Influence of the oral cavity on insulin release in the rat

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Blood glucose and insulin levels were measured in undisturbed and free-moving rats. The insulin level rose in the 1st min after the start of food intake; the glucose level began to release in the rat. Am. J. Physiol. 230(5): 1411-1415. 1976.

Blood glucose and insulin levels were measured in undisturbed and free-moving rats. The insulin level rose in the 1st min after the start of food intake; the glucose level began to increase only in the 3rd min if a fluid carbohydrate-rich food was eaten. The insulin release followed a biphasic pattern. If the same quantity of food ingested orally was injected into the stomach in the same time as the animals needed to complete oral ingestion, delayed insulin release could be seen and the second phase of insulin release was exaggerated. The glucose level, which started to rise in the 3rd min, increased much more than during oral ingestion. With respect to insulin release the same phenomena could be observed if carbohydrate-free fluid food was used instead of carbohydrate-rich fluid food. It is argued that the oral cavity plays a major role in the first phase of insulin release, which in its turn seems to be important in the homeostasis of the blood glucose and insulin levels.

IN PREVIOUS WORK (14) it was shown that within 1 min after the start of food ingestion in rats there is a considerable increase of blood insulin concentration and that this precedes the rise of blood glucose. The resulting high levels of blood insulin and glucose are maintained for some time. The immediate increase of insulin can also be observed when the animals eat carbohydrate-free food, in which case the glucose level does not rise. In view of these findings it is tempting to assume that the oral cavity plays an important role in this early insulin response.

To investigate the role of the oral cavity, the following experiments were performed. Rats were provided with stomach and heart catheters and accustomed to eating a fluid diet. The changes in blood insulin and glucose were followed while the animals were eating this diet. Two days later the same experiment was performed; however, the same quantity of food ingested orally 2 days before was now injected into the stomach in the same time as the animals needed to complete oral ingestion. These two experiments were done at the same time of day. Both carbohydrate-rich and carbohydrate-free food were used in the experiments.

Methods

Subjects and Maintenance

Male Wistar rats weighing 300–350 g were maintained in individual Plexiglas chambers (25 x 25 x 30 cm) at a room temperature of 20°C and allowed food and water ad libitum. A standard diet, providing 20% protein, 53.5% carbohydrate, 4.5% fat, and 22% water, with added minerals and vitamins, was presented in the form of a bar that could slide easily through a dispensing tube attached to one of the walls of the cage. The bar could be removed from the dispenser and weighed without disturbing the animal. Practically no food was spilled. Weight and food intake of the animals were measured every day. Lights were on from 6 A.M. until 6 P.M.

Implantation of Stomach Cannulas

The animals were anesthetized with ether and provided with a stomach catheter, as originally described by Epstein and Teitelbaum (2). Some modifications were made. Instead of a polyethylene tube, a silicon tube (length 16.9 cm, ID 0.3 mm, OD 0.63 mm) was inserted through the nostril, nasal cavity, and esophagus by a technique proposed by Strubbe (personal communication). The length over which the tube was inserted was 13.6 cm, i.e., the distance between the tip of the nose and the body of the stomach. The remaining 3.3 cm was pulled under the skin from the tip of the nose and emerged from an incision on the top of the skull, where it was attached to a bent needle (23 gauge). The needle was fixed to the skull with three screws and methacrylic two-component glue. Finally, the protruding end of the needle was closed with a polyethylene cap. The use of a silicon tube provided an important advantage because there was virtually no drop either in body weight or in food intake. Most animals returned to preoperative weight 10 days after insertion of the cannula. This point may be important because considerable drops in body weight, as occur after implantation of a polyethylene tube, affect the release of insulin (15 and unpublished observations).

Implantation of Heart Cannulas

Nineteen days after implantation of the stomach cannulas all animals in which this implantation was satisfactory [i.e., the animals had regained preoperative weight (Fig. 1)] were provided with a heart catheter by a technique already described (11). This was attached to a second needle on the top of the skull. Blood sampling started 5 days after implantation of the heart catheter.

Experimental Procedure

All experiments were performed in the daytime (i.e., between 11 A.M. and 1 P.M.). Starting on the 3rd day
after stomach catheterization, the normal chow was removed every day at 9 A.M. At 11 A.M. a cup containing a mixture of 3 g Vivonex R (Eaton Laboratories, containing 85% carbohydrate, 7.5% protein, 5.5% fat) supplemented with water to 6 ml was put in the cage. The mixture was centrifuged before use for 10 min at 3,000 rpm to remove all larger solid particles (in order to avoid blocking of cannulas when the mixture was infused). The normal chow was returned at 12:00 h. This procedure was repeated every day throughout the whole series of experiments except when otherwise stated. After 10 days all animals ate the Vivonex mixture immediately after the cup was placed in the cage, without leaving any food. Five days after implantation of the heart cannula the first experiment was started.

Experiment 1. In this experiment water (at a temperature of 40°C) was injected into the stomach through the stomach cannula during 8 min, i.e., the average time needed to eat the mixture was recorded. Use of the procedure with the air bubble ensured that the Vivonex mixture first reached the stomach precisely at time zero. At the end of the observation the stomach cannula was flushed with water. All fluids injected into the stomach were heated to 40°C just prior to infusion.

Experiments 5 and 6. Experiments 2 and 3 were repeated with carbohydrate-free food, which consisted of a homogeneous emulsion of lecithin 1 g, casein 6 g, vegetable oil 10 g, and 50 ml water. The caloric density of this emulsion was nearly the same as that of the Vivonex mixture.

As stated previously, the blood sampling procedure is virtually free of stress. The same is true of the stomach infusions, as indicated both by the absence of signs of agitation in the animal and the stability of the glucose and insulin levels (see below) during infusion of water.

Chemical Determinations

The blood samples were immediately chilled at 4°C. Glucose was measured by the ferricyanide method of Hoffman (8) in a Technicon AutoAnalyzer on 0.05-ml blood samples that were taken from the 0.25-ml sample immediately after withdrawal. The remaining 0.2 ml of blood was centrifuged at 4°C and the plasma samples were stored at −30°C. Plasma insulin was determined according to the Hales-Randle method (7), with rat insulin as the standard. The assays were performed with a radioimmunoassay kit (Radiochemical Centre, Amersham). Duplicate assays were performed on 25-μl samples of plasma from experimental animals. To each of the standards were added 25 μl of plasma from alloxan-diabetic rats deprived of food for 36 h.

Anatomy

After termination of the experiments, the animals were killed and the placing of the tip of the stomach cannula was verified. In all animals in the experiments the tip ended exactly in the body of the stomach.

Statistical Methods

Wilcoxon's test was applied to determine significance of differences in blood glucose and insulin levels. Where P < 0.05 it was assumed that the difference was significant.
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RESULTS

Experiment 1: Water Infusion (7 Rats, 1 Infusion Each)

At the beginning of the infusion the rats usually were sleeping. During the infusion they often went on doing so and never showed any sign of agitation. The infusion of water did not cause a change in either the glucose or the insulin level (Fig. 2A). After termination of the infusion there was a suggestion of a slow decline in the insulin level, which may be attributed to the absence of food during the experiment (13).

Experiment 2: Control Meals (6 Rats, 1 Meal Each)

A spontaneous meal of normal chow (mean size 1.8 ± 0.2 g) resulted in an immediate increase of blood insulin whereas blood glucose rise showed a delay of 3 min (Fig. 2B). These effects were identical with those seen in animals without a stomach cannula (14).

Experiment 3: Vivonex Mixture Eaten (7 Rats, 1 Meal Each)

All animals finished eating 6 ml of the Vivonex mixture within 8 min after being offered the food cup and the start of ingestion. The glucose level began to rise in the 3rd min after the start of eating. It reached a plateau of about 130 mg/100 ml blood about 5 min after the start of eating. After 14 min it began to decline again (Fig. 3A). The insulin level rose within the 1st min to 51 ± 2.4 µU/ml plasma and reached a value of 80 ± 8.2 µU/ml plasma after 3 min. It stabilized at about this value for 15 min; it then showed a second increase to 110 ± 10.6 µU/ml plasma, where it stayed for the remaining 30 min of the experiment.

Experiment 4: Vivonex-Mixture Infusion (7 Rats, 1 Infusion Each)

All animals were asleep just prior to the start of infusion. After the start of infusion they woke up and...
began walking around in the cage during the whole infusion period, mostly nibbling on wood shavings. However, they did not show any sign of stress, as indicated either by piloerection or agitated moving like shoveling wood shavings or running around in an agitated way. The glucose level rose significantly above preinfusion levels for the first time 3 min after the beginning of the infusion and the rate of increase was the same as in the previous experiment until the 5th min. It then continued to increase much more, up to 150 mg/100 ml blood, 9 min after the start of infusion (Fig. 3B). The differences between experiments 2 and 3 in glucose levels 7, 9, and 14 min after the start of infusion were highly significant (P < 0.01 in all cases). Contrary to the previous experiment, there was no increase in insulin level at all in the 1st min after the start of infusion and it began to rise only in the 3rd min at the same time the glucose level was increasing too. However, it was significantly lower than in the 3rd min in the previous experiment (44 ± 7.7 μU/ml vs. 80 ± 8.2 μU/ml; P < 0.01). In the following minutes it increased extremely compared with the values reached in the previous experiment at 7, 9, and 14 min (P < 0.01 for all points). It remained significantly higher than in the previous experiment during the whole experimental period.

Experiment 5: Carbohydrate-Free Emulsion Eaten (6 Rats, 1 Meal Each)

All animals finished eating 6 ml of this emulsion within 8 min after being offered the food cup and the start of ingestion. The glucose level did not increase at all, but even started to decline 14 min after the beginning of the meal (Fig. 4A).

In this case too a significant increase in the insulin level could be observed in the 1st min from 12 ± 2 μU/ml plasma to 36 ± 2.0 μU/ml plasma (P < 0.01). The insulin level declined in the 5th min; thereafter it increased slowly to 28 ± 7.3 μU/ml and then declined again.

Experiment 6: Carbohydrate-Free Emulsion Infused (6 Rats)

As in experiment 4, all animals were asleep just prior to the start of infusion. After the start of infusion they woke up and began walking around in the cage during the whole infusion period, mostly nibbling on wood shavings. However, they did not show any sign of stress, as indicated either by piloerection or agitated moving like shoveling wood shavings or running around in an agitated way. The shape of the glucose curve was the same as in experiment 5 (Fig. 4B). The insulin level started to increase only in the 3rd min. In the following minutes the increase was much higher than that observed in the previous experiment at 7, 9, and 14 min (P < 0.01 in all cases).

DISCUSSION

In regard to the influence of oral ingestion of food on the blood levels of glucose and insulin, the results of the present experiments agree with those of earlier work (14). At first sight it might seem that there is a discrepancy in that the insulin level remains elevated for a much longer time after a Vivonex meal (Fig. 3A) than after a meal of normal chow (Fig. 2B), but this can be attributed to the larger size (3.0 g vs. 1.8 g of dry substance) and the higher concentration of carbohydrates of the Vivonex meal.

In particular, the earlier work has also shown that in the dog (3) and in the rat (14) the increase of the blood insulin concentration, which sets in while the meal is going on and declines gradually some time after its completion, proceeds in two phases. The early insulin response (EIR) occurs before there is any rise in the blood glucose level. Indeed, the EIR is also seen when the meal contains no digestible carbohydrates or even no nutrients at all (14). In the present study, too, the EIR always accompanies an oral meal, irrespective of the nature of the diet (solid or fluid, with or without carbohydrates).

The main new finding is that no EIR is ever seen if, under otherwise comparable conditions, the food is administered by stomach cannula instead of ingested orally. It is concluded that stimulation of the oropharyngeal area by food is a contributing factor in eliciting EIR in vivo. Presumably the pathway connecting the mouth cavity with the β-cells of the pancreas consists of 1) (gustatory) receptors and their afferent fibers, 2) a relay station in the brainstem, and 3) an efferent channel involving the vagal nerve, which may act directly on the islets (5) or through the intermediary of vagally re-
leased intestinal hormones (1). Our result to date (unpublished observations) indicate that the brainstem link is located in the ventromedial hypothalamus.

The EIR is followed by a second, more prolonged phase of insulin release. This can be plausibly ascribed to the action on the islets of absorbed nutrients, primarily glucose but—especially with carbohydrate-free food—also amino acids (4). In the case of orally taken meals the insulin release follows a biphasic pattern, just as described previously (14). However, in the case of intragastric feeding, only the second phase occurs and the first phase is completely absent.

It is pertinent to compare the present findings with the results of Grodsky (6). This author, working with the isolated perfused pancreas, found a biphasic insulin release when the glucose concentration of the perfusion fluid was raised according to a step function. He concluded that the β-cell has two compartments, one from which large amounts of insulin can be released on short notice but is soon depleted and another providing a slower but steadier release. When the glucose concentration was raised slowly according to a ramp function, Grodsky obtained a slow monophasic insulin release. It therefore is not surprising that a monophasic insulin response was seen in the present experiments with intragastric feeding, for here the blood concentration of glucose (and presumably that of other nutrients as well) went up according to a ramp function, with a slope comparable to that used by Grodsky. One may further ask whether the EIR is due to release of insulin from Grodsky’s first compartment. The only argument for this is the time course of the EIR. This can best be judged in the case where no second wave of insulin release overlaps the effects of the EIR in the blood concentration of insulin. Taking into account data on the removal of insulin from the blood in the rat that indicate a half-life of about 2 min (Strubbe, personal communication), one can derive from these data that the time course of insulin release during the EIR bears a marked resemblance to that of the release of Grodsky’s first compartment. If, on this basis, one accepts provisionally that the EIR is due to insulin release from this compartment, it follows that release from the first compartment can be elicited not only by a steplike increase of blood glucose as used in Grodsky’s in vitro experiment, but also, under in vivo conditions, by a rapid reflex response to (sensory) effects of food intake. It should be noted in this context that no steplike increases in blood glucose occur in vivo during the first 2 min of a meal in the rat (12).

Finally, the present findings contain some suggestion as to the possible function of the EIR. Comparison of Fig. 3A and 3B shows that oral ingestion causes far less violent deviation of blood glucose from the normal level than does intragastric infusion of the same quantity of the same food at the same rate. The control experiment in which water was infused shows that the strong hyperglycemia resulting from intragastric food cannot be due to the fact that the infusion washes nutrients from the stomach into the gut. If we accept that the supply of absorbable nutrients is equal for oral and intragastric feeding, we may conclude that it is the EIR that keeps blood glucose within reasonable bounds in the case of the oral meal. Since the rate of insulin release is an exponential function of the glucose level in the range of 100–180 mg/100 ml (9), potentially dangerous overstimulation of the β-cell may well occur in the case of intragastric infusion. In this light, the EIR accompanying normal meals may serve as a protection against overloading of the β-cells (which otherwise might create a risk of diabetes).

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