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Transport of Cortisol, Progesterone and Cholesterol Across Isolated Mesentery

Effect of Metyrapone

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Summary. Isolated rat mesentery, mounted in a diffusion cell, is used as a model for the study of vascular endothelium permeability characteristics. The passage of tracer molecules is measured in the absence of osmotic or hydrostatic pressure gradients across the mesentery. The permeability coefficient of the membrane for cortisol and progesterone is similar. When bound to transcortin, cortisol crosses mesentery at a significantly slower rate. Metyrapone ditartrate increases by 30% the passage of free and of transcortin-bound cortisol, but is without effect on the passage of progesterone or glucose in the same conditions. When the transfer of cholesterol across mesentery is studied, a high percentage of the tracer is trapped by the membrane.

Increasing attention has been paid for several years to the morphology, metabolism and permeability of endothelial cells. Since a direct approach to the functional properties and transport characteristics of vascular endothelium offers many methodological difficulties, a closely related structure like peritoneum is in several instances a more accessible and therefore more suitable material for this study. Several permeability characteristics of isolated mesentery *in vitro* have already been studied (Starling, 1896; Berndt & Gosselin, 1961; Brachet & Rasio, 1968; Brachet, Couturier and Rasio, 1969; Couturier & Metzger, 1971).

The recent finding of Bruno, Leclercq, Virasoro and Copinschi (1971) that metyrapone in man accelerates the disappearance of cortisol from plasma led us to investigate the effect of this drug on the transport of

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cortisol, either free or bound to transcortin, across isolated rat mesentery mounted in a diffusion cell. Control studies were performed with tritiated progesterone and glucose-^{14}C.

Materials and Methods

Method

The technique used in this laboratory for studying the passage of tracers across isolated rat mesentery has been previously published (Brachet & Rasio, 1969; Couturier & Metzger, 1971). A translucid part of rat mesentery is excised immediately after killing the animal (male albino rats fed ad libitum), rinsed in saline and inserted between the two chambers of a diffusion cell. The surface between the two chambers covered by mesentery measures 0.79 cm^2. Each chamber is then filled with 2 ml of Krebs-Ringer's bicarbonate buffer (pH 7.4) containing 300 mg/100 ml of glucose and the diffusion cell is placed in a water bath at 37 °C. Two channels allow the gasification of the buffer by a mixture of O_2 (95%) and CO_2 (5%) and a gentle stirring of the medium on both sides of the membrane. Preliminary studies (Couturier, *unpublished observations*) have shown that in our device, this stirring of the medium does not alter the passage of small molecules such as glucose.

At time 0, 0.2 μC of the tracer are added to one compartment (compartment IN). Diffusion of the tracer across the mesentery is then followed by sampling 0.1 ml of the medium simultaneously from both compartments, at regular time intervals, for 35 or 45 min. The samples are assayed for radioactivity in a liquid scintillation counter (Nuclear, Chicago), using Bray's solution as counting medium (Bray, 1960). The concentration gradient of the tracer, plotted on a semi-logarithmic scale *vs.* time, allows to estimate the t 1/2 value for the diffusion of the tracer, provided the phenomenon be linear and the recovery (sum of cpm in each compartment) constant during the whole experiment. The permeability coefficient P is then calculated according to the formula

$$P = \frac{1450 \cdot 10^{-5}}{t\,1/2} \text{ (}\textit{see}\text{ Appendix).}$$

Materials

Cortisol 1-2-^{3}H (specific activity [S. A.]: 40 C/mmole, CEN, Mol, Belgium), progesterone 1-2-^{3}H (S.A.: 41 C/mmole, CEN, Mol, Belgium) and cholesterol 1-2-^{3}H (S.A.: 30 C/mmole, Radiochemical Center, Amersham, England), dissolved in ethanol, were diluted in normal saline (2 μC/ml); the final concentration of ethanol in buffer was thus less than 3%. This concentration of ethanol does not alter the permeability of mesentery to radioglucose (Couturier, *unpublished observations*).

Transcortin-bound tritiated cortisol was prepared by mixing 3 ml of steroids-free plasma (obtained by treating a normal plasma pool with dextran-coated charcoal according to Heyns, Van Baelen and De Moor (1967) and 0.05 ml of an ethanolic solution of cortisol 1-2-^{3}H (S.A.: 40 C/mmole, 50 μC/ml). This preparation was introduced into compartment IN; the same preparation but without tritiated cortisol was introduced into compartment OUT.

The radiochemical purity of the labeled compounds was better than 98%.

Protocols

In a first series of experiments (protocol I), the permeability coefficient was measured for cortisol, transcortin-bound cortisol, progesterone and cholesterol by kinetic studies during 45 min. In a second series of experiments (protocol II), the effect of metyrapone on the permeability coefficient for cortisol, transcortin-bound cortisol, progesterone and glucose was tested in the following manner: after two samplings at a 10-min interval, allowing to estimate the permeability coefficient of each membrane in the absence of the drug, metyrapone ditartrate (Metopyrone® Ciba) was added to both compartments of the cell at a final concentration of 0.5 mg/ml and the diffusion was further measured during 20 min. Control studies using tartaric acid (final concentration 0.28 mg/ml, i.e. the same concentration as in the metyrapone experiments) were performed in similar conditions with tritiated cortisol.

Results

Protocol I

The recovery was fairly constant for cortisol, transcortin-bound cortisol, progesterone and glucose. For cholesterol, 27% was lost after 45 min of incubation (Table 1), probably because it was trapped in the mesentery. Therefore, the exchange of cholesterol across mesothelium could not be compared to the other tracers whose recovery was fairly constant over 45 min. For cortisol, transcortin-bound cortisol, progesterone and glucose, the concentration gradient of the tracer, plotted on a semi-logarithmic scale, was linear over the duration (45 min) of the experiment. This demonstrated that the permeability coefficient could be accurately estimated over a short (10 min) period of time.

Table 1. Recovery of cortisol-^{3}H, transcortin-bound cortisol-^{3}H, progesterone-^{3}H, cholesterol-^{3}H and glucose-^{14}C during incubations of 25 or 45 min in a diffusion cell[a]

Tracer	n	Recovery of radioactivity (cpm)			
		Time after addition of the tracer (min)			
		5	15	25	45
Cortisol-^{3}H	9	13,892 ± 2927	13,864 ± 3228	13,881 ± 3202	14,574 ± 3589
Transcortin-cortisol-^{3}H	12	5379 ± 671	5500 ± 700	5824 ± 756	5570 ± 739
Progesterone-^{3}H	14	5057 ± 851	5060 ± 878	4912 ± 822	4780 ± 838
Cholesterol-^{3}H	12	4490 ± 54	4219 ± 103	3743 ± 111	3278 ± 149
Glucose-^{14}C	14	9449 ± 1191	9150 ± 1012	9160 ± 1009	–

[a] Values given are mean ± SEM; n = number of experiments.

Table 2. Passage of tracer amounts of steroids and glucose across isolated rat mesentery [a]

Tracer	P (cm $sec^{-1} \times 10^{-5}$)	n
Cortisol-^{3}H	45.8 ± 6.7	24
Transcortin-cortisol-^{3}H	25.9 ± 2.4	30
Transcortin	7.3	
Progesterone-^{3}H	48.3 ± 4.5	28
Glucose-^{14}C	59.6 ± 3.4	34

[a] Permeability coefficient P is expressed in cm $sec^{-1} \times 10^{-5}$; values given are mean $\pm$ SEM; n = number of experiments.
Value for transcortin is extrapolated (Brachet & Rasio, 1968).

P values in the absence of drug were calculated by pooling all results obtained either over 10 or 45 min (Table 2). They were similar for cortisol and progesterone, but significantly lower for transcortin-bound cortisol ($p < 0.01$). On the contrary, P for glucose was significantly higher than P for cortisol ($p < 0.005$).

Protocol II

Results are shown in Table 3. The addition of metyrapone ditartrate to the incubation medium was followed by a 30% increase of the passage of free and of transcortin-bound cortisol; these effects were statistically significant (respectively, $p < 0.01$ and $p < 0.02$, paired t test). In contrast, metyrapone had no effect on progesterone nor on glucose. Control studies showed that tartaric acid was ineffective on the passage of cortisol (P before addition of tartaric acid: 39.1 ± 4.0 cm $sec^{-1} \times 10^{-5}$; after addition of tartaric acid: 40.7 ± 3.5 cm $sec^{-1} \times 10^{-5}$).

Table 3. Effect of metyrapone (0.50 mg/ml) on the permeability coefficient P of isolated rat mesentery to various tracers [a]

Tracer	P (cm $sec^{-1} \times 10^{-5}$)		n	Effect (%)	p
	Before addition of metyrapone	After addition of metyrapone			
Cortisol-^{3}H	41.4 ± 3.6	53.7 ± 3.7	17	30	<0.01
Transcortin-cortisol-^{3}H	24.1 ± 3.2	31.3 ± 3.7	18	30	<0.02
Progesterone-^{3}H	51.8 ± 4.9	52.1 ± 4.2	13	—	NS
Glucose-1-^{14}C	58.2 ± 2.5	56.8 ± 3.1	17	—	NS

[a] P is expressed in cm $sec^{-1} \times 10^{-5}$; values given are mean $\pm$ SEM; n = number of experiments.

Discussion

Very little is known about the mechanisms of transfer of solutes from plasma to the interstitial fluid. Even less is known about the factors which are able to modify the permeability of capillaries. This lack of knowledge is mainly due to experimental difficulties. Although several techniques have been developed *in vivo* for the study of capillary permeability (for review, *see* Crone, 1969), an *in vitro* approach of the phenomenon is obviously useful.

It has been recognized from electron-microscopic studies that mesentery cells have in common some morphological, although nonspecific, similarities with endothelial cells. The presence of many pinocytic vacuoles in the cytoplasm and the similarities of the intercellular junctions of both types of cells have been pointed out (Majno, 1965).

Fragments of mesentery from rats placed in different metabolic conditions can be isolated and inserted in a diffusion cell. The passage of tracer molecules across the membrane and the possible effect of several drugs and hormones on this passage is easily investigated in such a device and gives reproducible results. Although no evidence exists that an effect displayed on mesentery also occurs in capillaries, it is reasonable to assume that several functional properties are common to both structures. Therefore, a careful study of the permeability of mesentery to different molecules may throw some light on the process of diffusion across vascular endothelium.

It is believed that the transport of small hydrophilic molecules across capillaries takes place between the cells (Chambers & Zweifach, 1947; Pappenheimer, 1953; Karnovsky, 1967). On the contrary, because of the high content of lipids in the cell membrane, liposoluble molecules are supposed to pass through the endothelial cells (Pappenheimer, 1953). As far as mesothelium is concerned, the t 1/2 value of the concentration gradient of hydrosoluble molecules is proportional to their molecular weight (Brachet & Rasio, 1968) and the passage of one large molecule (horseradish peroxidase; mol wt 40,000) has been shown to occur between the cells (Cotran & Karnovsky, 1968). The present results show that cortisol (mol wt 362) and progesterone (mol wt 314) cross the mesentery significantly slower than glucose (mol wt 180). This indicates that the mesentery discriminates between small molecules of different chemical families. On the other hand, the coefficient P is similar for cortisol and progesterone, whereas it is significantly lower for transcortin-bound cortisol ($P = 25.9$ cm $sec^{-1} \times 10^{-5}$); this

last value is intermediate between the experimental figure found for cortisol ($P = 45.8$ cm sec$^{-1} \times 10^{-5}$) and the value estimated for transcortin (mol wt $= 53{,}000$; $P = 7.3$ cm sec$^{-1} \times 10^{-5}$) by extrapolating the data obtained with other macromolecules (Brachet & Rasio, 1968). This indicates that the binding of cortisol to transcortin slows down its passage across mesothelium.

Our experimental design was used to investigate the possible effects of metyrapone upon the transport of cortisol across isolated mesentery. Indeed, previous studies had shown that besides blocking the 11β-hydroxylation in adrenals, metyrapone also stimulates the release of immunoreactive growth hormone, causes hyperglycemia and accelerates the disappearance of cortisol from plasma (Bruno *et al.*, 1971). This last effect could be due to an increased vascular permeability to the hormone. Therefore, the transport of cortisol across isolated mesentery was tested in the presence and in the absence of metyrapone. The present results clearly show that metyrapone accelerates the transport across mesentery of tritiated cortisol, either free or bound to transcortin. This effect of metyrapone cannot be displayed either with a very closely related molecule like progesterone or with glucose, which suggests that this action is fairly specific for cortisol: metyrapone probably acts on some mechanism of transport of cortisol across the cell membrane. This could explain why the effect of metyrapone is evident not only on free but also on transcortin-bound cortisol. Indeed, if cortisol crosses the membrane faster in the presence of metyrapone, the overall equilibrium reaction between cortisol and transcortin will be displaced towards a more important dissociation of the protein-steroid complex.

Finally, the important loss of radioactivity observed with cholesterol, contrasting with the quantitative recovery of cortisol, must be emphasized. This suggests that the solubility of cholesterol in the mesentery – most likely in the cell membrane – is higher than that of cortisol.

Appendix

In a two-compartment closed system, the diffusion of a tracer can be expressed as

$$\ln \frac{C_{IN} - C_{eq}}{C_0 - C_{eq}} = \frac{-DA}{x} \cdot \frac{(V_1 + V_2)}{V_1 \cdot V_2} \cdot t \qquad (1)$$

where C_0 = initial concentration in compartment IN

C_{eq} = equilibrium concentration $= \frac{C_{IN}+C_{OUT}}{2} = \frac{C_0}{2}$

D = free-diffusion coefficient in water

A = surface of the membrane (0.79 cm^2 in this device)

x = thickness of the membrane; V_1 and V_2 = volume of each compartment. In this system,

$$V_1 = V_2 = 2 \text{ cm}^2, \text{ thus } \frac{V_1+V_2}{V_1 \cdot V_2} = 1$$

t = time.

P, the coefficient of permeability, is defined as D/x and is expressed in cm sec^{-1}. When $t = t\ 1/2$ (time for C_0 to become $C_0/2$) and by substituting C_{eq} by $\frac{C_{IN}+C_{OUT}}{2}$ and by $\frac{C_0}{2}$, Eq. (1) can be written as

$$\ln \frac{C_{IN}-C_{OUT}}{C_0} = \frac{-DA}{x} t\,1/2 = -\text{P.A.}\, t\,1/2 \tag{2}$$

or

$$\ln \tfrac{1}{2} = -\text{P.A.}\, t\,1/2 \tag{3}$$

whence

$$P = \frac{2.3 \log 2}{t\,1/2} \cdot \frac{1}{0.79} \tag{4}$$

and if $t\ 1/2$ is expressed in minutes,

$$P = \frac{1450}{t\,1/2} \text{ cm sec}^{-1} \times 10^{-5}.$$

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