NOTE

In Vitro Study on the Rate of Intestinal Absorption of Insulin

MOTOAKI SHICHIRI, KENKICHI KARASAKI, NOBUAKI ETANI, AKIRA OKADA AND YUKIO SHIGETA

First Department of Medicine, Osaka University Medical School, Osaka

Synopsis

Using an everted sac technique, the intestinal absorption of insulin in the rabbit has been investigated.

Insulin was shown to cross the intestinal wall, as demonstrated by the immunoreactive insulin inside the sacs (serosal fluid). The rate of insulin transference for a period of one hr was small, corresponding to approximately 0.1% of the insulin added to the mucosal fluid. This rate was not inhibited by the addition of 2, 4-dinitrophenol.

The small transference rate would indicate that the submucosal and muscular tissues of the intestinal wall, in the absence of vascular perfusion, present a barrier to the movement of large molecular weight substances.

It has been generally accepted that insulin can be absorbed from the intestine of mammals, but the fraction absorbed is considered to be small (Laskowski et al., 1958; Danforth and Moore, 1959; Speth and Christian, 1963; Crane et al., 1968). The capacity of the intestinal mucosa to absorb insulin as a polypeptide might be limited.

The sacs of everted intestine are suitable for the study of intestinal transference (Wilson and Wiseman, 1954 a and b; Crane and Wilson, 1958). Using everted sac technique, Danforth and Moore (1959), and Nakano and Igawa (1964) demonstrated insulin transference across the intestinal wall qualitatively. On the basis of hypoglycemic response to sac contents, approximately 2% of the insulin added to the medium was transferred through the intestinal wall (Danforth and Moore, 1959). The difficulty in quantitative measurement, however, might be due to the fact that the transference of insulin was too small to detect by the method of blood glucose lowering effect in rabbits. It is possible now to measure a small quantity of insulin by the use of immunological method.

The present experiments are designed to determine the rate of transference of insulin across the intestinal wall. The effect of 2,4-dinitrophenol on the rate of transference is also studied.

Materials and Methods

Male white rabbits, weighing from 2.0 to 2.5 kg, were fasted for 16 to 18 hr. The rabbit was stunned by a blow on the head and killed by bleeding from the carotid arteries. The abdomen was opened by a midline incision and the small intestine was washed out with a solution of 0.9% NaCl containing 0.3% glucose. The jejunum 20-30 cm below the pyloric ring was resected after stripping the mesentery from the jejunum. To evert the intestine, a glass rod was used to push the one end into the intestinal lumen.

Everted sacs of the jejunum approximately 7 cm in length were prepared by a modification of the method of Crane and Wilson (1958). Three milliliter of Krebs-Ringer bicarbonate buffer containing 90 mg/100 ml glucose was instilled into each sac (serosal side) which was then placed in the flasks containing 30
ml of the same glucose containing medium (mucosal side).

Crystalline bovine insulin (Shimizu Chem. Co., Shimizu, Japan) was added to the medium at a concentration of 25, 50 and 100 U/ml. In some flasks, 2, 4-dinitrophenol (DNP) at a concentration of $10^{-4} M$ was added to inhibit the oxidative phosphorylation. The sacs were incubated for 120 min at 37°C under an atmosphere of 95% O$_2$: 5% CO$_2$. During incubation, a small quantity of samples was withdrawn from the serosal and mucosal fluid, and analyzed for glucose and insulin concentrations. At the end of experiments, the sacs were emptied and the weight was determined after drying for 3 hr at 110°C.

Glucose was measured by the method of Somogyi and Nelson (1944) and insulin was determined immunologically by the method of Hales and Randle (1963).

### Results

The transference of insulin across the jejunal wall was directly proportional to the time of incubation over a period of 2 hr (Fig. 1). The effect of concentration on the rate of insulin transport is shown in Figure 2. The rate of insulin transport increased with increasing insulin concentration in the medium, but this was not associated with a detectable decrease in concentrations on the mucosal side.

As shown in Table 1, there was a small transference of insulin across the wall, corresponding to approximately 0.1% for a period of one hr. With an initial concentration of 50 and 100 U/ml, jejunal sacs gave transference values of 105 and 213 mU/100 mg dry weight/hr, respectively. This rate was not affected by the addition of DNP (Table 2).
Table 1. Rate of insulin transport through everted sacs of rabbit jejunum

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Mucosal insulin concentration (U/ml) 0 min</th>
<th>Serosal insulin concentration (mU/ml) 60 min</th>
<th>Insulin transferred through intestinal wall (mU/100 mg dry wt./hr) 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>22 ± 3.6</td>
<td>26 ± 9.4</td>
<td>60 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>(0.10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>(0.27%)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>51 ± 2.2</td>
<td>51 ± 10.5</td>
<td>105 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>(0.10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>(0.25%)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>123 ± 7.0</td>
<td>112 ± 23.7</td>
<td>213 ± 13.0</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>(0.11%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>(0.22%)</td>
<td></td>
</tr>
</tbody>
</table>

* Fraction of insulin transferred through intestinal wall is expressed as percent of the insulin added to the mucosal fluid. Values indicate mean ± standard error.

Table 2. Effect of 2,4-dinitrophenol on the rate of insulin transport through everted sacs of rabbit jejunum

<table>
<thead>
<tr>
<th>Initial mucosal insulin conc. (U/ml)</th>
<th>2,4-dinitrophenol added (M)</th>
<th>Exp. No.</th>
<th>Insulin transferred through intestinal wall (mU/100 mg dry wt./hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0</td>
<td>5</td>
<td>98 ± 18.4</td>
</tr>
<tr>
<td>50</td>
<td>10^-4</td>
<td>5</td>
<td>97 ± 14.2</td>
</tr>
</tbody>
</table>

No significant difference between experimental groups with and without addition of 2,4-dinitrophenol is observed. Values indicate mean ± standard error.

Discussion

The transference of glucose across the sac wall of the jejunum was studied with the same initial concentrations of glucose inside and outside the sacs. The concentration of glucose on the serosal side rose from an initial value of 0.20% to over 0.31%, while that on the mucosal side fell to 0.16%. The glucose transference was directly proportional to the time of incubation for as long as 120 min. The rate of glucose transference was 0.54 mg/100 mg dry weight/hr for jejunal sac. Thus, the validity of the preparation studied here was considered to be preserved.

Insulin is rapidly destroyed by the action of proteolytic enzymes (Sanger, 1960; Nicols, 1960; Carpenter and Baum, 1962). In this kind of experiments, it is, therefore, necessary to remove the proteolytic enzymes from the mucosal surface. A solution by which the everted sacs were rinsed out was incubated with 0.5μ moles p-toluenesulfonyl-L-arginine methyl ester as a substrate for 120 min at 37°C, and analyzed the rate of destruction of substrate by the method of Roberts (1958). No demonstrable destruction of this substrate was observed. Insulin concentrations on the mucosal side, also, did not significantly change.

Although the mature intestine is considerably impermeable to large molecules (Smyth et al., 1961; Kabacoff et al., 1963; Avakian, 1964; Pierce and Smith, 1967), at least small amounts of insulin can be shown to cross the intestinal wall in in vitro experiments using everted sacs of the intestine. The transference of insulin across the intestinal wall was proportional to the time of incubation and to the concentration of insulin added in the medium. As compared to the concentration of insulin outside the sac, its concentration inside the sac was extremely low. Thus, as far as the viability of the intestine is preserved, the rate of transference might remain constant. On the basis of hypoglycemic response
to sac contents, Danforth and Moore (1959) estimated roughly that insulin concentration inside the sac at the end of incubation (one hr incubation) was approximately 2% that of incubating medium. In the present study, the immunoreactive insulin inside the sac contents was small, corresponding to 0.1% for one hr incubation at 37°C. When intestinal sac contents with a higher insulin concentration were injected intravenously to rabbits, the blood glucose was decreased by 10 to 15%, but this was not enough to determine the biological unit of insulin. These results, however, demonstrated that insulin can be transferred across the intestinal wall in a biologically and immunologically active form. This small transference rate would indicate that the submucosal and muscular tissues of the intestinal wall, in the absence of vascular perfusion, might present a barrier to substrate movement. As far as absorption studies are concerned, therefore, the technique of perfusion of the intestinal vascular system appears to be a better tool. An attempt using intestinal perfusion technique is in progress.

Finally, the transference of insulin across the intestinal wall was not inhibited by the addition of 2,4-dinitrophenol, while the active transport of glucose was inhibited. The transference of insulin, therefore, might not be involved in the mechanism of active transport. Using an everted sac technique, Kimura et al. (1970) demonstrated that the transference of lysozyme was not inhibited by the addition of NaF, KCN and 2,4-dinitrophenol. On the other hand, Lecce (1966) showed the inhibition of the transport of r-globulin by mono-iodoacetate. The exact mechanism of the transport of protein or peptides across the intestinal wall is, at present, unknown. Recently, cytochemical studies (Clark, 1959; Cornell and Padykula, 1969; Cornell et al., 1971) have revealed that an exogenous macromolecule can be taken up by a characteristic membraneous subcellular system within the intestinal absorptive cells. For enhancing the absorption of the therapeutic large molecular weight substances, such as peptide hormone, it is of great importance to learn more about mechanism of transport of macromolecules through the intestinal wall.

Acknowledgment

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References