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Rapid insulin release after ingestion of a meal in the unanesthetized rat

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STRUBBE, J. H., AND A. B. STEFFENS. *Rapid insulin release after ingestion of a meal in the unanesthetized rat.* Am. J. Physiol. 229(4): 1019–1022. 1975.—Blood glucose and insulin levels were measured in undisturbed and free-moving rats. The insulin level rises already in the 1st min after the start of food ingestion, whereas the glucose level begins to increase only in the 3rd min if carbohydrate-rich food is eaten. This early rise in insulin level is observed also under conditions in which either carbohydrate-free food or even "food" without any caloric value is offered. The smell of food cannot produce this early insulin response. It is concluded that in the rat other factors besides a rise in nutrient content in the blood produce insulin release in the first minutes after food ingestion.

insulin secretion; food intake; autonomic nervous system; gastrointestinal hormones

ALTHOUGH MUCH IS KNOWN about the secretion of insulin, especially in the rat, precise data are absent on the mode of action of the most common and important stimulus for insulin secretion, ingestion of a meal.

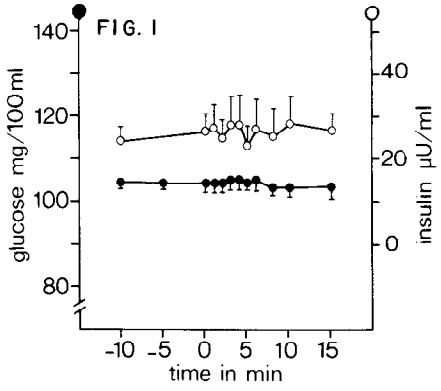
Fischer et al. (5) showed a reflex insulin secretion in conscious dogs after oral intake of glucose. The insulin level was enhanced before the arterial glucose level began to rise. In the present paper, phenomena of this kind will henceforth be termed "early insulin response" (EIR). Hommel et al. (10) showed that in dogs the mouth was the location of the receptors for the EIR. Others reported that with oral glucose tolerance tests more insulin is mobilized than after intravenous administration of glucose (4, 16, 17). From such findings it appears that besides several nutrients like glucose (13) or amino acids (6, 11), other fast and directly acting factors may mediate the effect of a meal on insulin release. The nature of the additional factor is not known exactly. Two possibilities have been considered: *1*) stimulation of the vagal innervation of the pancreas which can enhance insulin secretion (7), and *2*) a stimulating action of enterohormones has been reported (3, 22). So far no reports are available that in rats an EIR exists after the start of a meal. Therefore, experiments were made to investigate this point. It will be shown that in conscious stress-free rats ingestion of a meal results in a rapid release of insulin both in the ad libitum situation, i.e., the situation in which the food has been removed 2 h before the start of the experiment, and in the deprived situation, i.e., 24 h after the removal of food. This effect is not mediated by a change in glucose concentration of the blood. Some attempts were also made to investigate the nature of the stimulus that is responsible for the EIR.

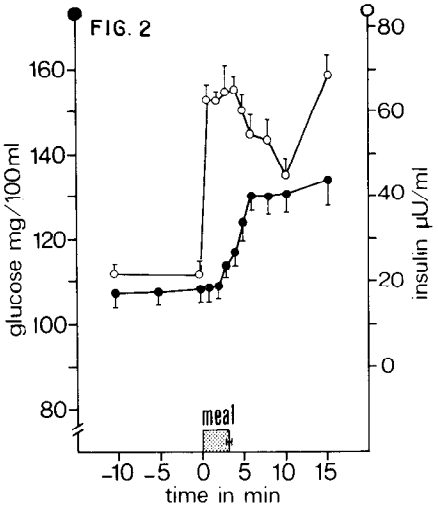
METHODS

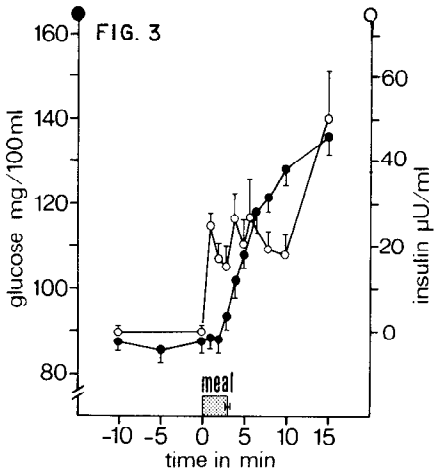
Male wistar rats weighing 400 g were maintained in individual Plexiglas chambers (25 x 25 x 30 cm) at a room temperature of 20°C and were allowed food and water ad libitum. Lights were on from 6 A.M. until 6 P.M. The food was presented in the same dispensers as previously described (18). A standard diet ("C-rich diet") containing 20% protein, 53.5% carbohydrate, 4.5% fat, and 22% water with added minerals and vitamins was available ad libitum. In some experiments a diet without digestible carbohydrates ("C-free diet") containing 20% protein, 43% fat, 37% cellulose with added minerals, and vitamins was used. The powdered C-free food was mixed with 50% water. There is evidence that cellulose cannot furnish nutrients to the rats (1).

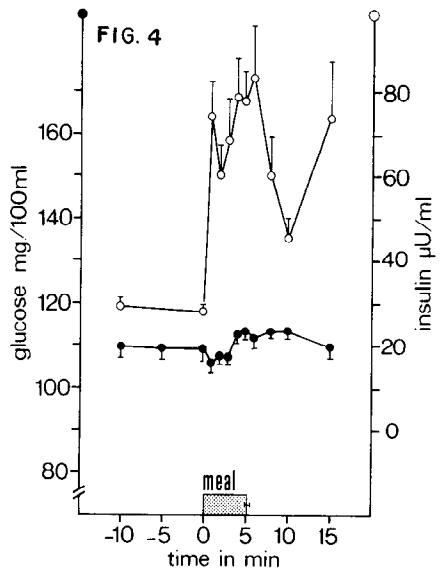
Blood glucose was measured by the ferricyanide method of Hoffman in a Technicon AutoAnalyzer on samples of 50 μl blood. Plasma insulin was determined according to the Hales-Randle method (9) using a rat insulin standard and an immunoassay kit (Radiochemical Centre, Amersham, England). Duplicate assays were performed on 25-μl samples of plasma in the way described earlier (21). An investigation of the present type requires that techniques do not disturb the animals. The blood samples were therefore taken via a heart catheter (20). Stress due to blood loss with repeated sampling was avoided by transfusing blood taken from a donor rat by heart puncture and warmed to 39°C. Rats were only used when neither the sampling procedure nor the presence of an experimenter disturbed their behavior.

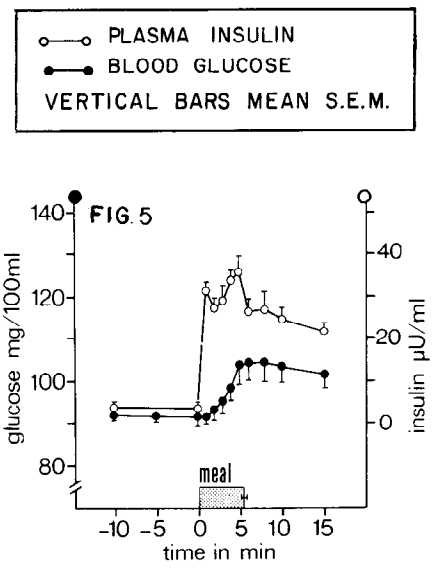
Either 2 or 24 h before the start of each observation, the food was removed; when it was returned, the rat always began to eat immediately. Blood samples were taken at −10, −5 (only a glucose sample), 0 (start of the meal), and after the meal onset at 1, 2, 3, 4, 5, 6, 8, 10, and 15 min in *experiments 1–5* and at 1, 2, 3, 4, and 5 min in *experiments 6* and *7*, and at 1 and 2 min in *experiments 8* and *9*. All observations were performed in the daytime about 4 h after light on. The Student t test was applied to determine significance of differences in blood glucose and insulin levels. It was assumed that the difference was significant if $P < 0.05$.

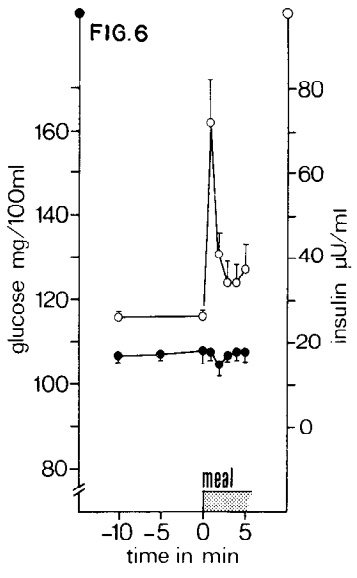


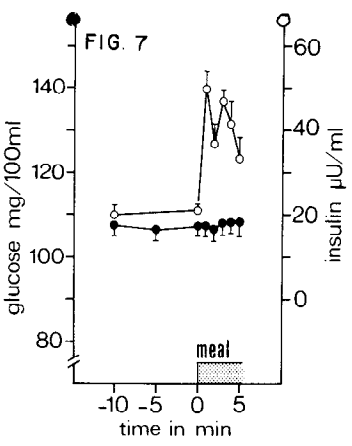


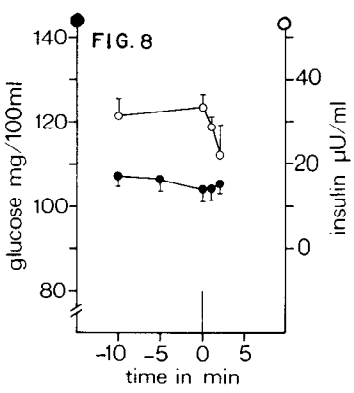












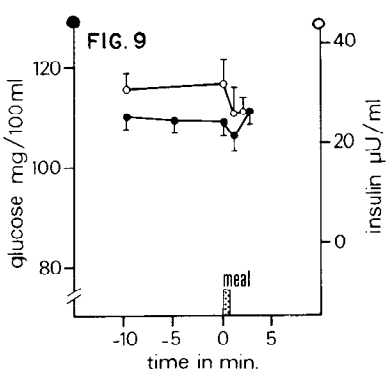


FIG. 1–9, Symbol: o——o, plasma insulins; ●——●, blood glucose.

FIG. 1. Effects of blood sampling on blood glucose and insulin levels without food intake (6 rats). Vertical bars mean SEM.

FIG. 2. Blood glucose and insulin levels during eating carbohydrate-rich food, ad libitum (6 rats, 1 meal each).

FIG. 3. Blood glucose and insulin levels during eating carbohydrate-rich food after 24-h food deprivation (6 rats, 1 meal each).

FIG. 4. Blood glucose and insulin levels during eating carbohydrate-free food, ad libitum (6 rats, 1 meal each).

FIG. 5. Blood glucose and insulin levels during eating carbohydrate-free food after 24-h food deprivation (6 rats, 1 meal each).

FIG. 6. Blood glucose and insulin levels following a change from carbohydrate-rich to carbohydrate-free diet (6 rats, 1 meal each).

FIG. 7. Blood glucose and insulin levels following a change from carbohydrate-rich to dummy food (6 rats, 1 meal each).

FIG. 8. Effects of smell of food on blood glucose and insulin levels.

FIG. 9. Effects of atropinization on blood glucose and insulin levels during an attempt to eat.

RESULTS

Experiment 1. In this experiment the influence of blood sampling on blood glucose and insulin levels was measured. No food was offered in these cases. Six rats (2 h deprived) were used which were accustomed to the C-rich diet ad libitum. Figure 1 shows that sampling alone does not change the insulin or glucose levels.

Experiment 2. The same six animals (2 h deprived), habituated to C-rich food ad libitum, were given 1.7 g of this food during the observation, i.e., an amount equal to the average size of an ad libitum meal of this food. As shown in Fig. 2 the glucose level remains constant until the 2nd min after which a rise sets in. The insulin level shows a sudden rise ($P < 0.001$) in the 1st min after the start of the meal.

Experiment 3. This experiment was similar to *experiment 2*, but the rats were deprived of food for 24 h before the observation. Starting levels of glucose and insulin are significantly lower ($P < 0.001$ and $P < 0.001$, respectively) than in the ad libitum situation. Here again the glucose level rises (Fig. 3) only in the 3rd min, but a significant insulin rise in the 1st min is observed ($P < 0.001$). The amplitude of this EIR, however, is smaller than in *experiment 2* ($P < 0.005$).

Experiment 4. The same six animals were then kept during 2 wk on the C-free diet ad libitum. During the observation the rats (2 h deprived) were given a standard amount of 3 g of this diet (equal to mean ad libitum meal size of this food). The starting levels of glucose and insulin do not differ from those on C-rich food (Fig. 4). However, the effect of the meal on blood glucose is much smaller, and in fact nonsignificant. The insulin level rises as in *experiment 2*.

Experiment 5. This experiment, carried out after *experiment 4* with the same six rats, was similar to *experiment 3*, but C-free food was used. Again the starting levels are the same as on the C-rich food under comparable conditions (Fig. 5). Although there are no digestible carbohydrates in the diet, there is a small increase in blood glucose. Almost the same insulin rise is observed as in *experiment 3*. Comparison of this EIR with that in *experiment 4* shows a significant decrease after 24 h fasting ($P < 0.005$).

Experiment 6. As the animals in *experiments 4* and *5* were habituated during 2 wk to the C-free diet, it could be that they had learned to perform the EIR during that time. For that reason the observations of *experiment 4* were repeated on six naive rats (2 h deprived) at their first contact with the C-free diet. In this experiment the speed of eating is very low as the sudden change to the new diet causes a novel diet reaction (2, 24). The normal meal taking is temporarily replaced by cautious sampling of small quantities of the new food. Yet a rapid insulin rise (Fig. 6) can be observed in the 1st min ($P < 0.001$).

Experiment 7. This experiment was designed to investigate which component of the diet gives the signal for the EIR. The same rats (2 h deprived of the C-rich food) were used as in *experiment 6*. A meal without nutritional value was offered for this purpose. The meal consisted of a mixture ("dummy food") of 56% paraffin oil, 8% Vaseline, and 36% cellulose. The mixture is very greasy and has almost the same consistency as the C-free diet. Here again the same novel diet reactions are observed as in the previous experiment, and the insulin level rises in the 1st min after meal onset ($P < 0.001$). No change in the glucose level could be observed during eating (Fig. 7).

Experiment 8. Whether the smell of food alone can evoke insulin release is investigated in this experiment. The same rats were used as in *experiment 2*. The animals (2 h deprived) could smell the normal C-rich food in the cage, but eating was impossible by preventing the animal from reaching the food by turning the dispenser toward the wall. They made vigorous efforts to reach the food during several minutes. A small nonsignificant decline in the insulin level is observed when the animals are able to smell but not ingest the normal C-rich diet (Fig. 8).

Experiment 9. In this experiment the effect of atropinization on the EIR was investigated. The same rats were used as in *experiment 2*. The animals (2 h deprived) were injected 10 min before the withdrawal of blood with 0.25 mg atropine sulfate. The animals tried to eat immediately when the food was offered, but were unable to swallow the food taken in the mouth and choked on it, which caused unmistakable stress. A small nonsignificant decline in the insulin level is observed while there is no change in glucose level (Fig. 9).

DISCUSSION

It has been shown (18) that [^{14}C]glucose from enteric absorption does not reach the blood until the 3rd min after food ingestion. This is confirmed in *experiments 2* and *3*, in which the glucose level at *time 1* is equal to or lower than the level at *time 0*. Yet the insulin level increases significantly above the base-line level at *time 1* in these experiments, and in *experiments 4* and *5*, in which the diet is C free. So in these experiments the peripheral blood glucose level can be excluded as a factor responsible for the EIR. Because it is unlikely that amino acids derived from proteins in the food are absorbed earlier than glucose, it is improbable that amino acids cause that EIR. If food of either diet is withheld for 24 h, the blood glucose and insulin levels are stabilized at lower levels than in the ad libitum situation. Several authors have shown that after 24 h of deprivation the insulin release is impaired after glucose administration (8, 12) or after a meal (21). It is of great interest, therefore, to investigate whether the EIR is absent or lower after a fast of 24 h than in the ad libitum situation. *Experiments 3* and *4* show that, although there is a significant EIR after 24 h deprivation this is significantly smaller than in the ad libitum situation (cf. *experiments 2* with *3*, and *4* with *5*).

Although there are no digestible carbohydrates in the C-free diet, there is a small increase of the glucose level in the second and following minutes after ingestion of this diet in the deprived condition, though not as high as after the C-rich diet. This glucose originates possibly from glycogenolysis, but the nature of the stimulus for its release is not known. Alternatively, an origin of this glucose from gluconeogenesis cannot be excluded. After a change in diet from C rich to C free (*experiment 6*), ingestion of the C-free meal already produces the EIR. It is concluded that no habituation to the C-free diet is required before the early response can be evoked. Also, digestible nutrients are not required to initiate the early response. *Experiment 8* demon-

strates that the smell of food alone cannot stimulate the EIR; however, the alternative explanation cannot be excluded at present that the strange situation that the animal was able to smell but not to eat might act as a psychological stress and that the resulting increase of sympathetic activity overruled and inhibited the effect of the factors responsible for the EIR (25).

The secretin concentration (which can enhance insulin release) measured radioimmunologically rises rapidly during the first minutes after oral glucose (3), whereas gastrin (23) and glucagon (15) increase only later after good ingestion. It has been reported (7) that insulin can be released within a very short time after vagus stimulation so that this mechanism can play a role in the observed phenomena. Both secretin and the vagus action are therefore the major candidates for the unknown stimulus to the pancreas. They may act separately or both together, and it might even be possible that the vagus action can release secretin (3). More studies about this problem can possibly reveal more precisely the factors involved and the physiological significance of the early insulin release after meal ingestion.

Administration of atropine in order to study the role of the vagus in the EIR suppresses the effects of eating on the EIR completely. However, this experiment is not appropriate to solve this question because the animals are not able to swallow the food after atropinization and choke on it with unmistakable behavioral symptoms of stress. The stress can decrease insulin release by sympathetic activation (14).

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