Preabsorptive insulin release and hypoglycemia in rats

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Preabsorptive insulin release and hypoglycemia in rats. Am. J. Physiol. 230(1): 56-60. 1976.—Peripheral blood glucose and immunologically reactive insulin levels were determined in freely moving normal rats which were submitted either to a free oral glucose load or to a gastric administration of the glucose load. Identical determinations were performed in ventromedial hypothalamic nucleus- (VMH) lesioned and vagotomized rats after the same oral intake. It was demonstrated that: 1) a free oral glucose intake was immediately followed by two peaks of insulin release and a resultant decrease in blood glucose; 2) a gastric glucose load resulted in a single peak of insulin release and the concomitant decline in blood glucose; 3) the recorded blood glucose level was the resultant of the insulin-induced hypoglycemia and the postabsorptive hyperglycemia; and 4) the responses were largely exaggerated in VMH-lesioned rats and abolished by vagotomy. It is concluded that the early prandial insulin release reflexly induced by food-related stimuli temporarily enhances the metabolic conditions which provoke feeding.

Blood glucose level; gastrointestinal hormones; vagus nerve; reflex

It is well known that the amount of insulin released is higher after an oral compared to an intravenous glucose load. Such differences were repeatedly investigated in man (4, 10, 22, 29, 32, 34) and confirmed in rat (3) and rabbit (26). They were explained either by the activity of gastrointestinal hormones on insulin release or by a neurally controlled insulin release elicited by food-related stimuli.

Important literature is available about the early changes in blood glucose level after an oral and particularly a sweet-tasting fluid intake. The discrepancy in the reported results is likely due to differences in the experimental procedures and to a lack of sensitivity and specificity in the methods of blood glucose determination.

Furthermore, it has been reported that when normal rats (38) or dogs (12, 13, 16) eat a meal, there is a biphasic insulin response: an initial response (5–10 min after the onset of the meal) and a later more prolonged one. When dogs were sham fed, only the initial response occurred, but when the meal was introduced into the stomach only the later response was exhibited. These findings suggest that the initial response is neurogenic and initiated by meal-related stimuli.

The experiments to be reported here were an attempt to compare and clarify these different kinds of experimental observations. The preabsorptive and part of the postabsorptive plasma insulin and blood glucose levels were followed continuously by means of improved methods of insulin assay and blood glucose determinations. These records were performed and compared after various oral or gastric intakes in normal, vagotomized, or ventromedial hypothalamic nucleus- (VMH) lesioned rats.

Methods

Female Wistar rats weighing 200–250 g, individually caged, had free access to food and water. Light was on from 6 A.M. to 6 P.M. Rats were randomly assigned to normal oral, normal gastric, VMH-lesioned, or vagotomized groups. All of them were provided with a cardiac cannula chronically implanted according to Steffens’s method (37).

At the time of the heart catheterization, a chronic intragastric cannula was implanted in those rats which had to be submitted further to intragastric administration of solutions. The method employed was a modification of Kohn’s technique (18, 25). Rats were allowed 1 wk for postoperative recovery.

VMH electrolytic lesions were produced through a 28-gauge stainless steel needle insulated except for 0.5 mm at the tip by a 2-mA anodal current passing for 15 s. The stereotaxic coordinates, according to the De Groot atlas, were A: 5.8, L: 0.6, D: −3.2. VMH-lesioned rats were tested only when the hyperphagia was well established.

Bilateral subdiaphragmatic vagotomies were performed according to the method of Snowdon and Epstein (36). At the conclusion of the experiment, the completeness of vagotomy was determined by the procedure of Legros and Griffith (21). Rats with incomplete vagotomy were discarded from the results. Vagotomized rats lost weight during some 10 days; then their body weight increased rapidly for about 5 days (31). Further, they continued to gain weight slowly. The experiments began when the rate of weight gain was stabilized.

Blood glucose determinations were performed by a glucose-oxidase method, using the Technicon Auto-Analyzer. In order to carry out a continuous recording of the blood glucose level, blood as well as reagents were pumped via a Tygon tubing by a proportioning pump. Ten minutes before the experiment, the rat was slightly heparinized. At the time of the experiment, a tube with a pumping capacity of 0.03 ml/min was fitted on the headpiece of the cardiac catheter of the rat freely moving in its home cage. The sample tube was firmly attached to a light lever which rotated freely and was...
counterbalanced with thin elastic thread. Ten minutes after the beginning of the pumping, the rat was submitted either to the free oral intake or to a gastric administration of a sweet solution. These continuous recordings of blood glucose level lasted up to 30 min.

Blood samples assigned to insulin determinations were not taken during the same experimental test. For the sampling, blood was pumped by a tube with a pumping capacity of 0.1 ml/min. Blood was collected in small cups of a rotating device which moved every minute. Blood was withdrawn continuously during 10 min (sometimes 12 min), 1 min before and 9 min after the beginning of the intake. The 10 samples so obtained were centrifuged at 7°C, and the plasma was stored at -24°C. Duplicate assays were performed on 25 μl of the plasma resulting from each 1-min sample, using a radioimmunoassay kit (Radiochemical Centre, Amersham, England), with a rat insulin standard.

The general procedure was constant over groups. At 6 A.M. corresponding to light onset, food was removed, and the test was carried out 5 h later at 11 A.M. Rats were offered the sweet-tasting solution; the free oral intake was performed within 1 min. The intragastric administration also lasted 1 min; in this case, the solutions were warmed up to 30°C in order to prevent pyloric disturbances.

RESULTS

Experiment 1. Fourteen normal rats were offered a free oral intake of 1 ml of a 50% glucose solution. They always drank the entire 1 ml which was offered drop after drop in 1 min.

Eight other rats were submitted to an intragastric administration of the same amount of the same solution.

The mean time courses of the concomitant plasma insulin (one sample every 1 min) and blood glucose levels (continuous determination) are represented in Fig. 1 for the oral intake and in Fig. 2 for the intragastric one. The first insulin point is the mean basal level. The second insulin point is the mean value during the minute of glucose intake. The third simultaneously represents the mean of the first peak insulin level (see Fig. 3) and the mean time of its occurrence (± SEM). The next point represents the first minimum and so on. The mean time course of the blood glucose is the mean of the individual curves. The width of the shaded area corresponds to the standard error to the mean of five horizontally equidistant points of the mean curve. The calculated values reported on the figure became significantly different from the 3rd min after the beginning of the oral intake. Figure 3 is an example of a single rat which was given an oral glucose intake as previously described.

At 11 A.M. the plasma immunologically reactive insulin (IRI) level of a normal rat food deprived since 6 A.M. was 15.15 ± 1.6 μU/ml. It was already increased during the oral intake and reached a first peak of about 53 ± 6 μU/ml in 2.5 min (148 ± 16 s) after the start of the intake. A second peak of 48.3 ± 3.4 μU/ml could be seen on about the 5th min (286 ± 16 s). Insulinemia decreased...
immediately before a new long-lasting increase which, as will be discussed further, resulted from the postabsorptive hyperglycemia.

After the intragastric administration of the same glucose solution, a single peak of \(56.6 \pm 4.7 \mu U\) was exhibited; it occurred about 3.5 min (216 ± 12 s) after the beginning of the gastric load. The extremely short half-life (2 min) of endogenous insulin in the rats accounts for the time course of the decreases following the peaks (Strubbe, personal communication).

The blood glucose level decreased as early as on the minute of intake; the lowest value corresponding to a 6.5 ± 0.46% decrease was observed on about the 3rd min (171 ± 6 s) after the start of the intake. Then the blood glucose level increased, reached the basal value on the 5th min (281 ± 5 s), and again increased. As is now well established (36), the intestinal absorption of a glucose solution begins as early as on the 3rd min after the start of the intake. So at every moment the recorded blood glucose level was likely the result of both the decrease due to the insulin secretion and the postabsorptive influx of glucose into the blood stream. In the case of the intragastric administration, the insulin-induced hypoglycemia was greatly masked by the absorption. A 3% decrease was obtained.

**Experiment 2.** The effects on the blood glucose level of the free oral intake of either 1 ml of the 50% glucose solution, either 2 ml of a 25% glucose solution (given within 1 min also), or 1 ml of 0.125% saccharin solution were compared.

Results are presented in Fig. 4. Whereas an oral intake of 1 ml of a 50% glucose solution resulted in a 6.5% decrease (6.5 ± 0.5), an oral intake of a more diluted glucose solution resulted in a 12% decrease (11.6 ± 1.1) of the blood glucose level, lasting about 7 min (426 ± 36 s). An oral intake of a saccharin solution brought about an 8% decrease (8.1 ± 1) lasting some 6.5 min (405 ± 9). An important point was that there is no further increase. The return to the basal level is likely due to the physiological riposte to hypoglycemia. The larger decrease obtained by the 25% glucose solution suggests that the induced hypoglycemia was counteracted by the rate of glucose absorption, slow in relation to its dilution.

**Experiment 3.** Seven hyperphagic, VMH-lesioned rats participated in this experiment. Five of them only were submitted to a 12 min plasma insulin determination.

Results illustrated in Fig. 5 were calculated as previously described. The plasma IRI level of 5-h food-deprived VMH-lesioned rats was 76.4 ± 11.7 \(\mu U/ml\). The two early peaks of IRI level already seen in normal rats were greatly increased up to 135.2 ± 18 and 151.4 ± 17 \(\mu U/ml\). The concomitant early decrease of blood glucose level reached 14%. The further insulin secretion likely stimulated by glucose absorption was also very high.

**Experiment 4.** Twelve rats were submitted to a bilateral subdiaphragmatic vagotomy.

The results of five of them did not differ significantly from the results of normal rats. Results in the other seven rats were completely different (Fig. 6). The
plasma insulin pattern was flat until the 8th min; the value of this 8th min point was the first to be significantly different from the others. It seemed likely that this late increase represented the insulin response to absorption. The blood glucose curve showed a steady increase beginning on about 2.5 min \((143 \pm 6 \text{ s})\) after the start of the intake. The mean preabsorptive level of these 5-h-fasted vagotomized rats was \(96.4 \pm 2 \text{ ng/ml}\); it reached \(125 \text{ mg/ml}\) on about the 7th min \((437 \pm 18 \text{ s})\). The denervation at the stomach level was tested as complete in the 12 rats. It was suggested by the differences in the results obtained that in the above five rats the small pancreatic vagal branch was left undamaged.

**DISCUSSION**

This work presents a detailed study of the time course of the early metabolic consequences of an oral intake. Such an intake is immediately followed by two initial peaks of insulin release and by a concomitant fall of the peripheral blood glucose level. The results of a number of similar studies on the blood glucose level are equivocal. A decrease has been reported by different authors \((1, 8, 9, 10, 17, 19, 40)\). Others reported an increase \((24, 33, 34, 39)\) or no change \((6, 11, 13, 16)\). The differences between paradigms and methods of determination make the comparison difficult. The continuous recordings of the peripheral blood glucose level permitted us to show that an early decrease occurs within the 1st min of the intake and is the obvious effect of insulin secretion. The pure phenomenon is seen in the absence of a caloric challenge after saccharine ingestion. Otherwise, the glucose influx due to absorption lessens and shortens the hypoglycemic effect of the insulin release.

In respect to the preabsorptive time course of plasma insulin level, the results reported here clearly confirmed the findings of Steffens in the rat \((38)\), those of Hommel \((16)\) and Fisher et al. in the dog \((12, 13)\), and several in man \((15, 27, 28)\). *Experiments 1 and 2* emphasize that the two early phases of insulin secretion are exhibited when blood glucose is low. So the peripheral blood glucose level can be excluded as a factor responsible for these initial IRI increases. Moreover, *experiment 3* shows that complete vagotomy abolishes the phenomenon. It is therefore possible to conclude that a reflex of insulin secretion occurs within the first minutes of an intake and is effective as early as on the 1st min.

In addition, from *experiments 1 and 2*, it may be concluded that the first peak originates from stimulation of oropharyngeal receptors, taste buds, or mechanical receptors involved in chewing or swallowing. The second response is initiated at the gastric or duodenal level, and intestinal receptors recently described by Mei et al. \((23)\) might be responsible. The meal-related stimuli possibly involved in the neurally induced insulin release have been particularly investigated in man. Smell, taste, chewing, sight, or even suggestion of a desirable meal under hypnagogic have been found effective. There are several reports that insulin secretion can become associated with arbitrary stimuli as an effect of a conditioning procedure \((37, 41)\). Recently in such a conditioning we used an olfactory stimulus as the only conditioned stimulus (unpublished data). Our results support Deutsch's hypothesis \((9)\) that stimuli associated with feeding, i.e., sight, smell, taste of food, result in hypoglycemia (reflect of insulin secretion) as a consequence of the animal's natural history. As a matter of fact, long-term access to saccharine was found to extinguish the hypoglycemic response. Nevertheless, that such a phenomenon be programmed in the animal's genetic inheritance is not excluded.

Regarding the efferent pathway of this reflexly induced insulin release, two possibilities may be considered. It has been reported that insulin can be released within a very short time after a vagal stimulation \((14, 30)\), and *experiment 3* showed that vagotomy prevents even the first peak to occur. Furthermore, it is well known that gastrointestinal hormones enhance insulin release, and secretin, the concentration of which increases rapidly after an oral intake of glucose \((7)\), is a plausible stimulus for the early responses. One of these two possibilities is not exclusive of the other, and gastrointestinal hormones may be a link between vagus and pancreas.

Booth and Miller \((5)\) reported that an oscillation in blood glucose level was induced in the mildly hungry rat by the occurrence of a signal normally associated with a brief opportunity to feed. They pointed out the role of the lateral hypothalamic area (LHA) in these conditioned blood glucose changes. Besides, electrical stimulation of the LHA has been reported to cause an increase of plasma insulin \((20, 38)\).

The *experiment 4* reported here showed that in VMH-lesioned rats with well-established hyperphagia, not only is the basal plasma insulin higher than in normal rats, but also the early reflexly induced insulin release is largely exaggerated. In our previous paper, we hypothesized that the uninhibited ventrolateral function might lead to a neurogenic hyperresponse of insulin secretion via perhaps the bundle of Schult (2) and the vagus nerve. Moreover, there are some indications \((D. N. Stephens, manuscript in preparation)\) that the central pathway of the insulin secretory reflex includes other brain sites, particularly posterior hypothalamus. The results of *experiment 4* explain some features of the hyperinsulinemic syndrome of VMH-lesioned rats. The first postlesion oral intake of such a rat results in an important insulin release and a high rate of glucose utilization. As a consequence, the subsequent meal is precipitated; this positive feedback accounts for the hyperphagia and the metabolic disturbances.

The question arises as to the physiological involvement of this reflex. From a behavioral point of view, a relation between this insulin secretion reflex and the feeding pattern of normal, hyperphagic, and vagotomized rats may be assumed. From a metabolic point of view, this reflex and its effect on the total prandial insulin release provides a good explanation of the evolution of the postabsorptive hyperglycemia respectively observed in normal, VMH-lesioned, and vagotomized rats. This will be the subject of a further study.

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**REFERENCES**

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