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Originals

Extrapancreatic Effect of Glibenclamide: Stimulation of Duodenal Insulin-Releasing Activity (DIRA) in Man

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Summary. Experiments were performed in order to study a possible participation of gastrointestinal factors in the insulinotrophic action of glibenclamide in man. Six healthy volunteers received 5 mg glibenclamide in 50 ml saline orally. Biopsies were taken from the duodenal mucosa before and after administration of the drug. The duodenal insulin-releasing activity (DIRA) was assayed in the extracts of the biopsy material by using an in situ pancreas preparation of rat. The corresponding drug, IRI and glucose levels were measured in peripheral blood. The values of IRI correlated with both the prior elevation of DIRA and the increasing levels of the drug in the blood. These data indicate that glibenclamide might stimulate the release of gut factor(s) which, in turn, could possibly sensitize the pancreas response to the drug.

Key words: Glibenclamide, DIRA, gastrointestinal hormones, duodenal mucosa extracts, insulin secretion, pancreas, B-cells, gastrointestinal factors, insulinotrophic action.

In 1964, three separate studies demonstrating higher levels of plasma insulin after an oral load of glucose than after intravenous administration, renewed interest in the concept of an enteropancreatic axis [3, 5, 15]. In previous publications [6, 7, 17], oral glucose administration in man or rat was shown to be followed by an increase in the insulinogenic activity extracted from the duodenal mucosa. Extracts of human or rat duodenal mucosa taken after a glucose load were shown to stimulate insulin release in an in situ rat pancreas preparation to a much higher degree than extracts of mucosa removed in the fasting state. It was suggested that the passage of glucose through the duodenum might activate some inactive factor(s) which would, in turn, stimulate insulin release from the pancreas.

The insulinogenic activity contained in the duodenum was called DIRA, for Duodenal Insulin-Releasing Activity [18]. It seems to play a role of mediator between the gut and the pancreatic B-cells.

In a recent experiment, glibenclamide, an antidiabetic sulfonylurea, was shown to enhance DIRA when administered intragastrically to rats [11]. The purpose of the present study was to investigate this effect in man.

Materials and Methods

A detailed description of the method was given in previous communications [6, 7, 18]. The study was performed with healthy, non-obese volunteers, aged 18 to 24. After an overnight fast, a Charles Debray biopsy capsule for multiple sampling was inserted into the duodenum under radioscopic control. The first biopsy from the duodenal mucosa and the first blood sample (10 ml from an antecubital vein) were taken for control. Immediately afterwards, the subject drank a solution of 5 mg glibenclamide (sodium salt) in 50 ml water. Blood samples and biopsies were taken at the times indicated in the diagrams. Each biopsy was weighed and homogenized after withdrawal in a hundredfold (w/v) amount of saline solution kept on ice. After centrifugation for 25 min at 3500 g, the supernatant was collected and stored at −20° C until DIRA estimation in an in situ rat pancreas preparation called "receptor rat". The constancy of the mucosal extraction was checked by repeating the same experiment over a three-year period (Table 1).

Table 1. Insulin concentration in portal blood of the receptor rats one minute after an injection of rat duodenal mucosa extract removed 45 min after intragastric glucose administration. Results during three years

Year	IRI (μU/ml)	n
1972	490± 79	12
1973	487±142	7
1974	461±128	7

Bioassay in 24-h Fasted Receptor Rat

Male Wistar rats were laparotomized under anaesthesia with intraperitoneal pentobarbital (25 mg/kg body weight). A catheter was then placed into the portal vein above its junction with the pancreaticoduodenal vein. The aorta and the coeliac trunk were exposed for injection. Ten minutes were sufficient to avoid interference with insulin discharge due to manipulation. 0.7 ml blood was taken from the portal vein for estimation of the basal IRI concentration. 200 μl of duodenal mucosa extract – prepared as described above – were injected within ten seconds into the coeliac trunk at the junction of the hepatic and superior pancreatico-duodenal arteries. Blood samples were taken from the portal vein 1, 5, 10, and 15 min after injection of the extract. Insulin was measured in heparinized plasma by a double-antibody radioimmunoassay [9]. The standard curve was prepared, using a pure rat insulin preparation (Novo). The glucose levels were measured by the O-toluidine method [2].

In the receptor rat, the maximal IRI response to the injected extract occurred one minute after the injection, and this one-minute peak has been used as the value for Duodenal Insulin-Releasing Activity (DIRA). Glibenclamide was determined by radioimmunoassay [12].

Results

Figure 1 shows the values of DIRA in extracts of mucosal biopsies, together with the corresponding concentrations of glucose, IRI and glibenclamide in peripheral plasma. Glibenclamide levels increased linearly up to 45 min following drug administration, and the maximal drug level was not reached by the end of the experiment. IRI increased from 10 μU/ml to a maximal concentration of about 30 μU/ml at 60 min. The basal value of DIRA was 86 ± 17 μU/ml; within 15 min it increased to a maximum of 209 ± 25 μU/ml, which was significantly different from the basal value ($p < 0.0025$). There-

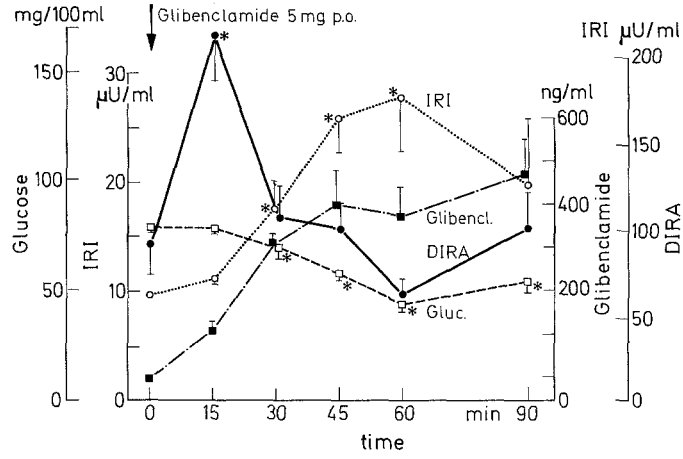

Fig. 1. DIRA in extracts of duodenal mucosa fragments taken from 6 normal subjects following oral administration of 5 mg glibenclamide in 50 ml water, and corresponding levels of plasma IRI glucose and glibenclamide. Mean ± SEM. Values are compared with the zero time value, and statistical significance is expressed by the symbol: black star, $p < 0.01$

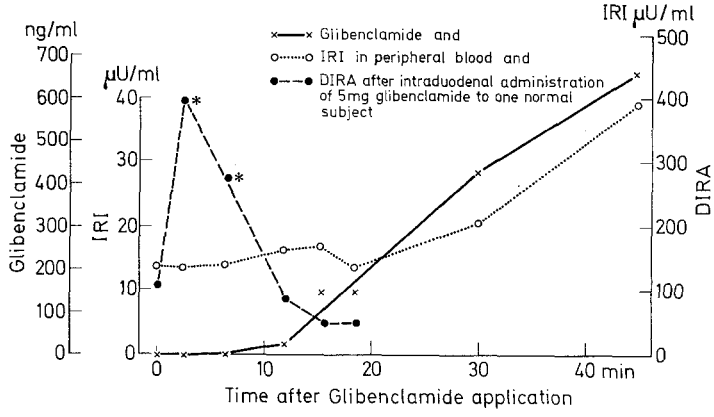

Fig. 2. DIRA glibenclamide and IRI in duodenal fragments from one normal subject following direct duodenal administration of 5 mg glibenclamide in 50 ml water. Star indicate that the value is significantly ($p < 0.05$) different from 0 value

Table 2. Variations of portal insulin (IRI) in the receptor rat one minute after injection of saline, duodenal mucosa extracts from normal fasted subjects or duodenal mucosa extracts from fasted subjects with addition of 0.5 μg/ml glibenclamide

Injection of	IRI (μU/ml)	n
Saline	77±17	7
Control duodenal mucosa extract	106±18	24
Control duodenal mucosa extract + 0.5 μg/ml glibenclamide	109±23	7

after DIRA decreased to a minimum at 60 min, at which time its level was 32% below the original value. Forty-five minutes after oral administration of glibenclamide the minimal glucose concentration was significantly below the value measured before drug administration.

In order to study the effect of glibenclamide during the first minutes following drug administration, one subject received 5 mg glibenclamide in 50 ml saline directly into the duodenum through an endoscope. As shown in Figure 2, the maximal DIRA value was observed 2.5 min after administration of the drug, whereas detectable concentrations of glibenclamide in the plasma were observed only after 12 min. The plasma drug levels were below the detection limit (10 ng/ml) at 2.5 and 6.5 min, and glibenclamide levels increased between 12 and 45 min. Peripheral plasma IRI levels started to increase after 18.5 min.

In control experiments, administration of 50 ml saline without addition of glibenclamide to normal subjects did not alter the value of the items mentioned in Figure 1.

The levels of glibenclamide in the mucosal extracts, estimated by radioimmunoassay, were always below 0.2 μg/ml. Injection of a human duodenal mucosa extract, with the addition of 0.5 μg/ml glibenclamide, into the receptor rat displayed no significant additional effect, compared with the injection of a similar duodenal mucosa extract without glibenclamide (Table 2).

Discussion

In 1967 [8], it was observed that an oral glucose load increased the insulinotrophic activity contained in the human duodenal mucosa. Later, administration of both glucose and arginine was shown to enhance the duodenal insulin-releasing activity (DIRA) in rat duodenal mucosa. Neutral fat was shown to decrease this activity [7, 17]. These observations suggested the presence of a specific enteropancreatic mechanism for insulin release after ingestion of food components.

In 1969 [10], it was suggested that glibenclamide might exert its insulinotropic effect partly through the release of gastrointestinal mediators besides the well-known direct effect on the pancreas [13, 14]. This suggestion was based on the observation that oral administration of the drug to the dog was as effective in increasing peripheral insulin levels as an intravenous injection of the same dose, in spite of a much lower drug concentration after intragastric administration.

Recent studies [11] with glibenclamide in the donor-receptor rat system have shown that intragastric application of glibenclamide to rats caused a rapid enhancement of DIRA in the mucosa. The increase observed in peripheral IRI correlated with the rise in the concentration of the drug in the mucosa as well as with the elevation of mucosal DIRA. Further, enhancement of the insulinotrophic activity could be indirectly demonstrated in plasma of rats treated intragastrically with glibenclamide. These observations suggest that glibenclamide might exert two different releasing activities: one releasing insulin at the pancreas level directly, the other inducing the release of gastrointestinal factor(s) which amplify the first effect.

The present data show that glibenclamide induces an elevation of DIRA in man. The increase of DIRA precedes the elevation of IRI and the decrease of blood glucose levels. The maximum duodenal insulin-releasing activity in the mucosa was observed before any significant alteration of IRI and glucose occurred. The observation made after intraduodenal administration of glibenclamide to one single subject indicates that DIRA was elevated before the appearance of the drug in peripheral blood.

The correlation between increasing IRI levels and the increasing drug concentration indicates that insulin release depends on the presence of the drug in the blood circulation. However, IRI has been reported to reach its maximal level in man as late as 30 min after intravenous injection of glibenclamide in spite of the very high levels of the drug immediately after injection [1, 14, 16]. This indicates that the drug is not likely to be the sole signal for inducing insulin release from the pancreas. The delay in insulin secretion in response to intravenous glibenclamide, together with the enhancement of DIRA in man and rat [11] before the elevation of IRI, suggests that the drug might exert its action at the pancreas level only after sensitization of the B-cell by a gut factor. Studies in the rat [11] have suggested that the major effect of DIRA is to sensitize the pancreas to the direct insulinotrophic action of glibenclamide. That could explain the parallelism observed between the rises in IRI and glibenclamide concentrations in the plasma as well as the effect of a rather low plasma concentration of glibenclamide on IRI secretion.

It should be further noted that the addition of a relatively large amount of glibenclamide to a duodenal mucosa extract of an untreated subject did not ensure a significant elevation of portal IRI in the receptor rat. This proves that the observed rise in DIRA did not result from the presence of glibenclamide itself in the injected extract.

Glibenclamide has been recently reported to increase GIP levels in combination with food components [4]. Further work is being carried out in order to determine whether or not the duodenal insulin-releasing activity reported here shares identity with established gastrointestinal polypeptides such as GIP or GLI.

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