factors which cause the different result *in vivo* and *in vitro*. The unstirred layer may be one of them.

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The same problem was recently discussed from a different standpoint by Lieb, W. R. and Stein, W. D. (1972) *J. Theoret. Biol.* 36, 641–645

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