PRELIMINARY COMMUNICATIONS

The Displacement of Insulin from Blood Capillaries

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Summary. Normal anaesthetized bull-dogs were perfused with constant amounts of ^{125}I bovine insulin. During the control period, steady state arterio-venous gradients and lymph levels were achieved. Positive arterio-venous differences were observed across the hind limbs and the head. Lower levels of ^{125}I-insulin were found in cervical and leg lymph than in corresponding venous plasma. The rapid intravascular injection of unlabelled insulin resulted in an almost immediate reversal of the arterio-venous gradients of ^{125}I-insulin in the head and the hind limb. The injection of small amounts of glucose into the lower abdominal aorta induced a very rapid diminution of the arterio-venous gradient across the hind quarters; the effect was not mediated by the release of endogenous insulin. In both experiments, the lymph levels of ^{125}I-insulin remained relatively constant.

The data indicate that the vascular pole of the capillary endothelial cells can adsorb labelled insulin and either exchange it with unlabelled insulin or release it under the direct influence of glucose.

Introduction

The binding of insulin to tissues has been demonstrated *in vitro* [8] and *in vivo* [14]; it has led to the theory that insulin combines with a specific receptor at the cell membrane [11]. Whether the insulin-receptor bond is stable or labile is still controversial: in rat heart, insulin can be rapidly washed off with an insulin-free perfusate [1], whereas in rat diaphragm its binding is not reversible [20]. Insulin also penetrates the plasma membrane [9, 19]; within the cell, the hormone either retains its unaltered structure or undergoes enzymatic degradation. Insulin has been recovered from intracellular fractions in its immuno-reactive form [6]; on the other hand, insulin-degrading activity has been demonstrated in nearly all tissues [12].

It is generally assumed that the binding *in vivo* of insulin to tissues and its subsequent degradation do not allow any extrapancreatic storage of the hormone. This opinion is challenged in the present study. It is entirely possible, indeed, that if a tissue were able to adsorb more insulin than it could inactivate, it would store insulin; moreover, if the adsorption were labile, the stored insulin could be somehow released. The vascular endothelium was tested as a possible site for peripheral insulin storage and release; its large surface in close contact with circulating insulin is ideally located for an optimal adsorption; moreover the absence of a concentration gradient of insulin between vascular and extravascular space in baseline conditions, indicates a low insulin degrading activity across capillary membranes [15].

The experiments were performed in normal anaesthetized bull-dogs. During a constant infusion of radioiodinated insulin, steady state arterio-venous gradients and lymph levels were achieved. In some dogs, exogenous crystalline insulin was injected through a foreleg vein, in order to induce a competitive displacement of labelled insulin in the hind limb and in the head. In other dogs, glucose was injected slowly into the hind limbs, in such minute amounts as to avoid the release of endogenous insulin. In both groups, the positive arterio-venous gradients of labelled insulin were rapidly reversed or diminished.

The constant levels of labelled insulin in lymph suggested that no major insulin displacement occurred at the cell surfaces in the interstitial space.

Material and Methods

Fourteen bull-dogs were anaesthetized with intravenous sodium pentobarbital, 35 mg/kg after a 16 h fast. Controlled respiration (50 per cent O_2, 50 per cent air) was maintained through an intratracheal tube, with an automatic respirator. An isotonic sodium chloride solution was perfused through a left foreleg vein at a constant rate of 500 ml/h.

The lower portion of the abdominal aorta and of the inferior vena cava, the femoral vein and the jugular vein were cannulated with No 280 polyethylene tubing. Along the external saphenous vein, or the jugular vein, a main lymphatic vessel was cannulated distally by means of No 50 polyethylene tubing; all the other surrounding lymphatics were ligated proximally. One hour after the surgical procedures, a priming dose of 6 ml of a labelled insulin solution was given through a right foreleg vein; it was immediately followed by an infusion of 20 ml/h, which was maintained constant throughout the experiment. The insulin solution was a mixture of 0.6 ml of bovine ^{125}I-insulin (Sorin; concentration 9–29 μγ/ml; specific activity 9.4–13.5 mCi/mg; pH 3), 3 ml of the dog plasma and isotonic saline in a total volume of 100 ml; this solution contained less than 5 per cent of non-TCA precipitable activity. Ninety minutes after the beginning of the infusion, a mixture of pork and beef insulin (Novo Actrapid) 0.1 U/kg was administered in 15 seconds into the left foreleg vein. In other experiments, isotonic glucose 0.05 g/kg was injected into the abdominal aorta during a one minute period of time.

1.5 ml of arterial and of venous blood were simultaneously drawn, every 15 min during the first 90 min of the perfusion, and at 15 sec, 1, 3, 5, 10, 20 and 30 min following the end of the insulin or glucose injection. Lymph collections consisted of the entire volume obtained by gravity from the catheter during a 15 or 10 min period of time. Lymph flow was occasionally stimulated by gentle massage. The heparinized blood and lymph samples were centrifuged at 4°C; 0.2 ml of plasma or cell-free lymph was added with a drop of 20 per cent KI solution in distilled water and 3 ml of a 30 per cent trichloracetic acid solution in distilled water. The precipitate was washed twice and its radioactivity measured in a γ wellcounter. The activity of each sample of plasma or lymph was determined in duplicate.

Results

Fig. 1 shows the effects of an intravascular injection of exogenous insulin upon the arterio-venous and the blood-lymph concentration gradients of labelled
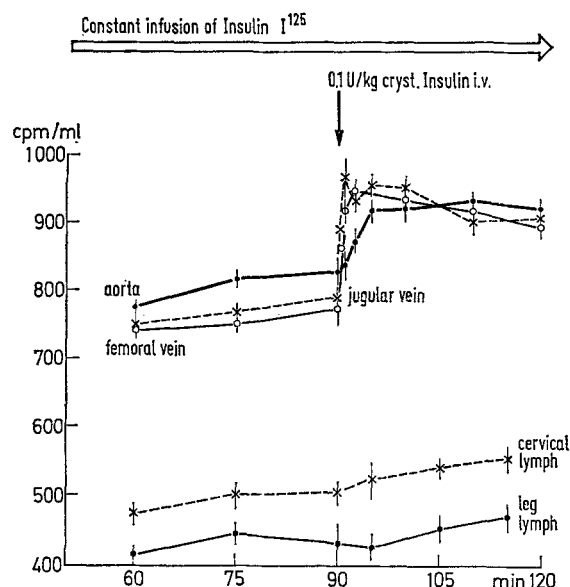

Fig. 1. The effect of crystalline insulin upon the arterio-venous and blood-lymph gradients of ^{125}I-insulin across the hind limb and the head.
N = 6; mean values $\pm$ S.E.M.

insulin in the hind limb and the head. The TCA-precipitable radioactivity increased almost immediately in the vascular compartment; the arterio-venous gradients were reversed within seconds and remained so for 10 min. The changes in the concentration gradients are statistically significant ($p < 0.05$). Only a minor increase in lymph radioactivity was noticeable.

Fig. 2 shows the effects observed with glucose administered via the abdominal aorta. There was no change in the arterial plasma radioactivity, whereas a

rapid rise was observed in the inferior vena cava plasma. The arterio-venous concentration gradient in the hind limbs was significantly reduced for 20 min ($p < 0.05$). No such an effect was detected in the head.
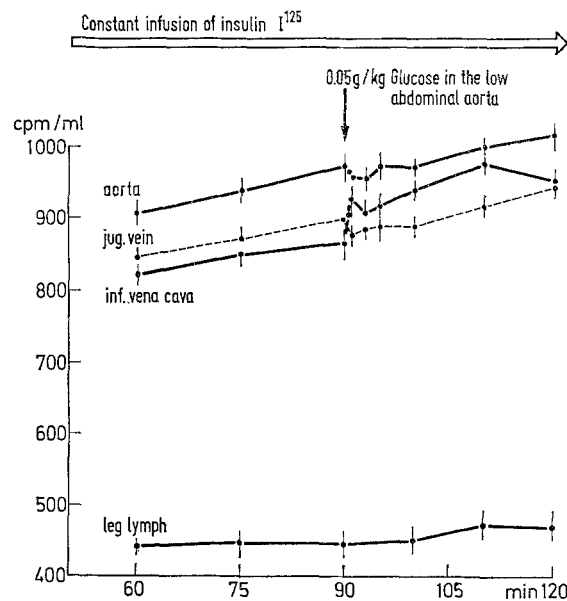

Fig. 2. The effect of an injection of glucose into the low abdominal aorta upon the arterio-venous and blood-lymph gradients of ^{125}I-insulin across the hind limbs and the head
N = 8; mean values $\pm$ S.E.M.

Discussion

During the first 90 min of the perfusion, an equilibrium was achieved between the inflow and the removal of labelled insulin in the hind limb and the head. The equilibrium was disrupted by the injection of exogenous insulin: the TCA-precipitable radioactivity increased in the vascular compartment, and the arterio-venous gradients were reserved. Current experiments in our laboratory have shown that the displacement of TCA-precipitable radioactivity by insulin can also be obtained in the vascular bed of intestine, liver and lungs.

An increased fluid loss in relation to labelled insulin cannot be involved in the effect since the haematocrit value in the arterial and venous blood was not modified by the insulin injection. Nor is the effect merely accounted for by a diminished rate of labelled insulin degradation; in such a case, the arterio-venous gradients would have been at the most suppressed.

As the gradients became negative, some labelled insulin has been displaced from receptor sites and has been released into the venous blood. The sites of insulin adsorption and release are conceivably the blood cells, the capillary membranes in the vascular bed and the cell surfaces in the interstitial fluid. There is no evi-

dence that insulin is present in blood cells; moreover, the addition of an excess of insulin to blood samples obtained during control period and incubated at 37°C, did not change the plasma TCA-precipitable radioactivity. If insulin had been displaced in the extravascular space, the radioactivity in lymph would have been increased; lymph capillaries are indeed permeable to insulin [17]. The constant level of lymph radioactivity and the very rapid reversal of the arterio-venous gradients indicate that insulin was displaced at the vascular pole of endothelial cells.

The binding of insulin to capillaries may prevent to some extent the passage of physiological amounts of insulin from vascular to extravascular space. Thus following the rapid intravenous injection of glucose in normal dogs, there is a small rise, if any, in leg lymph insulin [17]. Moreover in the human forearm preparation [2, 21, 22] or in the perfused rat adipose tissue [18], a transient and physiological rise in arterial serum insulin does not increase the cell glucose uptake. In some of these experiments, the arterial and venous concentrations of serum insulin were measured, and insulin was cleared by the perfused tissues.

The release of insulin by capillaries is also a matter of some interest; in this study the previously adsorbed labelled insulin was displaced by the unlabelled insulin. The displacement of insulin from capillaries could account for the reversal of the arterio-venous gradient across the human forearm, in the course of rapidly decreasing concentrations of serum insulin [13].

Some characteristics of insulin binding were further investigated in the experiments where glucose was slowly injected in the hind limbs; the positive arterio-venous gradient was rapidly reduced; again the levels of labelled insulin in lymph remained constant. The effect of glucose was a direct one; indeed it was not seen in the head, as it should have been if endogenous insulin had been released [16]. Since in this group of dogs the arterio-venous gradient was not reversed, one can only speculate whether glucose has diminished the removal of ^{125}I-insulin or has increased its release. There is no evidence that the insulin disappearance rate is diminished by glucose. It is possible therefore that capillaries may release insulin into the blood under glucose stimulation. This hypothesis is compatible with the following observations; the infusion of small amounts of glucose or ribose into the portal vein or the right atrium in dogs induces a rise in circulating insulin despite the absence of a change in arterial blood sugar levels [4, 5]. The intravenous injection of glucose in dogs immediately after total pancreatectomy increases the levels of serum insulin provided the pre-operative concentrations of insulin were kept high [3]. Finally, the initial peak of serum insulin observed after a glucose injection can be reproduced with the perfused rat pancreas preparation [7]; however, with pieces of pancreatic tissue incubated *in vitro* in the presence of glucose, a rapid insulin secretion is lacking [10]. It is therefore possible that the initial rise of circulating insulin is due, at least in part, to a direct effect of glucose upon capillaries.

It is concluded from this study that the vascular endothelium should not be merely considered as a membrane for insulin diffusion, but also as a site for extrapancreatic storage and release of insulin.

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