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Hypothalamically-Induced Insulin Release and its Potentiation During Oral and Intravenous Glucose Loads

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Male Wistar rats were provided with bilateral cannulas in the lateral hypothalamic area (LHA) and cannulas in the left and right jugular vein. Freely moving rats provided in this way with cannulas were infused with transmitters in the LHA and with various substances in the blood circulation during simultaneous sampling of blood without disturbing the animals. Infusion of norepinephrine (NE) in the LHA resulted in increased insulin levels while plasma glucagon and blood glucose were nearly not affected. This LHA mediated insulin release was suppressed by atropine injection in the blood circulation suggesting a vagal contribution to the observed phenomenon. Administration of either an oral or i.v. glucose load during noradrenergic stimulation of the LHA elicited an exaggerated insulin response when compared to their controls. This LHA potentiated insulin response during an oral and i.v. glucose load could be suppressed by atropinization of the rats. It is concluded that meal-related stimuli are relayed to the NE-stimulated area of the LHA and that these stimuli modulate the output from this area of the LHA that is concerned with the release of insulin.

INTRODUCTION

A large body of evidence has accumulated indicating that apart from circulating blood nutrients the autonomic nervous system plays an important role in the regulation of the release of insulin and glucagon from the endocrine pancreas[6,10,15,17].

Electrical stimulation of the vagal branches to the pancreas elicits insulin release[6,15] though vagal stimulation results in some species (dog and calf) in concomitant glucagon release[5,17]. Electrical stimulation of the splanchnic nerve, on the other hand, causes glucagon release and suppression of insulin release[4]. Not only electrical stimulation of the vagus and the splanchnic nerve but also neurotransmitters involved in the action of the peripheral part of the autonomic nervous system are capable to influence the islet of Langerhans. Norepinephrine (NE), the sympathetic neurotransmitter and epinephrine (E) released concomitantly with NE by the adrenal medulla influence insulin release via direct stimulation of the α- and β-receptors of the B cell of the islets of Langerhans[23]. Stimulation of the α-receptors inhibits insulin release while β-receptor stimulation elicits insulin release[23]. The catecholamines inhibit glucose induced insulin release[19,23]. The B-cell is influenced both by circulating NE and NE released at nerve endings which are in contact with these cells[14]. On the other hand, both in vivo and in vitro studies show that the parasympathetic neurotransmitter acetylcholine applied to the pancreatic islets has the same effect as electrical stimulation of the vagus[13,16]. Because glucagon and insulin release elicited by vagal or cholinergic stimulation can be blocked by atropine, muscarinic receptors are present on the A and B cells[5,16].

Though much is known about the effects of peripheral manipulations of the autonomic nervous system on the islets of Langerhans, our knowledge on how central mechanisms are involved in peripheral autonomous effect is rather limited. Electrical stimulation of the ventromedial hypothalamic nucleus (VMH) of the anesthetized rat evokes a large rise of plasma glucagon and glucose levels with a simultaneous decrease in plasma insulin levels[9]. On the other hand

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electrical stimulation of the lateral hypothalamus (LHA) and medial forebrain bundle induces an increase of plasma insulin[3]. Not only electrical but also chemical stimulation of VMH and LHA can change hormone release from the pancreatic islets. Injection of 0.1 μg of NE bilaterally in the VMH of freely moving rats results in a glucagon and insulin peak which lasts for 3 min[8]. On the other hand injection of 0.1 μg of NE into the LHA causes an immediate insulin secretion without concomitant glucagon release[8]. Comparable results were reported by Shimazu and Ishikawa[25] in unrestrained rabbits.

Not only experimental stimulation of the VMH and LHA causes glucagon and insulin release, spontaneous food intake also elicits an early cephalic insulin response (EIR) that starts in the first minute after the onset of food intake and precedes any change in blood nutrient levels caused by the newly ingested food[30]. This EIR appears to be a similar phenomenon as the insulin release elicited by NE injection into the LHA since during feeding an efflux of NE can be measured for 60–90 min in hypothalamus and preoptic area of rats starting immediately after meal onset[21]. Regarding the pathway between the hypothalamus and the islet of Langerhans both a neural and a humoral system should be taken into consideration. Humoral factors isolated from the hypothalamus are demonstrated to elicit insulin release in vitro and in vivo[22]. The existence of a neural pathway follows from the observation that atropinization of rats suppresses the EIR[30].

Since plasma insulin, glucagon and blood glucose levels were studied only after a pulse injection of NE into the LHA the purpose of this study was to investigate those levels during infusions of small amounts of NE into the LHA during 20 min in order to study the effects of prolonged increase of NE levels in the LHA. As a pronounced rise of insulin was found during NE infusion it was investigated if this increase of insulin could be suppressed by muscarinic antagonistic agents like atropine. Since eating causes EIRs and concomitant secretion of NE in the hypothalamus the influence of increased NE levels in the hypothalamus on above-mentioned blood parameters after an oral and i.v. glucose tolerance test (OGTT and IVGTT, respectively) was studied. We further studied the influence of atropinization of the rats on the effects of an OGTT and IVGTT during NE infusion in the LHA.

MATERIALS AND METHODS

Subjects and maintenance

The animals in all experiments were male Wistar rats ranging in weight between 250 and 400 g at the beginning of the experiments. They were kept in individual plexiglass cages (25 × 25 × 30 cm) at room temperature (20 °C ± 2 °C) and had continuous access to food and water, unless otherwise stated. They were maintained on a 12-h light–dark cycle (06.00–18.00 h light). The standard C-rich diet (Muracon Lab chow) contained 20% protein, 53.5% carbohydrate, 4.5% fat and 22% water with added vitamins and minerals (4.1 kcal/g). The rats were handled and weighed every morning at about 09.00 h.

Surgery and control of body weight

All surgery was performed under ether anesthesia. A 1–2 week recovery period was allowed between subsequent surgeries. The experiments started as soon as the rats returned to pre-operative weight. There were no significant differences in body weight of the rats at the beginning of an experiment.

Implantation of brain cannulas

Permanent stainless-steel cannulas (length 22.0 mm, o.d. 0.3 mm, i.d. 0.1 mm) for chemical stimulation were stereotactically implanted bilaterally in the LHA according to he coordinates of de Groot (AP + 5.4, V —2.3, L ± 1.7 mm)[7]. A sterile stainless-steel obturator (length 23 mm, o.d. 0.08 mm) flush with the tip of the cannula was used between experiments to ensure that the cannula remained open and pyrogen free. The cannula was protected with a 21-gauge needle except for the last 4 mm at the top of the cannula. The protective sleeve was affixed to the skull with acrylic. Sterile polyethylene tubing (length 7.0 mm, o.d. 0.61 mm, i.d. 0.20 mm) was put around the cannula and obturator. A polyethylene cap (length 6.0 mm) was placed at the protruding end of the 21-gauge needle to cover the free end of the cannula.

Implantation of heart catheters

All animals were provided with a heart catheter according to the techniques earlier described[28]. This method allows blood sampling in unanesthetized, undisturbed, free moving rats[27]. Whenever i.v. infusions were performed, the rats were provided with a

second heart catheter implanted in the contralateral (left) jugular vein. This smaller second catheter (o.d. 0.64 mm, i.d. 0.28 mm) permits blood flow from the acromiodeltoid cephalic and anterior jugular vein to the external jugular vein. In general, rats did not loose weight after implantation of the second catheter indicating that no serious blockade in venous return from the head occurred after surgery.

Blood sampling procedures

All experiments were performed between 11.00 h and 14.00 h, i.e. during the rats light cycle. Food was removed 2 h before the start of an experiment. Blood samples of 0.06 (glucose only) and 0.3 ml were taken for determination of blood glucose, plasma insulin and glucagon. The sampling schedule is stated separately for each experiment. A transfusion of citrated blood obtained by means of a heart puncture from a donor rat was given after each 0.3 ml sample unless otherwise stated.

LHA infusion procedure

After removal of the cap and the obturator, a sterile polyethylene tube (length 400 mm, o.d. 0.61 mm, i.d. 0.20 mm) was connected to each cannula. The tube was filled with sterile test fluid except the last 8 mm at one of the ends of the cannula which remained filled with air. This end of the tube was sealed off to prevent any unwanted leakage into the brain. Under the sealed end remained an air bubble with a length of 4 mm. After attachment of the cannulas the animals were returned to their cages 30 min prior to the start of the experiments. The far end of all tubing remained outside of the cage so as not to disturb the animal[27]. At the start of the infusion, the sealed ends of the tubes were cut off and the tubing was quickly connected to the infusion pump. The infusion schedule is stated separately for each experiment and the start of moving of the air bubble was designated as time 0. The duration of all LHA infusions was for a 20 min period (0.25 μl/min). Constant moving of the air bubble originally under the sealed end of the tube ensured for a uniform infusion rate.

Norepinephrine (NE) solutions were diluted with sterile saline from a stock solution of 2 mg/ml solvent immediately before the start of the experiment. One criterion for satisfactory implantation in the LHA was the occurrence of an immediate rise of plasma insulin one minute after the start of an NE infusion.

Chemical determinations

The blood samples were transferred immediately to chilled (0 °C) centrifuge tubes, containing 5 μl heparin solution (500 U/ml) as anticoagulant and 5 μl aprotinin solution (120 kIU/μl, NOVO) to prevent proteolysis of glucagon.

Blood glucose was measured by the ferricyanide method of Hoffman (Technicon Autoanalyzer TM II) with 0.05 ml blood taken from the 0.3 ml sample. The remaining 0.25 ml blood was centrifuged at 4 °C and the supernatant stored at —30 °C. Rat specific plasma immunoreactive insulin (IRI) and plasma immunoreactive glucagon (IRG) were determined by means of a radioimmunoassay kit (NOVO). Guinea pig serum M8309 served as antiserum for the insulin assay. Antiserum K964 was used for the glucagon assay and has very little cross-reaction with gut glucagon. Duplicate assays were performed on 25 μl plasma samples. Both in the insulin and glucagon assay the bound and free ^{125}I-labeled hormone was separated by means of a polyethylene-glycol solution (23.75% w/w) as suggested by Henquin et al.[11].

Histology

Cannula placement was investigated. The rats were anesthetized with ether and perfused with 10% formalin. The brains were removed and stored overnight in 10% formalin and 4% glucose. The brains were frozen quickly in melting isopentane (—80 °C) and cut at 40 μm on a cryostat microtome. Brain slices were stained with cresyl fast violet, examined under a light microscope and compared with the atlas of De Groot[7]. Only the results of rats with correct cannula placements were used.

Infusion of NE in the LHA

The purpose of this experiment was to investigate the plasma insulin, glucagon and blood glucose levels during infusions of different quantities of NE (at a rate of 6.25, 12.5 and 25 ng/min) in the LHA. At the most appropriate dose the NE infusion was administered in the absence and presence of atropine.

Two groups of rats were used. Group 1: rats (n = 7) with an initial mean weight of 250 g randomly received 4 treatments, i.e. 3 doses of NE or a NE-solvent solution. NE was administered at a low (6.25),

medium (12.5), or high, 25.0 ng/cannula/min dose. The NE or its vehicle was given over a 20 min period, total volume infused was 5 μl at a rate of 0.25 μl/min. Each rat received each of the 3 doses but once with an interval of at least 2 days in between. Group 2; rats (n = 6) with a mean weight of 400 g, at the beginning of the experiment, randomly received two treatments, i.e. a NE-infusion 12.5 ng/cannula/min with or without atropinesulfate (0.5 mg/kg). The atropine pulse was administered via the heart catheter 10 min prior to the NE-infusion. Blood transfusions of 1.2 ml were given at t = —11, +9 min, and after the completion of the experiment. An interval of at least two days was allowed between treatments. The blood sampling procedure for both groups was t = —11, —6 (blood glucose only) —1, +1, +3, +5, +7, +9, +14, +19, +24 and +34 min. NE infusions started at time point 0.

An oral glucose tolerance test during NE infusion in the LHA

In the previous experiment we observed that NE infusion in the LHA resulted in a significant increase of plasma insulin. The aim of this experiment was to determine whether or not there was any interaction between glucose-stimulated insulin release and NE-stimulated insulin release. In this experiment rats provided with LHA cannulas and a heart catheter were infused with NE at a rate of 12.5 ng/min/cannula. During this infusion rats received an oral glucose tolerance test (OGTT) of 135 mg glucose dissolved in 1.5 ml of water.

Seven rats with a mean body weight of 300 g at the beginning of the experiment were used for the oral glucose tolerance test. Blood samples were taken at t = —11, —1 (start infusion in the LHA was at time 0), +1, +3, +5 (start of OGTT was at time 5), +6, +8, +10, +12, +17, +22, +32 and +42 min. Blood transfusions were given at t = —11, +8 and after the completion of the experiment. One week prior to the experiment, the rats were conditioned to lick 1.5 ml of a 9% glucose solution (135 mg). The freshly prepared glucose solution was offered in a porcelain dish. Five minutes before the presentation of the glucose the NE infusion was begun. All animals received an OGTT. Each rat was given randomly glucose + buffer infusion, or glucose + NE infusion in the LHA.

An intravenous glucose tolerance test during NE infusion in the LHA

In the previous experiment it was clearly demonstrated that an OGTT during NE infusion in the LHA elicited an exaggerated insulin release. The question arises as to the cause of this exaggerated insulin release. It can be hypothesized that stimulation of receptors in the oral cavity by glucose strongly enhances the effects of the already NE stimulated LHA. It might also be hypothesized that an increased sensitivity of the B cell of the islet of Langerhans to a certain glucose load is responsible for the observed phenomenon. To discern between both possibilities rats were subjected to an i.v. glucose tolerance test (IVGTT) during either NE infusion or buffer infusion in the LHA. If the exaggerated insulin release during NE infusion in the LHA is caused by an increased sensitivity of the B cells to a certain oral glucose load a similar phenomenon must occur during an IVGTT.

In this experiment a new group of 7 rats (mean weight 300 g) provided with LHA cannulas and two heart catheters (one for the withdrawal of blood and the other smaller one for the infusion of fluids) received infusions in the LHA with either NE at a rate of 12.5 ng/min/cannula or buffer. During this infusion rats were subject to an i.v. glucose tolerance test (IVGTT). An i.v. glucose load of 80 mg glucose, dissolved in 1.5 ml of saline was administered at a rate of 0.1 ml/min via the smaller of the two catheters. This glucose load was chosen because this load leads to a moderate increase of both glucose and insulin levels[29].

The infusion tube was filled with the glucose solution except for the last 2 cm which were filled with saline. The glucose solution and saline were separated by a small air bubble (1 mm). Disappearance of the small air bubble in the needle attached to the skull of the rat which took only 5 s after the start of the infusion was termed as the start of the IVGTT. Five minutes before the start of the IVGTT the NE infusion was begun. All animals received an IVGTT. Each rat was given randomly i.v. glucose + buffer infusion in the LHA, or i.v. glucose + NE infusion in the LHA. Blood samples were taken at —11, —1 (start infusion in LHA was at time 0), +1, +3, +5 (start of IVGTT was at time +5), +6, +8, +10, +12, +17, +22, +32 and +42 min.

Effects of atropinization on an OGTT and IVGTT during NE infusion in the LHA

In the previous experiments it was clearly demonstrated that insulin release was strongly augmented during both an OGTT and an IVGTT by a simultaneous infusion of NE in the LHA. The question arises as to which pathway is involved regarding this potentiation of insulin release. Humoral factors can be released by the hypothalamus during noradrenergic stimulation of the LHA[22]. On the other hand, it is most likely that neural pathways are involved in the observed phenomenon. In this respect the dorsal motor vagus nucleus and its efferent fibers are prominent relays since vagal stimulation elicits insulin release[6,15,17] and the suppression of insulin release by atropinization during noradrenergic stimulation of the LHA as described above leads to the same conclusion. For that reason the following experiment was performed in which an OGTT and an IVGTT was given to rats during NE infusion in the LHA with and without atropine administration prior to the NE infusion.

A group of 9 rats were used in this experiment (7 out of the previous experiment and 2 additional ones). The effect of atropine was tested in these animals. The rats randomly received two treatments, i.e. an NE infusion of 12.5 ng/cannula/min with or without atropine sulfate (0.5 mg/kg). An atropine pulse was administered via the heart catheter 10 min prior to the NE infusion in the LHA which was given during a period of 20 min. In the control situation a saline injection was given via the heart catheter instead of an atropine pulse. Five minutes after the start of NE infusion the OGTT was started. Blood samples were taken as in the OGTT and IVGTT exeriments, however, 10 and 1 min before atropine injection two additional blood samples were taken. It was measured that the rats were eating the glucose at the same speed after atropinization as in the situation without atropinization. All animals finished their glucose meal within 1 min. In addition in the same rats comparable experiments were performed but instead of an OGTT they received an IVGTT.

RESULTS

Infusion of NE into the LHA

The results of the parametric study are presented

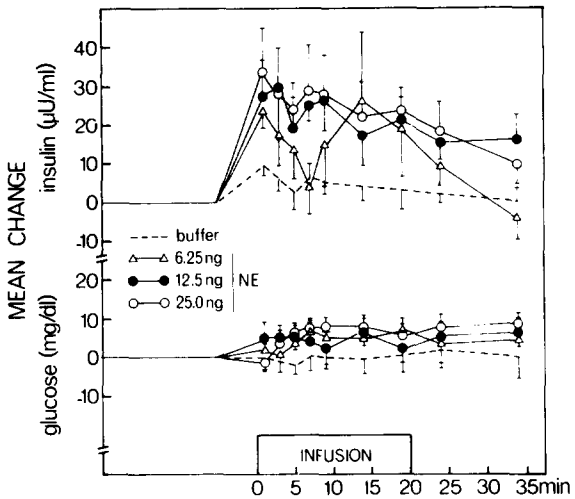

Fig. 1. Effect of infusion of different doses of NE in the LHA on blood glucose and plasma insulin. Data are expressed as means ± S.E. mg/dl and μU/ml respectively, changes from preinfusion levels of glucose and insulin which were the average of time points —11 and —1 min levels.

in Fig. 1. Average basal insulin level, i.e. before the start of NE infusion in the LHA was 20 ± 4.3 μU/ml plasma. Analysis of variance applied to these means revealed significant differences among the 4 treatments (F (3.35) $P < 0.01$). All 3 doses of NE resulted in a significant rise in plasma insulin levels within the first minute when compared to the infusion of the vehicle alone and remained elevated during the infusion with the exception of the low dose. The insulin levels remained elevated during infusion of the high dose (25.0 ng/min) and middle dose (12.5 ng/min) and differences between these two doses regarding insulin levels could not be measured. No significant changes in blood glucose levels were observed although a slight increase was noted when NE was infused at all 3 doses.

As shown in Fig. 2, atropine completely inhibited the NE stimulated insulin response within the first minute and continued to suppress this response throughout the experiment with mean insulin levels returning to pre-infusion levels at —34 min. In this group of rats insulin levels i.e before the start of NE infusion in the LHA was 45 ± 6.8 μU/ml plasma in the nonatropinized group and 39 ± 5.3 in the atropinized group. No significant differences were observed between the two treatment groups in either plasma glucagon or blood glucose levels.

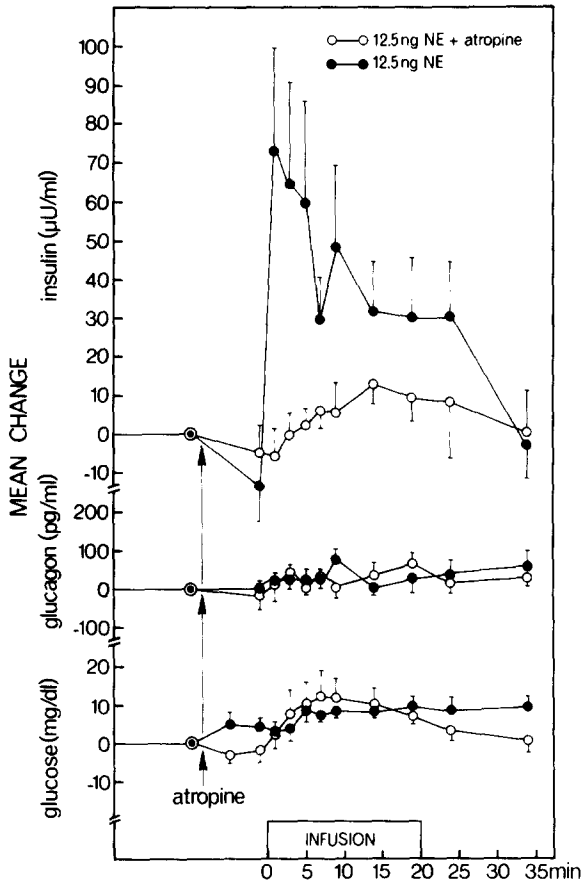

Fig. 2. Effect of NE infusion in the LHA (12.5 ng/min at rate of 0.25 μl/min during 20 min) on blood glucose, plasma insulin and glucagon either with atropinization (0.5 mg/kg), O—O, or without atropinization, ●—●. Data are expressed as means ± S.E. mg/dl, μU/ml, pg/ml respectively, changes from preatropinization levels of glucose, insulin and glucagon which were the average of those of time point 1 min before atropinization.

An oral glucose tolerance test during NE infusion in the LHA

Ingestion of glucose (135 mg) elicited an early insulin response (EIR) in both treatment groups eating glucose and receiving either NE or buffer solution in the LHA. However, a much greater response was measured in the rats eating glucose during infusion of NE in the LHA (Fig. 3). Mean change in the first minute compared to baseline insulin levels for the two experimental situations were oral glucose + NE in the LHA 79 ± 20.8 μU/ml; oral glucose + buffer in the LHA 13 ± 8.9 μU ($P < 0.02$ Student's t-test). Prior to glucose ingestion NE infusion into the LHA resulted in an immediate rise in insulin within the first minute (mean change from baseline 45 ± 5.4 μU/ml). Four minutes later, i.e. 1 min before the start of the OGTT, the mean change above baseline was 15 ± 9.3 μU/ml.

Oral glucose elicited a small increase in glucagon levels within the first minute for the two treatment groups. Mean change in glucagon levels in the first minute for the treatments were: oral glucose + NE in the LHA 51 ± 26.0 pg/ml; oral glucose + buffer in the LHA 85 ± 21.3 pg/ml. No reliable difference in glucagon levels was observed among the two treatment groups prior to the ingestion of glucose. A small nonsignificant rise in glucose was measured 3 and 5 min after the start of NE infusion in the LHA as compared to the situation with buffer infusion in the LHA: (third min 9 ± 3.1 mg/dl vs 2 ± 3.9 ml/dl, fifth

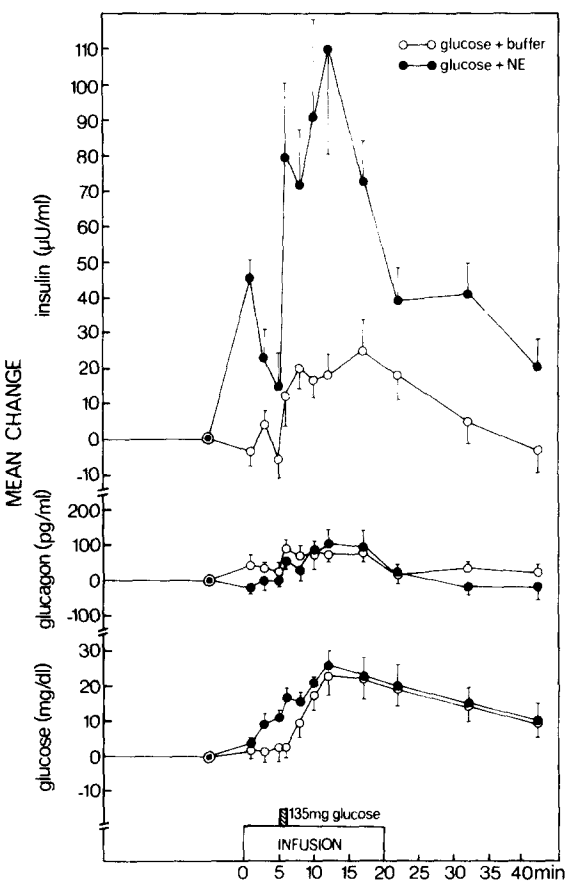

Fig. 3. Mean changes ± S.E. of blood glucose (mg/dl), plasma insulin (μU/ml) and plasma glucagon (pg/ml) during ingestion of 135 mg glucose which was ingested during either buffer infusion at a rate of 0.25 μl/min during 20 min, O—O, or NE infusion in the LHA (12.5 ng/min at a rate of 0.25 μl/min during 20 min), ●—●.

min 11 ± 2.5 mg/dl vs 2 ± 3.4 mg/dl). During and after ingestion of glucose an increment in blood glucose was present in both experimental situations and at min 17 (i.e. 12 min after the start of glucose ingestion) a peak value was reached of 26 ± 4.2 mg/dl in the situation in which NE was infused in the LHA vs 23 ± 5.1 mg/dl in the situation in which buffer was infused in the LHA. Then glucose declined in both situations in exactly the same way.

An intravenous glucose tolerance test during NE infusion in the LHA

The results in Fig. 4 show that infusion of buffer in the LHA before infusion of glucose in the general circulation did not lead to any changes in plasma insulin. The superimposed glucose infusion elicited insulin release in the first minute. Peak values were reached in the first and third minute of 22 ± 5.4 μU/ml and 13 ± 6.3 μU/ml plasma, respectively.

On the other hand, infusion of NE in the LHA caused a small increase of insulin before the IVGTT.

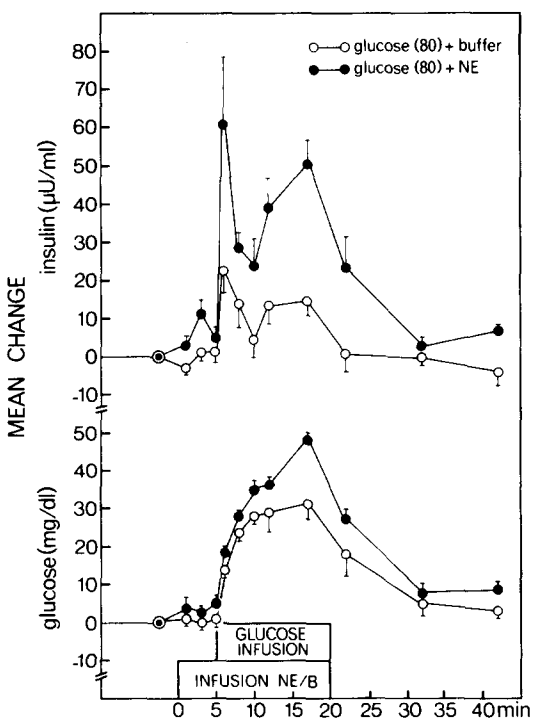

Fig. 4. Mean changes ± S.E. of blood glucose (mg/dl) and plasma insulin (μU/ml) during i.v. infusion of 80 mg glucose, which was infused during either buffer infusion at a rate of 0.25 μl/min during 20 min, O—O, or NE infusion in the LHA (12.5 ng/min at a rate of 0.25 μl/min during 20 min), ●—●.

The IVGTT elicited a peak in insulin release reaching 62 ± 15.8 μU/ml in the first minute and 30 ± 5.0 μU/ml plasma in the third minute. The observed difference between the two treatments in the first and third minute after glucose infusion are statistically significant ($P < 0.05$ and $P < 0.05$ respectively, Student's t-test). After the first peak a second peak followed which was much larger during NE infusion in the LHA than during buffer infusion in the LHA.

Regarding blood glucose, virtually no differences could be observed before the IVGTT and also during the first and third minute of the IVGTT only minimal differences were found in blood glucose: in the first minute an increase of 13 mg/dl with NE treatment and 13 mg/ml with buffer treatment; and in the third minute 23 mg/dl and 22 mg/dl respectively. After this initial simultaneous rise, blood glucose reached higher values in the NE treatment than in the buffer treatment situation. In the seventeenth min after the start of the IVGTT the concentrations were 48 ± 2.3 mg/dl and 31 ± 3.6 mg/dl respectively.

Effects of atropinization on an OGTT and IVGTT during NE infusion in the LHA

As depicted in Fig. 5 in the 9 animals insulin rose in the first and third minute of the OGTT during NE infusion in the LHA and without atropinization 49 ± 11.8 μU/ml and 37 ± 8.6 μU/ml plasma above baseline respectively. The insulin rise with atropinization was at the same time points 5 ± 2 μU/ml and 6 ± 1.7 μU/ml plasma respectively. These differences were statistically significant; in the first min $P < 0.005$, in the third min $P < 0.005$. Also after the third minute a considerable suppression of insulin release could be observed in the atropinized rats. Prior to glucose ingestion NE infusion in the LHA resulted in a rise in insulin which was smaller after atropine administration just as described above (cf. Fig. 2).

Regarding glucose, an immediate rise was observed with NE infusion in the LHA + an OGTT + atropine treatment after the start of NE infusion. Glucose reached its zenith of 29 ± 2.7 mg/dl above baseline level in the seventeenth min in this situation. Without atropine, however, glucose started to rise only after the beginning of glucose ingestion, a zenith was reached of 26 ± 2.9 mg/dl above baseline level in the seventeenth minute. Atropinization of the rats caused a significant increase of glucose in the

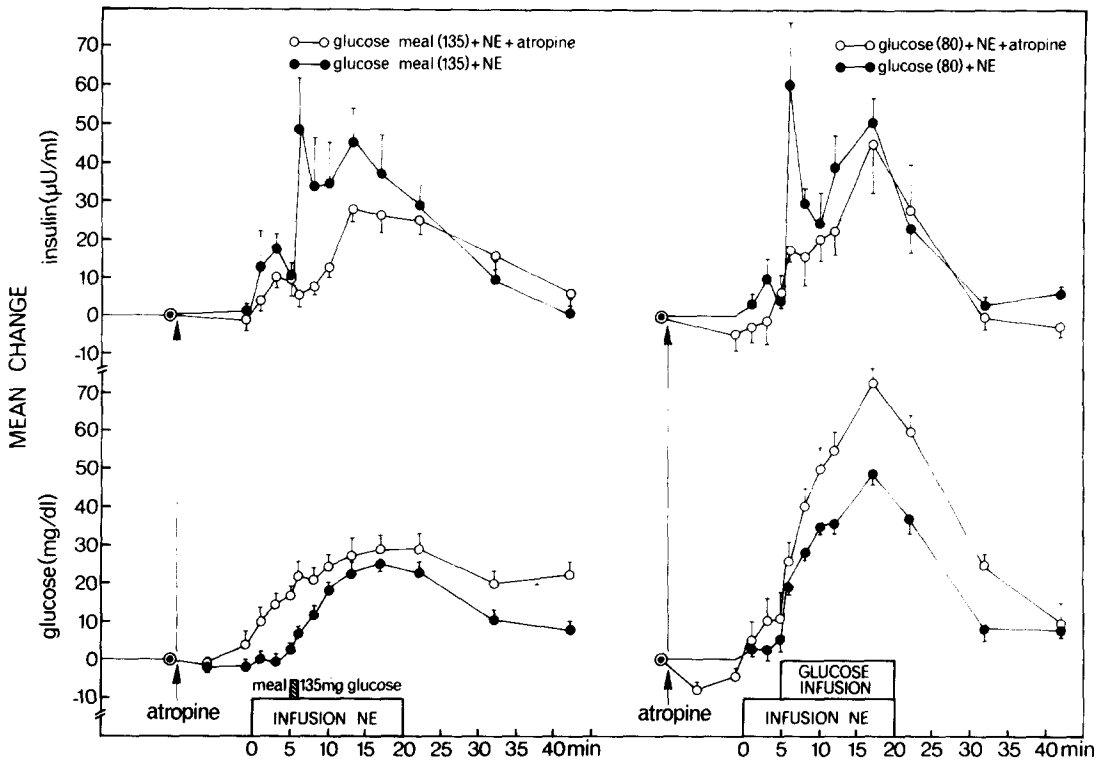

Fig. 5. Left panel : effect of ingestion of 135 mg glucose on blood glucose (mg/dl) and plasma insulin (μU/ml) during infusion of NE in the LHA (12.5 ng/min during 20 min) with atropinization (0.5 mg/kg) O—O, or without atropinization ●—●. Right panel: effect of i.v. infusion of 80 mg glucose on blood glucose (mg/dl) and plasma insulin (μU/ml) during infusion of NE in the LHA (12.5 ng/min during 20 min) with atropinization (0.5 mg/kg), O—O, or without atropinization, ●—●. In both cases data are expressed as means ± S.E. mg/dl and μU/ml, respectively, changes from preatropinization levels of glucose and insulin which were the average of time points 10 and 1 min before atropinization.

first, third, fifth, sixth and eighth min after the start of NE infusion in the LHA.

As Fig. 5 shows, atropinization of the rats before infusion of NE in the LHA and an IVGTT resulted in a considerable suppression of insulin during the first min after the start of the IVGTT: 62 ± 15.8 μU/ml without atropinization vs 17 ± 3.2 μU/ml plasma with atropinization ($P < 0.02$ Student's t-test). Thereafter, insulin levels did not differ significantly in the situation with atropinization as compared to that without atropinization. After an initial comparable level in both situations glucose rose much more after the first min of the IVGTT with atropinization. Glucose reached a zenith in the 12th min of the IVGTT: 72 ± 5.5 mg/dl above baseline level with atropinization vs 47 ± 4.7 mg/dl blood without atropinization $P < 0.005$ (Student's t-test).

DISCUSSION

The results of these experiments indicate that noradrenergic stimulation of the LHA causes insulin release and that cholinergic pathways are involved in the mediation of these chemically applied stimuli.

In the parametric study (experiment 1) and subsequent replications (experiment 2, 3 and 4) NE infusions over a 20 min period elicited an insulin response within the first minute. This immediate rise in insulin was observed in the 6.25 (low) as well as in the 12.5 (middle) and 25.0 ng/min (high) dose in addition to the 5 replications using the NE dose of choice, 12.5 ng/min.

These data are in agreement with studies in which it is reported that injections of NE into the hypothalamus, both the LHA and VMH elicit an insulin re-

sponse within the first minute[8]. Although the NE stimulatory effect on insulin was highly significant, the magnitude of the increase of insulin levels within the first minute was not as large as reported previously[8]. Δ Insulin = 62 in that study vs 28 reported here. However, in the earlier study[8], NE was injected as a pulse (0.1 μg/μl), whereas in the present investigation NE was applied over 20 min at 12.5 ng/min (total 0.25 μg/5 μl). Both the dilution factor and the rate at which the NE was administered may have accounted for the lower curves in our study.

Another point merits attention here. Infusion of 12.5 ng/min during 20 min elicited a larger insulin response in the rats participating in the NE infusion in the LHA experiment with atropinization than in those participating in the experiment in which several doses of NE were administered in the LHA (cf. Figs. 1 and 2). This may be due to the difference in body weight of both groups of rats. Also, the basal insulin level was higher in the group of heavier rats. This is in agreement with the idea of Porte and Woods[1] who reported firstly a correlation between basal insulin levels and body weight both in man and rats and secondly that the increase of insulin after an insulinogenic stimulus is proportional to the basal insulin level.

In spite of the higher insulin levels during NE infusion in the LHA no change in glucose levels could be observed though a decline should be expected. Probably counterregulatory mechanisms prevent such an expected decline. The nature of these mechanisms is not clear as yet. As glucagon does not rise an increased sympathetic tone which augments the glucose output from the liver[20,24] is less probable. Moreover, an increase in insulin level would not be expected with increased sympathetic tone. Most likely candidates for this counterregulation are growth hormone and adrenal cortical hormones released via ACTH. Also changes in insulin receptor sensitivity cannot be excluded. This needs further elucidation.

Atropine sulphate suppressed insulin release by NE infusion in the LHA. This finding suggests that the vagal nerve is involved in the release of insulin after NE stimulation of the LHA and that the vagal nerve takes part in the efferent circuit between the LHA and the islets of Langerhans (experiment 1). One problem may arise: in the presented experiments atropine sulphate was administered as an anticholinergic drug. In contrast with methylatropine, atropine is able to pass the blood–brain barrier. Therefore it can be argued that atropine does not act on cholinergic receptors of the B cells of the islets of Langerhans but on cholinergic pathways in the CNS which connect the LHA with the dorsal vagal motor nucleus. However, preliminary results of a study investigating the neural circuitry between the LHA and the motornucleus of the vagus related to insulin release indicate that CNS cholinergic pathways are unlikely to be involved in such connections (Luiten, unpublished results).

Results of other studies confirm the idea of a vagal contribution to insulin release[6,10,15,17]. Hyperinsulinemia induced by lesions of the ventromedial hypothalamus (VMH) could be reduced by subdiaphragmatic vagotomy[2,12]. Also subdiaphragmatic vagotomy prevented the increased output of insulin in VMH lesioned rats during a glucose load administered immediately after induction of the lesion[3]. The most conspicuous result of experiment 2 was that though an oral glucose challenge elicits an immediate rise in insulin levels within the first and third minute of an OGTT in the experimental rats, it was in the NE in the LHA + an OGTT group that the largest increase of insulin levels was observed (Fig. 3) which continued during the whole period of NE infusion. This increase was significantly different from the control group.

At this point it is necessary to draw attention to the relatively small early insulin response (EIR) elicited by the OGTT in the control group which rose in the first minute to 13 ± 8.9 μU/ml plasma (Fig. 3). This small increase is due to the fact that in this experiment the rats ingested a small amount of a rather diluted glucose solution (135 mg in 1.5 ml). The customary rise in glucose due to eating was also observed. Though the glucose level was somewhat higher in experiment 2 (Fig. 3) at the start of glucose ingestion in the situation in which NE was infused in the LHA as compared to the situation in which buffer was infused, it is unlikely that this higher basal level is responsible for the potentiated insulin release because a comparable higher glucose level was not found in experiment 3, in which also a potentiation of insulin was observed albeit during an IVGTT (Fig. 4).

Regarding the insulin response to an IVGTT, it

was demonstrated that NE infusion in the LHA led to a considerable potentiation of insulin release. During the first part of the IVGTT the rise of glucose was the same during both NE and buffer infusion in the LHA consequently the potentiation cannot be due to an increased glucose level. The continuation of the exaggerated insulin response on the other hand in the NE infused rats can be partly due to an increased glucose level which occurs in the latter part of the IVGTT (see Fig. 4). The reason for the higher glucose level in the rats infused with NE in the LHA to that in the rats infused with buffer might be attributed to increased growth hormone and corticosterone levels which counteract the peripheral utilization of glucose. The diminished utilization leads to a rise of blood glucose which in its turn triggers insulin release. This supposition needs further experimental support.

Atropinization of the rats caused a considerable suppression of insulin during infusion of NE in the LHA and an OGTT (see Fig. 5). This suppression is the more noteworthy because it is accompanied by a concomitant higher glucose level which should stimulate insulin release. In the experiment with NE infusion in the LHA and an IVGTT with atropinization (see Fig. 5) only in the first minutes of the IVGTT a suppression of insulin could be measured. After that insulin rose in nearly the same extent as in the experiment without atropinization. This second phase insulin rise might be explained by the concomitant much stronger glucose rise in the experiment with atropinization. The increased augmentation of glucose both in the OGTT and IVGTT with atropinization as compared to the situation without atropinization might be due to increased glucose production or to decreased uptake of the either ingested or infused glucose in the splanchnic bed. The most obvious explanation is decreased cholinergic activation of the liver glycogen synthetase system[24,26] but this needs further experimentation. The much higher glucose increase during an IVGTT than during an OGTT with atropinization and during NE infusion in the LHA might be the result of decreased gastrointestinal motility and resorption after atropinization so that glucose does not rise as high as during i.v. infusion.

It can be concluded that artificially increased noradrenergic activity of the LHA has a powerful effect on the B cell of the islet of Langerhans. McCaleb et al.[21] demonstrated that feeding elicits already in the first minute an immediate increase in norepinephrine release from neurons in the hypothalamus and noradrenergic bundle in the preoptic area. From these data it is an obvious assumption that the early insulin response which occurs at the onset of eating before any absorption of newly ingested nutrients is caused by increased noradrenergic activity of the LHA. At the moment our concept is that the state of the noradrenergic activity of the LHA is responsible for the quantity of insulin released by a glucose load, i.e. low noradrenergic activity of the LHA is accompanied by a moderate insulin secretion and high noradrenergic activity of the LHA by a large insulin release after a certain glucose load. As appears from the results of this work insulin release elicited by the LHA is most probably mediated by parasympathetic pathways. That neural mechanisms are involved in an early insulin response as a result of food intake has been shown by Strubbe and Van Wachem[31]. These authors demonstrated that EIRs are completely absent in diabetic rats recovered by islet of Langerhans transplants which have no innervation, although blood glucose and plasma insulin profiles are normal in the intermeal periods. However, results of recent papers indicate that besides neural factors also humoral factors released by the hypothalamus might play a role in insulin secretion[3,22]. The reason of the coexistence of both a neural and a neurohumoral system that control insulin release needs further elucidation.

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