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THE PASSAGE OF [^{125}I]INSULIN ACROSS ISOLATED MESENTERY EFFECT OF ANTI-INSULIN SERUM

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SUMMARY

The exchange of insulin, inulin, dextran and albumin across isolated rat mesentery is inversely related to molecular weight. The kinetics of the exchange are compatible with the laws of passive diffusion. After a 2-h incubation, 1% of the diffusible insulin is adsorbed on the membrane.

An excess of guinea-pig anti-insulin serum prevents the passage of insulin by forming a complex of high molecular weight and by blocking the membrane pathways of insulin diffusion. By contrast, under specific experimental conditions, appropriate amounts of guinea-pig anti-insulin serum can increase the transmesenteric flux of insulin.

INTRODUCTION

The passage of molecules across endothelial membranes occurs mostly by passive diffusion, hence it is primarily determined by molecular weight[1]. However, endothelial cells can play an active role in the passage of selected compounds either by enzymic action[2] or by cytopempsis[3].

The qualitative and quantitative aspects of hormonal transport across capillaries are not known; these aspects may be of importance as they can determine to some extent the access of protein hormones to target tissues[4]. Some properties of the transmembrane passage of insulin were studied in a diffusion cell where isolated rat mesentery was mounted as a boundary membrane between two compartments. The mesentery is a symmetrical membrane consisting of two layers of mesothelium separated by loose connective tissue. Because of its many morphological[5,6] and functional similarities[7–10] with the vascular endothelium, the preparation seems useful for the study of insulin transport across a living membrane. The kinetics of insulin exchange were compared to those of inulin, dextran (mol. wt. 15 000–17 000) and albumin. Furthermore, guinea-pig anti-insulin serum was used to modify the transport of insulin in an attempt to explain the mechanisms of its diabetogenic action *in vivo*.

MATERIAL AND METHODS

The diffusion cell is made of plastic material and is composed of two symmetrical chambers, In and Out. Each chamber has a total volume of 5 ml, a window area of 0.79 cm^2, and includes two vertical channels: the central channel (13 mm in diameter) is utilized for sampling the medium; through the lateral channel (2 mm in diameter) a mixture of 95% O_2 and 5% CO_2 is fed into the medium at a constant rate. The channels are connected within the chamber in such a way that the gas, while escaping through the central well, oxygenates the membrane and mixes the solution. The diffusion cell is shown in Fig. 1.

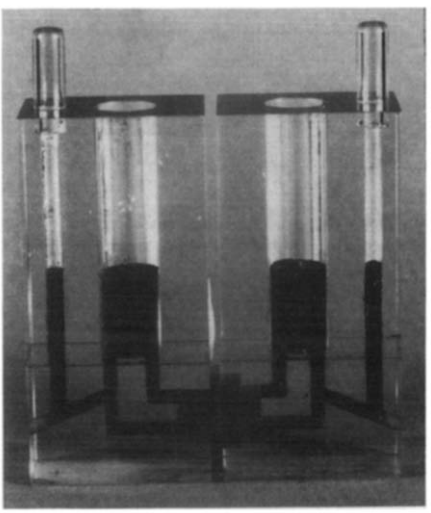

Fig. 1. Photograph of diffusion cell: the two parts have been placed next to one another and the device is ready for the experiment. Each chamber is filled with 2 ml of the medium (dark area) and is separated from the other by the isolated mesentery.

Male albino rats, weighing 250–300 g and fed on a standard diet, were killed by a blow on the head and bled through an incision in the neck. A portion of intact mesentery, free of visible fat and vessels, was excised, rinsed with a Ringer's solution and extended over the window of one chamber. Stretching of the membrane was carefully avoided. The surface utilized for the exchange was not touched; around this surface a rim of parafilm was applied to insure against leakage; the two parts of the cell were then brought together and secured with a rubber band.

The experimental apparatus was operated in a water bath at 38°. Each chamber was filled with 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 6 mg of glucose. Trace amounts of the radioactive molecules were added to the In compartment. No hydrostatic or osmotic pressure gradient existed across the mesentery. Aliquots of 0.1 ml of medium were simultaneously sampled from both chambers at various times during a 2- or 3-h incubation. For each tracer the logarithm of the concentration gradient across the mesentery (Δ = In — Out; ordinate) was plotted *versus* time (abscissa). A straight line was obtained; its $t_{1/2}$ value was arbitrarily used to estimate the magnitude of the exchange. The recovery of the radioactivity in both

chambers (Σ = In + Out) was measured in each experiment throughout the incubation.

The labelled molecules employed in this study were: bovine [^{125}I]insulin (Sorin; pH 3; specific activity, 9.0–13.5 mC/mg); [*carboxy*-^{14}C]inulin (New England Nuclear Corp.; crystalline solid; mol. wt., 5000–5500; specific activity, 1–3 mC/mg); [*carboxy*-^{14}C]dextran (New England Nuclear Corp.; crystalline solid; mol. wt., 15000–17000; specific activity, 0.5–2.0 mC/mg); human [^{131}I]albumin (Abbott; specific activity, 106 μC/ml). Inulin and dextran crystalline powders were dissolved in distilled water at 90°. All the radioactive solutions were diluted with the buffer prior to the incubation. The activity of radioiodinated insulin and albumin was counted as the trichloroacetic acid precipitate in a γ-well counter. The activity of the carbon-labelled inulin and dextran was counted as such in a scintillation β-well counter.

Guinea-pig anti-insulin serum, kindly provided by Dr. P. H. Wright (Indiana University, Indianapolis, Ind.), had an insulin-binding capacity determined by immunoassay of 2–3 units of crystalline insulin/ml (Lots No. 419-10 and No. 401): various amounts of this were added to one compartment, with or without [^{125}I]insulin. In other experiments both surfaces of rat mesentery were preincubated *in situ* at room temperature for 15 sec with the anti-insulin serum. The membrane was then thoroughly washed for approx. 1 min with large amounts of buffer, prior to being mounted in the apparatus. Guinea-pig anti-ferritin serum, kindly provided by Dr. E. Arquilla (University of California, Los Angeles, Calif.), was similarly utilized in control experiments.

RESULTS

Fig. 2 shows the patterns of the transmesothelial exchange of [^{14}C]inulin, bovine [^{125}I]insulin, [^{14}C]dextran and human [^{131}I]albumin. In the four sets of experiments, from the 10th or 30th min onward, a straight line was obtained when the logarithm of the concentration gradient was plotted against the time. The mean $t_{1/2}$ value of the insulin slope was 90 ± 5 min as compared with 65 ± 3 min for inulin

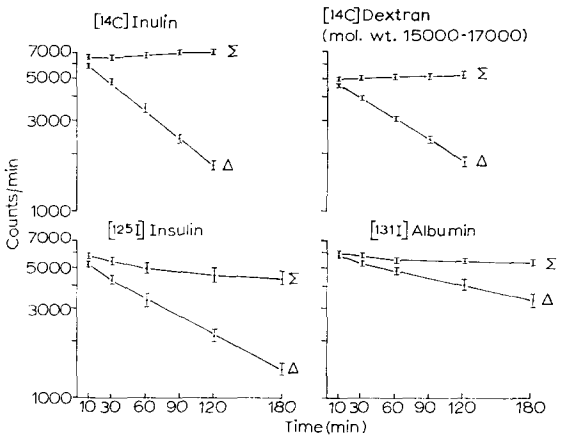

Fig. 2. Exchange of inulin, dextran, insulin and albumin across isolated rat mesentery. Δ, concentration gradient (In − Out). Σ, recovery (In + Out). Mean values with S.E. Number of experiments inulin, n = 12; dextran, n = 12; insulin, n = 14; albumin, n = 15.

TABLE I

EFFECTS OF GUINEA-PIG ANTI-INSULIN SERUM ON THE INSULIN EXCHANGE ACROSS ISOLATED RAT MESENTERY

Guinea-pig anti-insulin serum		[^{125}I]*Insulin*		*Number of experiments*	*Insulin exchange* $t_{1/2}$ *of slope* $\Delta \pm$ *S.E.* (*min*)
Neutralizing potency (*μunits insulin*)	*Site*	*μunits*	*Site*		
0	—	200–1400	In	14	90 ± 5
16000–24000	In	1000–1400	In	12	250 ± 24
20000–30000	Out	1400	In	6	260 ± 26
2000– 3000	Out	1400	In	6	49 ± 3
0; Anti-ferritin serum	Membrane	9000	In	6	83 ± 5
2000000–3000000	Membrane	9000	In	6	242 ± 30
200000– 300000	Membrane	9000	In	9	111 ± 5

and 83 ± 3 min for dextran. The passage of albumin was considerably slower: $t_{1/2}$ = 217 ± 14 min.

Table I summarizes the effects of guinea-pig anti-insulin serum on the insulin exchange. An excess of anti-insulin serum, wherever added, considerably delayed the exchange of insulin. However, when small amounts of anti-insulin serum were added to the Out compartment, opposite insulin, the exchange was increased ($t_{1/2}$ = 49 ± 3 min). The preincubation of mesentery with guinea-pig anti-insulin serum resulted in a diminution of insulin passage. The effect was striking with undiluted serum ($t_{1/2}$ = 242 ± 30 min); it was still significant with serum diluted 1:10 ($t_{1/2}$ = 111 ± 5 min). When undiluted guinea-pig anti-ferritin serum was used as a control for guinea-pig anti-insulin serum, no effect on the insulin exchange was observed ($t_{1/2}$ = 83 ± 5 min). Fig. 3 shows that negative values of Δ were obtained: (i) after a prolonged incubation when a small amount of anti-insulin serum was on one side of the membrane (Out) and insulin on the other (In); (ii) very rapidly when both chambers were intitially filled

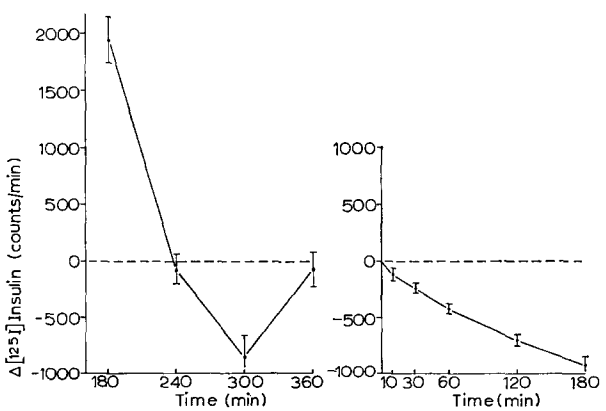

Fig. 3. Effect of guinea-pig anti-insulin serum on the concentration gradient of insulin. Left: 1400 μunits of insulin in the In compartment. 2000–3000 μunits of insulin antibody in the Out compartment. n = 6. Mean values with S.E. Right: 1400 μunits of insulin in the In compartment. 1400 μunits of insulin in the Out compartment. 2000–3000 μunits of insulin antibody in the Out compartment. n = 6. Mean values with S.E.

with similar concentrations of insulin and a small amount of anti-insulin serum was added to the Out chamber.

DISCUSSION

Solute transport across biological barriers is either active, with net migration against electrochemical gradients, or passive, in the direction of a chemical activity gradient. In this study the movement of inulin, dextran, insulin and albumin across isolated rat mesentery was inversely related to molecular weight and exhibited the kinetics of passive diffusion[11]: the instantaneous net molecular flux was proportional to the activity difference across the area of the membrane; it decreased progressively and ultimately would have reached zero[12]. The $t_{1/2}$ value of 90 min for insulin is comparable to the exchange of a molecule with a molecular weight higher than dextran (mol. wt. 15000–17000), most likely a trimeric form of insulin. Circulating insulin is also a small polymer with a molecular weight lower than albumin[13]. *In vivo*, endogenous insulin could also cross capillaries by the passive process of diffusion; this would account for the rapid appearance of the hormone in blood after an acute β-cell stimulation and its rapid passage from blood into extravascular spaces. While passing through the mesentery, insulin is partially adsorbed on the membrane: 1% of the total trichloroacetic acid-precipitable activity or antibody-bound activity of the medium is recovered from the mesentery at the end of the incubation (data not shown). This amount is twenty times higher than expected if insulin had merely diffused in the total water of the tissue. The adsorption of insulin on the mesothelium, and possibly on vascular endothelium[14], extends the property of hormonal binding to those tissues which are primarily involved in transport. In metabolic phenomena studied with either perfused or excised muscle and fat, due consideration must be given to the properties of endothelial barriers and mesothelial membranes enveloping the tissues.

The addition of an excess of guinea-pig anti-insulin serum to insulin results in the formation of an antibody–antigen complex of high molecular weight which diffuses through mesentery at a considerably lower rate than free insulin: the $t_{1/2}$ value of 250 min is slightly higher than the $t_{1/2}$ value of albumin and is compatible with the exchange of γ-globulins[9]. An excess of guinea-pig anti-insulin serum in the compartment opposite the one containing insulin also impedes the passage of the hormone: $t_{1/2} = 260$ min. Enough anti-insulin serum may have migrated across the mesentery, and again the diffusion of an antibody–antigen complex would be reduced. However, such a mechanism can be ruled out: the effect of insulin antibodies was indeed noticed within 10 min, a period of time too short to allow a significant flow of γ-globulins from one chamber to the other. The rapidity of insulin blockage suggested that guinea-pig anti-insulin serum exerted its action at the membrane. When the mesentery was preincubated for 15 sec with guinea-pig anti-insulin serum, the exchange of insulin was indeed impaired. Exposure of the membrane to undiluted antibodies had an effect similar to that observed with an excess of anti-insulin serum in either one of the two compartments. Under these experimental conditions the equilibration of insulin is at least as slow as the equilibration of albumin. When applied to the mesentery, the effect of anti-insulin serum does not result from a leakage of the antibody out of the membrane into the medium: at the end of the incubation no antibody–antigen complex was precipitated in the medium by a rabbit anti-guinea-pig serum. Moreover, the

effect was not related to a nonspecific obstruction by serum proteins of the cellular pathways of transport: guinea-pig anti-ferritin serum, used in control experiments, did not reduce the exchange of insulin. Finally, the effect of anti-insulin serum was not due to an extensive trapping of insulin within the mesentery: the recovery of insulin in the medium (Σ = In + Out) ranged from 99 to 92% throughout the incubation. It is concluded that guinea-pig anti-insulin serum is adsorbed on mesothelium at sites which are required for the insulin diffusion. The adsorbed antibody, whether combined with insulin or not, then prevents the entry of the hormone into the membrane.

In some instances, an entirely different effect of anti-insulin serum on insulin exchange is observed when the antibody is used at a concentration approximating that of the antigen. Table I shows that insulin added to the In compartment is recovered more rapidly in the Out compartment when the latter contains a small amount of the antibody. This effect is ascribed to the induction by anti-insulin serum of a high concentration gradient of free insulin: insulin is indeed bound as it flows into the Out compartment and little if any free hormone is available for backward diffusion. If such were the mechanism, negative values of Δ insulin should be obtained in the course of a prolonged incubation, since no attempt was made to distinguish between free and antibody-bound insulin. This was indeed observed (Fig. 3, left). Assuming there is no movement of antibodies across mesentery, the theoretical equilibrium would correspond to the passage of the total amount of insulin from the In compartment to the Out compartment. However, this was not observed: the finding is compatible with a slow diffusion of antibodies into the In compartment. Similar comments apply to the experiments where identical amounts of insulin were initially added to each side of the membrane and anti-insulin serum was added to the Out compartment (Fig. 3, right).

The experiments with anti-insulin serum show that the location and potency of the antibody affect the exchange of insulin between two compartments separated by a living membrane in different ways. The *in vitro* effects of insulin antibodies might elucidate so memechanisms of their diabetogenic action *in vivo*. The intravascular injection of anti-insulin serum into some experimental animals induces either a transient hyperglycaemia[15] or a permanent and fatal diabetic syndrome[16]. The deprivation of insulin at the target tissues may result from: (i) the binding of plasma insulin; (ii) the establishment of a positive concentration gradient of diffusible insulin between extravascular and vascular spaces; (iii) the shift of interstitial insulin into blood and its subsequent neutralization. It has been reported that some dogs injected with guinea-pig anti-insulin serum develop hyperglycaemia which occasionally does not respond to the intravenous injection of insulin[17]. As following the insulin injection the amount of free serum insulin was not determined, one can only speculate whether the lack of response to insulin was due to its binding to circulating antibodies or to a block of its transcapillary diffusion. Another report may support the latter hypothesis[18]. In alloxan-diabetic rats injected intravenously with guinea-pig anti-insulin serum, the subsequent intravenous injection of 3.4 units of insulin did not lower significantly the plasma sugar concentration despite the fact that the amount of unneutralized antibodies present in body fluids was only able to bind less than 2 units of insulin. Among other possibilities, it is conceivable that free insulin was prevented from reaching the peripheral tissues by a mechanism other than intravascular binding to circulating antibodies.

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REFERENCES

1 E. M. LANDIS AND J. R. PAPPENHEIMER, in W. F. HAMILTON AND P. DOW, *Handbook of Physiology, Section 2: Circulation*, Vol. II, Am. Physiol. Soc., Washington, 1963, p. 961.
2 D. S. ROBINSON, in R. PAOLETTI AND D. KRITCHEVSKY, *Advances in Lipid Research*, Vol. I, Academic Press, New York, 1963, p. 113.
3 G. MAJNO, in W. F. HAMILTON AND P. DOW, *Handbook of Physiology, Section 2: Circulation*, Vol. III, Am. Physiol. Soc., Washington, 1965, p. 2324.
4 E. RASIO, E. MACK, R. EGDAHL AND M. G. HERRERA, *Diabetes*, 17 (1968) 668.
5 A. A. MAXIMOW AND W. BLOOM, in A. A. MAXIMOW AND W. BLOOM, *A Textbook of Histology*, Saunders, Philadelphia, 1957, pp. 80, 47, 35.
6 J. E. FRENCH, H. W. FLOREY AND B. MORRIS, *Quart. J. Exptl. Physiol.*, 45 (1960) 88.
7 W. O. BERNDT AND R. E. GOSSELIN, *Am. J. Physiol.*, 200 (1961) 454.
8 J. CASCARANO, A. D. RUBIN, W. L. CHICH AND B. W. ZWEIFACH, *Am. J. Physiol.*, 206 (1964) 373.
9 E. BRACHET AND E. RASIO, *Arch. Intern. Physiol. Biochim.*, 76 (1968) 152.
10 D. L. ODOR, *J. Biophys. Biochem. Cytol.*, 2 (Suppl.) (1956) 105.
11 W. O. BERNDT AND R. E. GOSSELIN, *Biochem. Pharmacol.*, 8 (1961) 359.
12 J. S. ROBERSTON, in W. F. HAMILTON AND P. DOW, *Handbook of Physiology, Section 2: Circulation*, Vol. I, Am. Physiol. Soc., Washington, 1962, p. 628.
13 S. A. BERSON AND R. S. YALOW, *Am. J. Med.*, 40 (1966) 676.
14 E. RASIO AND V. CONARD, *Diabetologia*, 5 (1969) 53.
15 J. ARMIN, R. T. GRANT AND P. H. WRIGHT, *J. Physiol., London*, 153 (1960) 131.
16 J. ARMIN, R. T. GRANT AND P. H. WRIGHT, *J. Physiol., London*, 153 (1960) 146.
17 J. R. M. FRANCKSON, Y. ARNOULD, W. MALAISSE AND V. CONARD, *Diabetes*, 13 (1964) 532.
18 P. H. WRIGHT, L. R. CALIMLIM AND W. J. MALAISSE, *Am. J. Physiol.*, 211 (1966) 1089.