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Hypothalamic influence on insulin and glucagon release in the rat

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DE JONG, A., J. H. STRUBBE, AND A. B. STEFFENS. *Hypothalamic influence on insulin and glucagon release in the rat*. Am. J. Physiol. 233(5): E380-E388, 1977 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 2(5): E380-E388, 1977. — Blood glucose, plasma insulin, and glucagon levels were measured in undisturbed and free-moving rats. The insulin and glucagon levels rise in the 1st min after the beginning 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 and glucagon level is also observed under conditions in which carbohydrate-free food is eaten. A similar release of insulin and glucagon can be obtained by injection of 0.1 μ g of norepinephrine into the ventromedial hypothalamus, but the same injection made into the lateral hypothalamus causes release of insulin only, whereas injections in other hypothalamic areas are nearly without effect. Similar injections of isoproterenol did not cause changes in insulin, glucagon, and glucose levels. It is suggested that the early insulin and glucagon responses are of reflex origin and that the ventromedial and lateral hypothalamic areas are relay stations in the reflex pathways. The lack of effect of atropine to block the insulin and glucagon responses to noradrenergic stimulation of the ventromedial hypothalamus indicates that the efferent pathway is not cholinergic.

insulin and glucagon secretion; autonomic nervous system; food intake; ventromedial hypothalamus; lateral hypothalamus; chemical stimulation

IN A PREVIOUS PUBLICATION (36) it was shown that as early as 1 min after the beginning of eating, plasma insulin increases considerably in the rat, whereas blood glucose does not begin to rise until the 3rd min (provided that digestible carbohydrates are present in the food). The overall pattern of insulin release during a meal can be analyzed in two phases, a first phase independent of absorbed nutrients (the early insulin response or EIR) and a second, more prolonged phase certainly dependent on increases of blood nutrient levels. The EIR is also seen in experiments in which rats eat carbohydrate-free food or even food without any caloric value (36).

Two questions were left open by these studies. The first question concerns the origin of the EIR. Because the EIR is absent if the food is injected directly into the stomach (33), it follows that the stimulus for the EIR is independent of the absorption of nutrients and that the stimulus is perceived proximal to the stomach. Because electrical stimulation of the hypothalamus, especially of the ventromedial hypothalamus (VMH) and the lateral

hypothalamus (LH), can induce changes of insulin release (11) and because there is evidence that the VMH and LH receive information from taste and smell receptors (6, 20), it is tempting to assume that these areas are involved in the causation of the EIR. We attempted to verify this by selective activation of hypothalamic neural tissue, using injections of norepinephrine to mimic normal activities in the VMH and LH, which receive important noradrenergic terminals (22, 39).

The second question is why, in spite of the EIR, the glucose level does not decline in the case of a carbohydrate-free meal. After 24 h of food deprivation, glucose rises when carbohydrate-free food is eaten (33). One might speculate that this rise is due to the action of glucagon. If there is an early glucagon response (EGR) in addition to an EIR, this response might explain the behavior of blood glucose during a carbohydrate-free meal because glucagon is a potent glycogenolytic agent. If this explanation should turn out to be correct, the origin of the EGR must also be explored.

We therefore ran three experiments. In *experiment I*, the responses of plasma insulin and glucagon to both normal and carbohydrate-free food were examined. It was shown that the EIR and EGR occur simultaneously. In *experiment II*, the influences of intrahypothalamic injections of norepinephrine on insulin and glucagon release were investigated. Strong stimulatory effects were found, and it will be argued that these can be attributed to mechanisms also subserving the EIR and EGR. In *experiment III*, the animals were atropinized before stimulation of the VMH in order to investigate the contribution of parasympathetic efferent pathways to EIR and EGR.

METHODS

Subjects and Maintenance

Male Wistar rats weighing 250 g were maintained in individual Plexiglas chambers (25 × 25 × 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 standard diet ("C-rich diet") contained 20% protein, 53.5% carbohydrate, 4.5% fat, and 22% water with added minerals and vitamins. 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. The caloric value of this mixture was exactly half of that of the

standard diet. There is evidence that cellulose cannot furnish significant amount of nutrients to the rats (1).

Implantation of Brain Cannulas and Testing

Brain cannulas for chemical stimulation were placed in VMH and LH and in two other hypothalamic areas, dorsomedial hypothalamus (DMH) and preoptic area (POA) as controls.

A rat was anesthetized with ether and placed in a stereotaxic apparatus. Stainless steel cannulas (length 17 mm, gauge 23) were implanted bilaterally and closed at the upper end with a polyethylene cap. Coordinates for the tips of the cannulas for the four groups (ten animals each) according to De Groot (9) were: LH group: A, 5.8; L, 1.7; V, -2.4; VMH group: A, 5.9; L, 0.6; V, -2.7; DMH group: A, 3.8; L, 0.6; V, -1.6; POA group: A, 7.6; L, 0.6; V, -1.8. Ten days after recovery the first injections were made. The procedure was as follows: after removal of the polyethylene cap, each cannula was connected to a sterile polyethylene tube (length 40 cm, ID 0.28 mm, OD 0.61 mm) in the following way. A needle (OD 0.3 mm, ID 0.12 mm, length 22 mm) was pushed into one end of the tube so that it protruded exactly 17 mm. The tube was filled with a sterile methylene blue solution in saline except for 2.0 cm near the needle. The 2.0 cm of the tube and the needle contained 1 μ l saline with the test substance. The methylene blue solution and the test solution was separated by a small air bubble (length 1 mm). The other end of the tube was closed by a small pin. The protruding end of the needle was inserted into the brain cannula in such a way that the end was just in contact with the brain tissue. The animals were then replaced in their cages and the far end of the tubes remained outside the cage (35). By pushing the pin farther into the end of the tube until the air bubble came to the tip of the needle, 1 μ l of fluid was injected into the brain.

Fifteen days after cannula implantation, the animals were provisionally tested for correct cannula placement by means of bilateral injection of 20 μ g Nembutal (10 μ g in 1 μ l of saline through each cannula). The criterion for satisfactory implantation in the VMH implanted animals was: beginning of food intake by a previously nonfeeding rat within 2 min after injection; in the LH implanted animals, ending an ongoing meal within 10 s after injection; in the dorsomedial hypothalamic and preoptic area groups, no change of eating behavior after injection. Moreover, by the time of testing the weight of the animals had to be the same as unoperated controls because chronic reduction of body weight diminishes insulin release (33).

Six animals of the VMH group, six of the LH group, five of the DMH group, and five of the POA group passed this test. Their cannula placements were subsequently confirmed by histological analysis. The 22 experimental animals selected in this way received heart catheters.

Implantation of Heart Cannulas

Sixteen days after implantation of the brain cannulas, heart catheters were implanted by a technique al-

ready described (32). This method allows blood sampling in unanesthetized, undisturbed, free-moving rats (30). In addition to the 22 experimental animals, six animals without brain cannulas were provided with heart cannulas to serve as controls.

Experimental Procedures

General. All experiments were performed between 11 A.M. and 1 P.M., i.e., during the rats' day. Food was removed from the cage either 2 or 24 h before beginning the observation. In *experiment I*, the beginning of the first meal during the observation and, in *experiments II* and *III*, the moment of injection were termed *time 0*. Blood samples of 0.06 or 0.30 ml were taken, respectively, for determination of glucose only or for determination of glucose, insulin, and glucagon. These were withdrawn at -11, -6 (this one only for the determination of glucose), and -1 min, and at +1, +3, +5, +7, +9, +14, +19, +29, +39, and +49 min.

Chemical determinations. The blood samples were transferred immediately to chilled centrifuge tubes containing 5 μ l heparin solution (500 U/ml) as anticoagulant and 10 μ l aprotinin solution (Novo Industri A/S., Copenhagen) (1,200 KIU/ml) to prevent proteolytic degradation of glucagon. Glucose was measured by the ferricyanide method of Hoffman in a Technicon Auto-Analyzer on 0.05 ml blood samples taken from the 0.3-ml sample immediately after withdrawal. The remaining 0.25 ml blood was centrifuged at 4°C and the plasma after separation stored at -30°C.

Plasma immunoreactive insulin (IRI) was estimated by means of a radioimmunoassay kit (Novo) with a rat insulin standard. Duplicate assays were performed on 25- μ l samples of plasma from experimental animals. To each of the standards, 25 μ l of plasma were added from alloxan-diabetic rats that had been deprived of food for 36 h.

Plasma immunoreactive glucagon (IRG) was determined using a Novo radioimmunoassay kit. Antiserum K 964 was used. This antiserum has very little cross-reaction with glucagonlike activity released from the gut. Duplicate assays were performed on 25- μ l samples. Both in the insulin assay and in the glucagon assay the separation of bound and free ¹²⁵I-labeled hormone was performed by means of polyethylene glycol solution 23.75% (wt/wt) as suggested by Henquin, et al. (15).

Experiment I. Six normal animals without brain cannulas but with heart catheters were used to investigate the blood levels of glucose, insulin, and glucagon before, during, and after a meal. This was tested after 2 h of food deprivation and after a fast of 24 h. A standard amount of 1.7 g C-rich diet or 3.4 g C-free (i.e., the mean amount ingested in an ad libitum meal) was offered under both deprivation regimes. Each rat was tested only once with each diet at each deprivation level. The experiments with the C-rich diet were completed first. Next each rat was allowed 8 days to become accustomed to the C-free diet before the test session.

Experiment II. At *time 0*, the animal received one simultaneous bilateral injection of 0.2 μ g (i.e., 0.1 μ g in 1 μ l/cannula) of one of the following substances dis-

solved in saline: norepinephrine (pH 6.0) or isoproterenol (pH 5.7). The injected solutions were prepared from a solution of catecholamines by diluting 100 times with saline. An injection of 1 μ l of pure solvent for catecholamines, diluted with saline in the same way, served as a control. The solutions were prepared just before attachment of the tubes to the brain cannulas. Food (C-rich) was removed from the cage 2 h before injection and returned after termination of the experiments. A rat never received more than one bilateral injection per day. Each rat was used only once for each type of injection.

Whether the effects obtained were actually due to intracerebral action of the injections was checked as follows. Five rats of *Experiment I* were used to investigate the effects of systemic injection of 0.2 μ g norepinephrine on glucose, insulin, and glucagon levels.

Experiment III. In the six rats with VMH-implanted brain cannulas, used in *experiment II*, the parasympathetic efferent pathway to the islets of Langerhans was blocked by systemic injection of 0.5 mg atropine sulfate 15 min before bilateral injection of norepinephrine into the VMH. This is enough atropine sulfate to suppress completely all vagal cardiac reflexes. It also blocks food intake because the swallowing reflex is inhibited. Food was removed 2 h before the atropine injections. This experiment was repeated with five other animals with VMH-implanted cannulas. These animals were injected with 3 mg atropine sulfate 15 min before bilateral injection of 0.1 μ g norepinephrine into the VMH. This quantity of atropine sulfate was chosen in addition because Woods et al. (42) described a suppression of insulin release by this quantity of atropine sulfate when insulin release was elicited by a reflex mechanism.

Histology

All animals with brain cannulas were sacrificed with ether and perfused with 10% Formalin. The brains were removed, embedded in paraffin, and sectioned at 50 μ m. The sections were stained with hemalum and examined under a microscope with the aid of the atlas of De Groot (9) to determine the position of the tip of the brain cannulas.

Statistical Methods

The Student *t* test was applied to determine significance of differences in glucose, insulin, and glucagon levels. $P < 0.05$ was considered significant.

RESULTS

Experiment I: Food Experiments

In presenting and discussing our results we shall use the following terms: beginning value, the value of a variable at time -1 ; peak value, the highest value reached at any time after time 0; amplitude of response, the difference between beginning and peak values.

Experiment Ia (C-rich food, 2 h fasted, $n = 6$). As shown in Fig. 1A, the blood glucose level remained constant until the 3rd min after meal onset, whereafter a rise occurred. Plasma IRI suddenly rose in the 1st min after the beginning of eating. In the 5th min, it reached

a peak value of 67 ± 8 μ U/ml, significantly higher than the beginning value of 35 ± 4 μ U/ml ($P < 0.005$). Plasma IRG began to rise by the 1st min and culminated at 230 ± 15 pg/ml in the 3rd min, which was significantly above the beginning level of 147 ± 24 pg/ml ($P < 0.025$). The peak plasma IRI declined gradually and plasma IRG somewhat more rapidly to premeal levels.

Experiment Ib (C-rich food, 24 h fasted, $n = 6$). When the rats were deprived of food for 24 h, the beginning levels of glucose and IRI were significantly lower than after 2 h deprivation (glucose, 76 ± 2.2 mg/100 ml vs. 102 mg/100 ml, $P < 0.001$; IRI: 13 ± 4 μ U/ml vs. 35 ± 4 μ U/ml, $P < 0.005$); whereas IRG levels were nearly the same (163 ± 22 pg/ml vs. 147 ± 24 pg/ml). Here again, the glucose level rose only by the 3rd min (Fig. 1B), whereas significant rises of IRI and IRG were observed by the 1st min. Peaks occurred for insulin in the 9th min (38 ± 8 μ U/ml, $P < 0.025$) and for glucagon in the 3rd min (360 ± 41 pg/ml, $P < 0.005$). The amplitude of the IRI response was smaller after 24 h than after 2 h deprivation (25 ± 9 μ U/ml vs. 38 ± 9 μ U/ml); on the other hand, the amplitude of the IRG response was larger (197 ± 47 pg/ml vs. 83 ± 28 pg/ml). The peak plasma IRI declined gradually and plasma IRG very rapidly to the premeal levels.

Experiment Ic (C-free food, 2 h fasted, $n = 6$). The same six animals after 8 days habituation to the C-free diet were given 3.4 g of this diet after 2 h deprivation. The beginning levels of blood glucose, plasma IRI and plasma IRG did not differ from those on C-rich food (Fig. 1C). However, the effect of the meal on blood glucose was much smaller, and in fact not significant. A sudden rise of IRI was again observed in the 1st min and plasma IRI culminated in the 7th min. The amplitude of the IRI response was significantly higher than after 2 h deprivation in the case of C-rich food (86 ± 17 μ U/ml vs. 38 ± 9 μ U/ml, $P < 0.025$). After the peak value, plasma IRI declined rapidly but remained above the premeal level. Plasma IRG showed a significant rise after the beginning of eating and culminated in the 5th min.

Experiment Id (C-free food, 24 h fasted, $n = 6$). The same six rats were subsequently observed after 24 h deprivation. Again the beginning levels of glucose, IRI, and IRG were the same as on the C-rich food under comparable conditions (Fig. 1D). Insulin and glucagon began to rise in about the same way as in *experiment Ib*, but the glucose level did not change significantly. After the peak values of IRI and IRG in the 5th min, both parameters declined rapidly but remained above the premeal levels. Comparison of the amplitude of the IRI response with that in *experiment Ic* shows a significant decrease after 24 h deprivation (86 ± 17 μ U/ml vs. 32 ± 8 μ U/ml, $P < 0.025$), whereas the amplitude of the IRG response was larger than in *experiment Ic*.

Experiment II. Intrahypothalamic Chemical Stimulation

Experiment IIa (norepinephrine). In this experiment 0.1 μ g of norepinephrine per cannula was injected.

VMH group ($n = 6$). Upon injection of norepinephrine in the VMH all investigated parameters rose and culminated in the 1st or 3rd min (Fig. 2A and Table 1).

FIG. 1. Mean blood glucose, plasma insulin, and plasma glucagon levels during eating of carbohydrate-rich food, after 2 h food deprivation (A); carbohydrate-rich food after 24 h food deprivation (B); carbohydrate-free food after 2 h food deprivation (C); and carbohy-

drate-free food after 24 h food deprivation (D). Thin line represents plasma glucagon (—); normal line represents plasma insulin (—); thick line represents blood glucose (—). Vertical bars represent 1 SE.

FIG. 2. Blood glucose, plasma insulin, and plasma glucagon levels after injection of norepinephrine at time 0 into VMH (A), LH (B), DMH (C) and POA (D).

In all six animals, the glucagon level increased after noradrenergic stimulation. Because one of the animals showed a very high beginning value (700 pg/ml), this one was not included in the results. After the peaks the levels declined rapidly to preinjection levels. Control injection with saline induced no reliable change either of blood glucose, plasma IRI or IRG (Fig. 3A).

LH group ($n = 6$). Upon norepinephrine administration in the LH a large increase of plasma IRI levels occurred, whereas there was no change in either blood glucose or plasma IRG (Fig. 2B and Table 1). Again, control injection with saline did not reliably change either blood glucose, plasma IRI or IRG (Fig. 3B).

DMH group ($n = 5$). There was a small rise of plasma

TABLE 1. Beginning and peak values of blood glucose, plasma insulin, and plasma glucagon after intrahypothalamic stimulation with norepinephrine

	Glucose, mg/100 ml				Insulin, μ U/ml				Glucagon, pg/ml			
	Beginning value	Peak value	P	Peak value reached after min	Beginning value	Peak value	P	Peak value reached after min	Beginning value	Peak value	P	Peak value reached after min
VMH	105 $\pm$ 3	120 $\pm$ 3	<0.02	3	24 $\pm$ 6	77 $\pm$ 17	<0.02	1	240 $\pm$ 45	480 $\pm$ 36	<0.005	3
LH	96 $\pm$ 4	106 $\pm$ 6	NS	7	25 $\pm$ 7	96 $\pm$ 14	0.005	1	167 $\pm$ 58	287 $\pm$ 70	NS	14
DMH	104 $\pm$ 2	110 $\pm$ 2	NS	5	29 $\pm$ 4	48 $\pm$ 22	NS	1	272 $\pm$ 85	280 $\pm$ 65	NS	9
POA	103 $\pm$ 4	110 $\pm$ 4	NS	5	40 $\pm$ 22	35 $\pm$ 15	NS		272 $\pm$ 66	328 $\pm$ 53	NS	5
VMH*	101 $\pm$ 4	125 $\pm$ 5	<0.025	5	16 $\pm$ 4	68 $\pm$ 17	<0.005	1	200 $\pm$ 40	448 $\pm$ 50	<0.01	3

Values are means $\pm$ SE. * After atropine injection.

FIG. 3. Blood glucose, plasma insulin, and plasma glucagon levels after injection of saline at time 0 into VMH (A) or LH (B).

IRI after injection of norepinephrine, whereas blood glucose and plasma IRG did not change (Fig. 2C and Table 1).

POA group ($n = 5$). Norepinephrine injections did not affect blood glucose, plasma IRI, or plasma IRG levels (Fig. 2D and Table 1).

Experiment IIb (isoproterenol). To determine the nature of the receptor mediating the effects of norepinephrine upon insulin and glucagon levels, we also injected isoproterenol, which is known to have potent β -adrenergic actions, whereas norepinephrine acts principally on α -receptors. Isoproterenol was administered in all groups of rats used in *experiment IIa*. Although all groups showed small rises of all three substances, none were reliable (Fig. 4: A, B, C, and D).

Experiment IIc (systemic control). In order to exclude action of the intrahypothalamically administered norepinephrine via the circulation on the islets of Langerhans, we injected five rats with norepinephrine systemically with 0.2 μ g, the same amounts as used intracerebrally. This caused a small nonsignificant increase of blood glucose, plasma IRI, and plasma IRG (Fig. 5).

Experiment III. Blockade of Vagal Influences

Because efferent nervous pathways presumably mediate insulin and glucagon responses to norepinephrine in VMH and LH, the vagus may be the major candidate for this function. Therefore, vagal activation was blocked by means of systemically administered atropine sulfate. Two doses of atropine sulfate were used, 0.5 and

3.0 mg. Fifteen minutes after these injections, norepinephrine was injected in the VMH. Both doses of atropine did not result in any suppression of the increase of blood glucose, plasma IRI, and plasma IRG concentrations (compare Figs. 2A and 6; Table 1).

Anatomical Analysis of Location of Brain Cannulas

Figure 7 presents the locations of the brain cannulas for all animals.

DISCUSSION

Experiment I

Previously demonstrated effects of food intake on insulin and glucose levels (36) have been confirmed. The additional demonstration of a glucagon rise provides one mechanism by which glucose levels may be maintained during insulin release preceding or in the absence of glucose absorption (Fig. 1). In this regard, it is interesting that the amplitude of the glucagon response is larger in the deprived than in the ad libitum condition. As basal levels in the deprived condition are almost equal to those in the ad libitum condition, it is likely that the α -cells of the pancreas are more sensitive to the meal stimulus. This might be due to the lower basal level of insulin and smaller response of the β -cells to a meal in the deprived condition (Fig. 1) because insulin is known to inhibit glucagon release (37).

In these as in previous experiments (33, 36), we never observed a decrease of blood sugar levels when rats began eating as described by Louis-Sylvestre (19). A decrease seems unlikely in view of the EGR, simultaneously occurring with the EIR. The cause of these differences in the results is not clear, but it may be due to differences in experimental setup.

In regard to the cause of the EIR and EGR, it is most unlikely that either effect is due to absorption of nutrients into the blood. On the C-free diet, the EIR can obviously not be due to absorption of glucose, and there is evidence that the same is true for the C-rich diet (31). Because amino acids can stimulate both insulin and glucagon release (10), absorption of amino acids cannot be excluded with certainty as a cause of the EIR and EGR, but because both responses occur so rapidly (latency less than 1 min), this seems unlikely.

The following suggestions can be made about the function of the EIR and EGR. In the first place, absence of the EIR (as occurs when food is injected directly into the stomach) is associated with a decreased glucose tolerance and an increased late insulin response (33).

FIG. 4. Blood glucose, plasma insulin, and plasma glucagon levels after injection of isoproterenol at time 0 into VMH (A), LH (B), DMH (C), and POA (D).

FIG. 5. Blood glucose, plasma insulin, and plasma glucagon levels after injection of norepinephrine systemically at time 0.

Therefore, the EIR seems to be important for blood sugar homeostasis. Secondly, it is relevant that in human prediabetics (7) there is a decreased glucose tolerance and impairment of the first phase of insulin release in response to an increase of blood glucose. In view of all this, presumably the function of the EIR is to stabilize blood glucose levels in the face of the disturbance caused by increased glucose absorption after meals.

Our view in regard to the function of the EGR can only be speculative at present, but it seems possible that the EGR may serve to safeguard the blood sugar level against the hypoglycemic action of the EIR until absorption of glucose from the beginning of feeding. Moreover, the EGR may potentiate insulin release (26).

Experiment II

The absence of EIR after food injection in the stomach indicates that the receptors triggering the EIR are situated proximal to the stomach (33). For that reason, a reflex origin of the EIR seems plausible, but the pathway (presumably nervous) linking the receptors to the β -cells remains to be elucidated.

Because information from taste and smell receptors

FIG. 6. Blood glucose, plasma insulin, and plasma glucagon levels after injection of norepinephrine at time 0 in ventromedial hypothalamus of atropinized rats.

reaches the VMH and LH (6, 20), and because these areas are also known to be involved in insulin and glucagon release (11), they are candidates for the role of relay station for the EIR and EGR. Unit activities in both areas can be modulated by norepinephrine (39). We, therefore, applied norepinephrine for a further investigation of their role in insulin and glucagon release.

We observed a significant increase of insulin and glucagon after noradrenergic stimulation of the VMH (Fig. 2A), whereas control injections induced no change of hormone levels (Fig. 3A). Noradrenergic stimulation of the LH induced a high insulin response but a nonsignificant increase of glucagon (Fig. 2B). Injections in the DMH elicited only a small insulin increase and injection in the POA none at all. Glucagon was not reliably changed by either DMH or POA injections (Fig. 2C and D).

To exclude the possibility that the observed effects were due to leakage of norepinephrine into the general

FIG. 7. Locations of tips of brain cannulas of rats used in experiments. ●, location in VMH; ○, location in LH; ▲, location in DMH; △ location in POA.

circulation, norepinephrine was also injected systemically. This caused insignificant responses of insulin or glucagon. It can be concluded, therefore, that the observed insulin and glucagon responses to norepinephrine administered to the LH and VMH were caused by action inside the brain.

To explain the EGR in response to noradrenergic stimulation of the VMH, we see no other possibility than to assume that a neural network for the control of glucagon is represented in the VMH. The simultaneously occurring EIR can be explained in three different ways. First, because noradrenergic stimulation of the LH elicits a large increase in insulin level, diffusion of norepinephrine from the VMH into the LH may explain the insulin release (14). Second, the VMH may contain separate structures for insulin and glucagon release. Third, NE injection into the VMH may cause only release of glucagon, but this in its turn elicits an imme-

diately release of glucose from the liver, which eventually provokes insulin release. The first mentioned possibility is not very likely because hormonal responses were nearly absent after NE injection into the DMH and POA, also areas in the neighborhood of the LH.

Shimazu et al. (27, 29) demonstrated increased levels of active hepatic glycogen phosphorylase after noradrenergic stimulation of the VMH and increased levels of active hepatic glycogen synthetase after cholinergic stimulation of the LH. Because glucagon and insulin are potent agents initiating conversion of hepatic glycogen phosphorylase and hepatic glycogen synthetase, respectively, into their active forms, it is worthwhile to compare our results with those of Shimazu. The increase of active hepatic glycogen phosphorylase by noradrenergic stimulation of the VMH might be mediated by the augmented level of glucagon. How to incorporate the simultaneously occurring increase of insulin in this

picture is not yet clear. The question remains why in Shimazu's experiments only phosphorylase was activated. Does this mean that, contrary to our findings, he elicited only glucagon release by noradrenergic stimulation of the VMH? Further investigations have to be performed to unravel the role of the VMH in both the enzyme systems involved in glycogen synthesis, glycogenolysis, and insulin glucagon release, respectively. The release of insulin after LH stimulation is in accordance with the increased levels of active hepatic synthetase as described by Shimazu (27). However, the fact that he obtained increases of active synthetase levels after cholinergic stimulation and we obtained increases of insulin levels after noradrenergic stimulation of the LH remains inexplicable at the moment.

Finally, let us compare these results with data in the literature on electrical stimulation of the VMH and LH. Some data indicate that electrical stimulation of the LH (11, 34) does not greatly change insulin, glucagon, and glucose levels. Other data, however, show that electrical stimulation of both VMH and LH elicits hyperglycemia and hyperglucagonemia, whereas insulin levels are suppressed (12, 24, 28), indicating strong sympathetic activation that may obscure the effect of possible specific mechanisms for insulin and glucagon release in these areas.

Because norepinephrine acts principally on α -receptors, the receptor involved in the causation of insulin and glucagon release would presumably be of the α -type. This was tested by injection of a β -sympathomimetic. Injection into the hypothalamic areas with equimolar quantities of isoproterenol resulted in nonsignificant increases of blood glucose, plasma insulin, and glucagon levels (Fig. 4). Hence, the receptors mediating the insulin and glucagon responses to intrahypothalamic norepinephrine are probably not of the β -, but possibly of the α -type.

Meal intake caused insulin and glucagon release within 1 min after the beginning of eating. Norepinephrine in the VMH induced both insulin and glucagon release within 1 min after injection, whereas norepinephrine in the LH induced insulin release within 1 min. We suggest, therefore, that the VMH is a relay station in the elicitation of the EGR and the LH in eliciting the EIR, whereas the EIR occurring after noradrenergic stimulation of the VMH is possibly a secondary effect as mentioned before.

Experiment III

In regard to the efferent part of the reflex arc, the VMH and LH contain many roots of the autonomic

nervous system (3), and the islets of Langerhans are provided with a rich sympathetic and parasympathetic innervation (40). Electrical stimulation of the right vagal branch to the pancreas induces both insulin (13, 17) and glucagon release (5). Stimulation of the splanchnic nerve, however, inhibits glucose-induced insulin release (4). Hence, the most likely candidate for elicitation of the EIR and EGR is the vagus nerve.

Inhibition of vagal transmission by atropine, however, did not abolish the insulin and glucagon responses to norepinephrine in the VMH (Fig. 6). Thus, it is unlikely that these responses are mediated by the vagus. The following alternatives deserve consideration.

First, modulation of sympathetic activity may influence insulin or glucagon release (41). It can not be excluded that activation of adrenergic β -receptors in the islets of Langerhans can activate both insulin and glucagon release (16, 25). We are now exploring this alternative.

Second, noradrenergic VMH or LH stimulation may induce the release of a hypothalamic neurosecretion acting directly or indirectly (via another humoral factor) or insulin and/or glucagon release. Evidence for such a neurosecretion was shown by Lockhart-Ewart et al. (18).

Third, insulin release can be activated by intestinal humoral factors, and also glucagon release can be stimulated by at least one of them (38). However, this is a useful alternative only insofar as the release of these intestinal hormones is not governed by the vagus.

In conclusion, eating elicits an EIR and EGR, regardless of whether the food does or does not contain carbohydrates. Because the EIR and EGR are absent when food is injected immediately into the stomach, the stimulus for the EIR and EGR must act proximal to the stomach. The responses must be of a reflex origin because they precede almost certainly any change in nutrient level in the blood. The relay stations of the reflexes underlying the EIR and EGR are probably situated in the LH and VMH, respectively, because noradrenergic stimulation of these areas mimics the EIR and EGR. The rise of insulin after noradrenergic stimulation of the VMH may be a secondary effect. The functions of the EIR and EGR are probably to safeguard the blood sugar level during and immediately after a meal.

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