EFFECT OF A COMBINATION OF PROTEINASE INHIBITORS ON INSULIN ABSORPTION FROM THE SMALL INTESTINE IN DOGS

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A combined preparation of plant proteinase inhibitors, when introduced together with insulin (3:1) into the duodenum of adult dogs, considerably increases both the degree and duration of the hypoglycemic effect. If insulin (125 i.u./kg) is introduced into an isolated segment of jejunum, plant proteinase inhibitors do not modify the hypoglycemic effect.

Views on the ability of proteins to penetrate through cell membranes and, in particular, through the intestinal mucosa have changed in recent years [4, 11]. The high permeability of the intestinal mucosa to proteins, especially in newborn animals, has been demonstrated by McCance [7]. Similar results have been obtained for proteolytic enzymes [5, 6, 8], insulin [2, 3, 9, 10], and other proteins. Insulin is a convenient object for such investigations because of the relative ease of detection of its specific physiological effects. In the study of absorption of insulin from the intestine its hydrolysis by digestive proteinases must be prevented. For this purpose some workers have used a trypsin inhibitor from the soy bean and other protease inhibitors [3].

The object of the present investigation was to obtain additional facts on the absorption of insulin from the intestine and also to study the protective action of a preparation consisting of a combination of proteinase inhibitors against the destruction of insulin by pancreatic proteinases [1].

EXPERIMENTAL METHOD

Experiments were carried out on four dogs weighing about 20 kg. A metal fistula tube was implanted into the duodenum and into an isolated segment of small intestine of the animals.

Crystalline bovine insulin with an activity of 25.7 i.u./mg was used. The combined preparation of proteolytic inhibitors (PPI), obtained from haricot beans, produced 50% inhibition of trypsin and chymotrypsin in an enzyme-inhibitor ratio of 1:1 and 1:2 respectively [1]. The physiological effect of the absorbed insulin was determined from the change in the glucose concentration in the circulating blood estimated by Somogyi's method [12].

In each experiment the blood glucose concentration was determined in the fasting dog and this was taken as the initial level. Insulin, in various doses, together with the PPI and separately, was then injected through the fistula into the duodenum or isolated segment of the jejunum in 10 ml physiological saline, and the blood glucose concentration was determined over a period of several hours.

Changes in the glucose concentration with time in response to parenteral injection of 1 mg insulin were determined for the purpose of comparison in each animal (Fig. 1).

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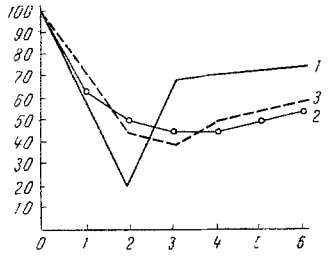

Fig. 1

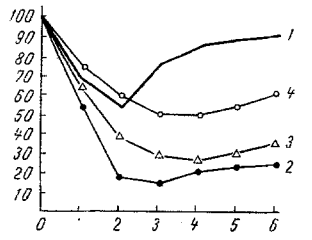

Fig. 2

Fig. 1. Changes in blood glucose concentration of dogs after intramuscular injection of 1 mg (25.7 i.u.) insulin (1), injection of 100 mg insulin into isolated segment of jejunum (2), and combined injection of 100 mg insulin and 300 mg PPI (3). Here and in Fig. 2: abscissa, time (in h); ordinate, blood glucose concentration (in percent of initial level). Mean results obtained in experiments on four dogs.

Fig. 2. Changes in blood glucose concentration of dogs after injection of 100 mg insulin (1), 100 mg insulin plus 300 mg PPI (2), 100 mg insulin plus 200 mg PPI (3), and 50 mg insulin plus 150 mg PPI (4) through the fistula into the duodenum.

EXPERIMENTAL RESULTS AND DISCUSSION

Proteolytic enzymes of the stomach and pancreas are among the barriers to the absorption of proteins from the intestine. For instance, a hypoglycemic effect after injection of 40 units insulin in 1 ml physiological saline per 100 g body weight has been observed in rats aged 2 and 8 days but not in rats aged 21 and 30 days [9]. In rats aged 2 days the blood sugar level was reduced from 39.3 ± 2.4 to 25.1 ± 2.1 mg%, and in rats aged 8 days from 75.0 ± 2.5 to 28.0 ± 2.5 mg%. The gastric contents in rats aged 2 and 8 days were found not to possess proteolytic activity, and the activity of the intestinal contents was 5-10 times below that of the intestinal contents of rats aged 21 and 30 days.

The results described above are in good agreement with those obtained in the present experiments on dogs. If 100 mg insulin was injected as an emulsion in physiological saline into the isolated segment of jejunum, a definite hypoglycemic effect was observed, only slightly less than the effect of intramuscular injection of 1 mg insulin (Fig. 1).

In this case a low glucose concentration in the blood was observed over a period of 3 h, but this was not observed after intramuscular injection. The difference in all probability was due to the relatively slow absorption of insulin from the intestine. The PPI, added to the insulin in this series of experiments, had no effect on the character of the changes in the blood glucose concentration (Fig. 1).

A smaller effect from the same dose of insulin (100 mg) was obtained if the injection was given into the duodenum, probably because of destruction of the hormone by pancreatic proteinases (Fig. 2). In this case the blood glucose concentration fell for only 2 h, after which it quickly increased.

Addition of the combined PPI from haricot beans to the insulin in this series of experiments considerably increased the hypoglycemic effect of the hormone. A marked effect also persisted if the dose of insulin was reduced to 50 mg, with a ratio of 1:3 to the inhibitor (Fig. 2). As the results show, a low glucose concentration was maintained in these experiments during observations for 4 h, but this was not found in the experiments in which the injection was given into an isolated loop of small intestine (Figs. 1 and 2).

Absorption of insulin from the intestine has been demonstrated in sucking pigs [2] and in newborn calves [10]. In the latter investigation it was found that more insulin, given by mouth, enters the blood stream than the lymph.

The effect of proteinase inhibitors – trypsin inhibitor from the soy bean, di-isopropylfluorophosphate (DPP), indole-3-acetate – has been studied on an isolated segment of jejunum in adult rats [3]. The soy trypsin inhibitor did not give a positive effect, as was also observed in the present experiments (Fig. 2),

for in this case the pancreatic proteinases must have been absent and the hormone could have been destroyed by peptidases of the intestinal mucosa. Injection of insulin in a dose of 160 i.u./kg (40 i.u. per animal) together with 0.075 mg DFP in this case did not cause any decrease in the glucose level during observations for 2.5 h. However, if the same animals were given an intraperitoneal injection of indole-3-acetate $2\frac{1}{2}$ h after the first injection, and 4 h later a further injection of half the dose of insulin combined with 0.075 mg DFP, the sugar concentration was reduced from 70 to 40 mg%.

The results cited above from the literature, together with those of the present experiments, can be regarded as evidence that absorption of insulin from the intestine continues for several hours, and that the hormone is destroyed not only by pancreatic proteinases, from which it can protected by proteinase inhibitors, but also by peptidases of the intestinal mucosa, which are not inactivated by proteinase inhibitors.

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