Circulating free fatty acids, insulin, and glucose during chemical stimulation of hypothalamus in rats

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STEFFENS, A. B., G. DAMSMA, J. VAN DER GUGTEN, AND P. G. M. LUITEN. *Circulating free fatty acids, insulin, and glucose during chemical stimulation of hypothalamus in rats.* Am. J. Physiol. 247 (Endocrinol. Metab. 10): E765–E771, 1984.—The aim of this study was to investigate plasma free fatty acids (FFA), insulin, and blood glucose during chemical stimulation of the lateral and ventromedial hypothalamic areas (LHA and VMH) in rats. Therefore male Wistar rats were implanted with bilateral cannulas in the LHA or the VMH and into the left and right jugular veins. Freely moving rats were then infused into the LHA and VMH with norepinephrine (NE), epinephrine (E), or acetylcholine or intravenously with NE or E. Before, during, and after the infusions, simultaneous blood samples were taken without disturbing the animals. Infusion of NE into the LHA resulted in a decrease of plasma FFA and a simultaneous increase of insulin. NE infusion in the VMH elicited an increase of plasma FFA, plasma insulin, and blood glucose. E infusion into the LHA did not lead to a change of plasma FFA, whereas insulin and glucose showed an increase. E infusion into the VMH evoked increases of plasma FFA and insulin. Peripheral administration of NE led to a sharp increase of FFA, whereas plasma insulin and blood glucose did not change. E in the periphery elicited an augmentation of plasma FFA and blood glucose and a suppression of insulin during infusion. After termination of E infusion, plasma FFA and glucose levels decreased, whereas plasma insulin showed a sharp increase. It is concluded *1*) that the effects produced by administration of NE and E are dependent on hypothalamic localization and local receptor population characteristics; *2*) that there are striking differences regarding the effects on the investigated blood parameters between hypothalamically infused NE and E and peripherally infused NE and E; and *3*) that the LHA and VMH are able to alter plasma FFA levels independently of blood glucose and insulin levels.

lateral hypothalamus; ventromedial hypothalamus; noradrenergic stimulation; adrenergic stimulation; cholinergic stimulation

IN THIS STUDY THE ROLE of chemical stimulation of the lateral and ventromedial hypothalamic areas (LHA and VMH) on plasma free fatty acids (FFA), insulin, and blood glucose is investigated. It is well established that both the LHA and VMH can strongly influence plasma insulin, glucagon, and glucose levels. Electrical stimulation of the VMH of rat and rabbit elicits a rapid increase of plasma glucagon and blood glucose (7, 19). Microinjections of norepinephrine (NE) into the VMH of the rat cause a sharp increase of plasma glucagon, plasma insulin, and blood glucose, whereas a similar injection into the LHA leads to an immediate increase only of plasma insulin (6). A comparable response (i.e., increase of both glucagon and insulin) is obtained in the rabbit after an NE injection into the VMH. However, an equimolar injection of epinephrine (E) is much more potent in this respect.

Noradrenergic stimulation of the LHA of rabbits has no effect on plasma levels of insulin, glucagon, and glucose, but adrenergic stimulation of the LHA in these animals results in an increase of insulin but is without effect on plasma glucagon and glucose (19). These data indicate the existence of species differences in the release of glucagon and insulin to catecholaminergic stimulation of the VMH and LHA.

Bolus injections as well as 20-min infusions of small amounts of NE profoundly influence plasma insulin and glucagon levels. Bilateral infusion into the VMH of 12.5 ng NE/min for 20 min leads to an increase of plasma glucagon and glucose throughout the infusion but is without effect on insulin (24). A similar infusion into the LHA, on the other hand, stimulates insulin release and strongly potentiates the insulin release to oral and intravenous glucose loads (25). The results of these experiments suggest an important role for the hypothalamus in the release of those hormones primarily involved in the regulation of blood concentrations of fuel substrates; i.e., glucose and FFA. The hypothalamus not only affects the function of the islets of Langerhans, which produce among other hormones glucagon and insulin, but also the liver by a direct neural influence (20).

Electrical or chemical stimulation of the VMH leads to glycogenolysis, whereas chemical stimulation of the LHA results in glycogenesis. In this respect the neural and hormonal influences exerted by the VMH and LHA on blood glucose levels are well documented (e.g., Ref. 18). However, comparable data regarding plasma FFA levels are virtually absent, and certainly none are available from unrestrained freely moving rats. The finding that dose-dependent increases of plasma FFA follow an injection of NE into the lateral brain ventricles of rats suggests that central nervous system (CNS) mechanisms are involved (2). In another study (18), an increase of plasma FFA was found in anesthetized rats during electrical stimulation of the VMH. However, this increase

was absent during electrical stimulation of the VMH in freely moving rats.

The aim of this study was to clarify the role of the LHA and VMH regarding the regulation of plasma FFA levels in relation to the effects of these areas on plasma insulin and blood glucose in unrestrained freely moving rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–300 g at the beginning of the experiments were used. They were kept individually in Plexiglas cages (25 × 25 × 30 cm) at room temperature (20 ± 2°C) and had continuous access to standard carbohydrate-rich food (Muracon laboratory chow) and water unless otherwise stated. They were maintained on a 12-h light-dark cycle (7:00 A.M.–7:00 P.M., light). The rats were handled and weighed every morning at about 9:00 A.M.

Surgery

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

Implantation of Brain Cannulas

Permanent stainless steel cannulas (21.0 mm L; 0.3 mm OD; 0.1 mm ID) for drug infusion were stereotactically implanted bilaterally into either the LHA or the VMH according to the coordinates of De Groot (5) (+5.4 mm A; −2.3 mm V; ±1.7 mm L; and +5.8 mm A; −2.7 mm V; ±0.7 mm L, respectively). A sterile stainless steel obturator, flushed with the tip of the cannula, was inserted into each cannula between experiments to ensure that the cannula remained patent and pyrogen free. The cannula end protruding from the skull was protected with a 21-gauge protective sleeve, except for the last 4 mm at the top of the cannula. The protective sleeve was affixed to the skull with acrylic. A piece of polyethylene tubing was put around the cannula and obturator. A polyethylene cap was placed at the protruding end of the protective sleeve to cover the free end of the cannula.

Implantation of Heart Catheters

All animals were provided with a silicon heart catheter according to the techniques described earlier (23). This method allows blood sampling in unanesthetized undisturbed freely moving rats (22). Whenever intravenous infusions were to be performed, the rats were provided with a second silicon heart catheter implanted in the contralateral (left) jugular vein. This smaller second catheter (0.64 mm OD, 0.28 mm ID) allows blood to flow from the acromiodeltoid cephalic and anterior jugular veins to the external jugular vein. In general, rats did not lose 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 10:00 and 12:30 A.M., i.e., during the light cycle. Food was removed 1.5 h before the start of an experiment. Blood samples of 0.5 ml were taken for determination of blood glucose, plasma insulin, and FFA. Samples were taken at −11, −1, +2, +7, +12, +17, +22, +27, +32, and +42 min. Infusion into either the brain or blood circulation started at *time 0*. A tranfusion of citrated blood, obtained by means of a heart puncture from a donor rat, was given after each 0.5-ml sample. Between the withdrawal of blood samples, the tip of the heart catheter was filled with 4% citrate solution (instead of heparin solution) to avoid activation of endothelial lipase.

VMH and LHA Infusion Procedure

After removal of the cap and obturator, a sterile polyethylene tubing (400 mm L, 0.61 mm OD; 0.20 mm ID) was inserted into each cannula. The tubing had been previously filled with sterile test fluid except for the last 8 mm at the distal end of the tubing, which remained filled with air. This end of the tubing was sealed off at 4 mm. Thus any unwanted leakage into the brain before the beginning of the infusion was prevented. Under the sealed end remained an air bubble with a length of 4 mm. After attachment of the tubing to the cannulas, the animals were returned to their cages 30 min before the start of the experiments. The distal end of each infusion tubing remained outside the cage so as not to disturb the animal (22). At the start of the infusion, the sealed ends of the tubes were cut off and the tubing was immediately connected to the infusion pump. The infusion schedule was from *time 0* and continued for 20 min in all experiments. The beginning of movement of the 4-mm air bubble was designated as *time 0*. Constant movement of the air bubble verified that a continuous infusion at 0.25 μl/min was occurring. Solutions of administered substances were diluted with sterile saline from a stock solution immediately before the start of the experiments. The stock solutions consisted of 1.82 mg NE tartrate and E tartrate, respectively, (corresponding with 1 mg free base) dissolved in 1 ml distilled water containing 1 mg $Na_2S_2O_5$, 8 mg NaCl, and 0.1 mg Na EDTA. $Na_2S_2O_5$ and EDTA were added to prevent oxidation of NE and E. The pH of this solution was adjusted to 3.6 by adding 2 N NaOH. The animals received the infusions in random order with at least 1 day between subsequent infusions.

Chemical Determinations

The blood samples were transferred immediately to chilled (0°C) centrifuge tubes containing 10 μl heparin solution (500 U/ml) as an anticoagulant. Blood glucose was measured by the ferricyanide method of Hoffman (Technicon Auto-analyzer TMII) with 0.05 ml blood taken from the 0.5-ml sample. The remaining 0.45 ml of

blood was centrifuged at 4°C, and 0.15 ml of the supernatant was used for the FFA assay, and the remaining portion of the supernatant was stored at −30°C for the insulin assay. Plasma FFA were determined according to the method of Antonis (1). The method was adapted to 0.15 ml plasma. The plasma for FFA determination was extracted immediately and the evaporated extracts were stored at −30°C until FFA determination. Rat specific plasma immunoreactive insulin (IRI) was determined by means of a radioimmunoassay kit (Novo). Guinea pig serum M8309 served as antiserum for the insulin assay. Duplicate assays were performed on 25-μl plasma samples. The bound and free ^{125}I-labeled insulin was separated by means of a polyethylene glycol solution (23.75% wt/wt) as suggested by Henquin et al. (9).

Histology

Cannula placement was determined as follows: 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 quickly frozen 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 (5). Only the results of rats with correct cannula placements, i.e., in the VMH or LHA, were used.

Statistics

Wilcoxon matched-pairs signed-ranks test was used when levels of blood components obtained during neurotransmitter infusion in either the brain or the blood circulation were compared with basal preinfusion values. A Mann-Whitney *U* test was applied if levels of blood components obtained during neurotransmitter infusion into the brain were compared with those obtained during diluted solvent infusion into the brain. The criterion of significance was set at $P < 0.05$.

EXPERIMENTAL PROCEDURES AND RESULTS

Experiment 1

Infusion of NE in the LHA and VMH. NE was infused bilaterally into the VMH of six animals and into the LHA of nine animals at a rate of 25 ng/min for 20 min. This quantity of NE was chosen because the largest increase of insulin levels was obtained with this dose (25). As controls for *experiments 1* and 2, seven rats provided with VMH and LHA cannulas were infused with solvent for NE and E, diluted in the same way as the NE and E solutions, into the VMH and LHA at a rate of 0.25 μl/min for 20 min.

Results. The data from this experiment are presented in Table 1 and Fig. 1. Infusion of NE into the LHA led to a decrease of FFA during the whole period of infusion from a basal level of 0.19 ± 0.023 μeq/ml plasma, whereas infusion of diluted solvent into the LHA elicited a significant increase in FFA levels after termination of NE infusion. Insulin increased significantly from a basal level of 25 ± 2 μU/ml plasma during infusion and most time points after infusion. Diluted solvent infusion into the LHA did not result in an increase of plasma insulin. Regarding glucose, no change from basal preinfusion levels was present during either NE infusion or diluted solvent infusion in the LHA.

Infusion of NE into the VMH elicited a significant increase of FFA from a basal level of 0.13 ± 0.029 μeq/

TABLE 1. *Basal values ± SE of FFA, glucose, and insulin*

	FFA, μeq/ml plasma			Insulin, μU/ml plasma			Glucose, mg/dl blood		
	LHA	VMH	Peripherally	LHA	VMH	Peripherally	LHA	VMH	Peripherally
Solvent	0.11 ±0.014	0.12 ±0.012		40 ±2	33 ±2		113 ±4	112 ±4	
E	0.11 ±0.019	0.10 ±0.022	0.12 ±0.017	33 ±3	41 ±9	42 ±8	107 ±3	114 ±3	112 ±3
NE	0.19 ±0.023	0.13 ±0.029	0.13 ±0.015	25 ±2	33 ±2	43 ±5	111 ±3	111 ±3	109 ±4
ACh	0.12 ±0.018	0.08 ±0.018		36 ±3	35 ±9		114 ±3	117 ±6	

Basal values ± SE of free fatty acids (FFA), glucose, and insulin as measured at *min 1* before the start of infusion of one of the following solutions: solvent, epinephrine (E), norepinephrine (NE), or acetylcholine (ACh). LHA, lateral hypothalamus; VMH, ventromedial hypothalamus.

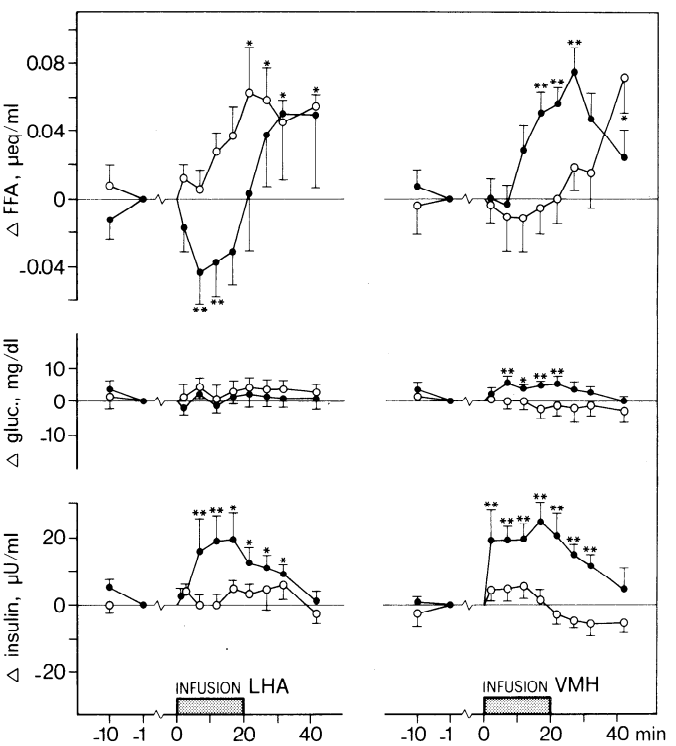

FIG. 1. Effects of norepinephrine (NE) infusion (25 ng·min^{-1}·cannula at a rate of 0.25 μl/min during 20 min) (●——●) and diluted solvent infusion (at a rate of 0.25 μl/min) (○---○) in lateral hypothalamus (LHA) (*left panel*) and in ventromedial hypothalamus (VMH) (*right panel*) on plasma free fatty acids (FFA), blood glucose, and plasma insulin. Data are means ± SE; μeq/ml plasma, mg/dl blood, and μU/ml plasma, respectively, changes from preinfusion levels of FFA, glucose, and insulin, which were average of those of *min 1* before infusion start. *Denotes a significant change from preinfusion level. **Denotes a significant change from diluted solvent infusion.

ml plasma during the later part of the infusion period and immediately afterward. Infusion of diluted solvent into the VMH did not result in a change of FFA during infusion. After termination of infusion, an increase was observed. Insulin increased significantly from a basal level of 33 ± 2 μU/ml during NE infusion and at most time points after termination of infusion. Diluted solvent infusion into the VMH did not cause a significant change in insulin. Glucose was slightly augmented during NE infusion into the VMH and immediately afterward, whereas blood glucose did not differ from basal values during diluted solvent infusion into the VMH.

Experiment 2

Infusion of E in the LHA and VMH. The same animals were used as in *experiment 1.* They received bilateral infusion of 25 ng E/min for 20 min.

Results. Table 1 shows the basal FFA, insulin, and glucose levels and Fig. 2 their changes during infusion of E into the LHA and VMH. Infusion of E into the LHA resulted in a nonsignificant increase of FFA, which did not differ from the FFA values obtained during diluted solvent infusion. Both insulin and glucose increased significantly above base-line levels at most time points during and after infusion of E.

Infusion of E into the VMH elicited an FFA increase in the later portion of the infusion period, which continued after termination of infusion. E infusion into the VMH caused an augmentation of insulin above base line. During E infusion into the VMH, glucose did not differ from basal values or from glucose values observed during diluted solvent infusion.

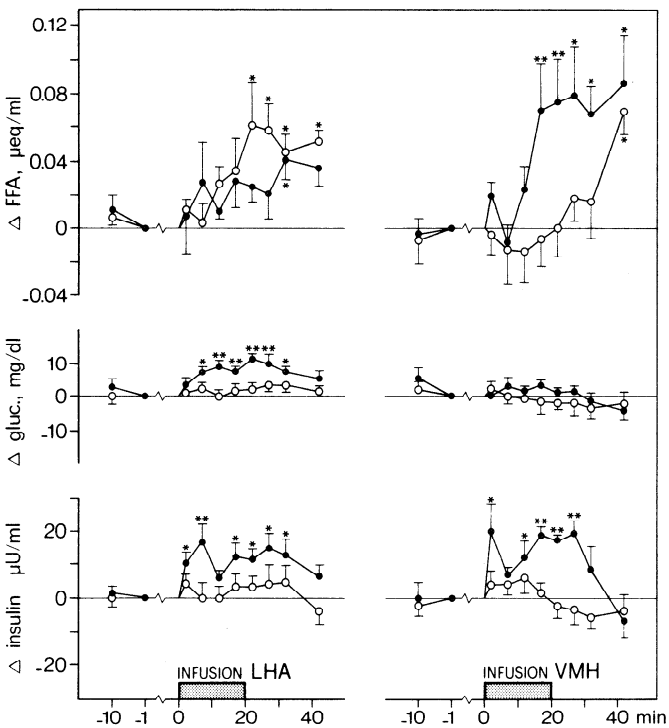

FIG. 2. Effects of epinephrine (E) infusion (25 ng·min^{-1}·cannula at a rate of 0.25 μl/min during 20 min)(●——●) and diluted solvent infusion (at a rate of 0.25 μl/min)(○---○) in LHA (*left panel*) and in VMH (*right panel*) on plasma FFA, blood glucose, and plasma insulin. See Fig. 1 for further explanation.

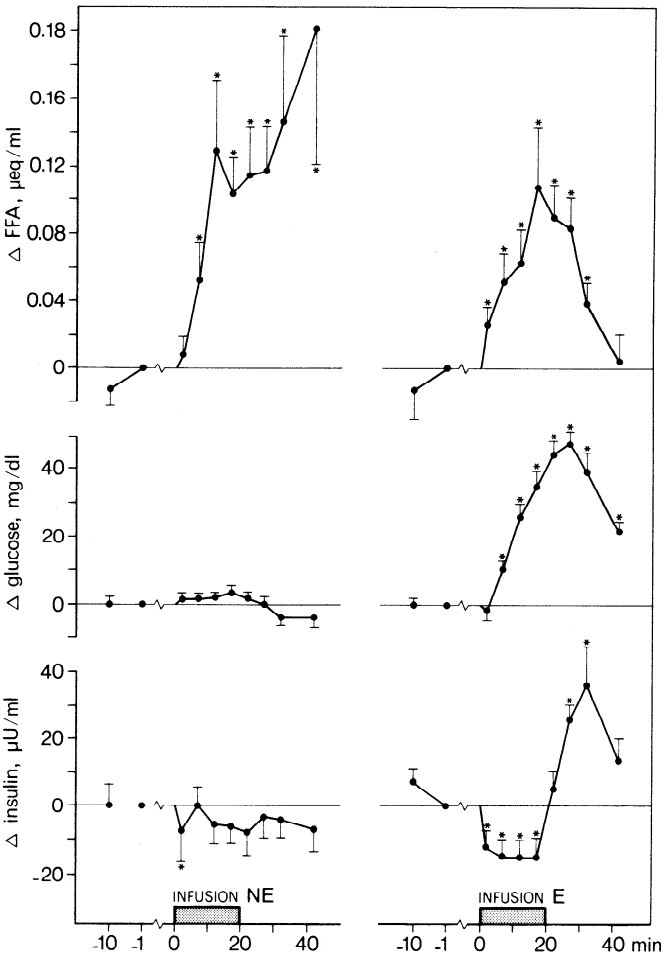

FIG. 3. Mean changes ± SE of plasma FFA (μeq/ml), blood glucose (mg/dl), and plasma insulin (μU/ml) from preinfusion levels, which are average of those of *min 1* before infusion start, during intravenous NE infusion (*left panel*) and intravenous E infusion (*right panel*) at a rate of 50 ng/min. See Fig. 1 for further explanation.

Experiment 3

Infusion of NE and E into the blood circulation. NE and E were infused into the blood of six rats at a rate of 50 ng/min and dissolved in 0.02 ml saline for 20 min.

Results. The results of this experiment are described in Table 1 and Fig. 3. Intravenous NE infusion for 20 min elicited a sharp increase in FFA during and after infusion. Insulin showed a small decrease, whereas glucose did not differ from basal values. On the other hand, an intravenous E infusion for 20 min caused a significant increase of FFA only during the E infusion period, and thereafter they decreased to preinfusion levels. Insulin decreased significantly during E infusion and increased significantly after termination of E infusion. Glucose showed a sharp increase during E infusion and reached a peak value at *min 27,* whereupon a decrease followed.

Experiment 4

Infusion of acetylcholine (ACh) in the LHA and VMH.

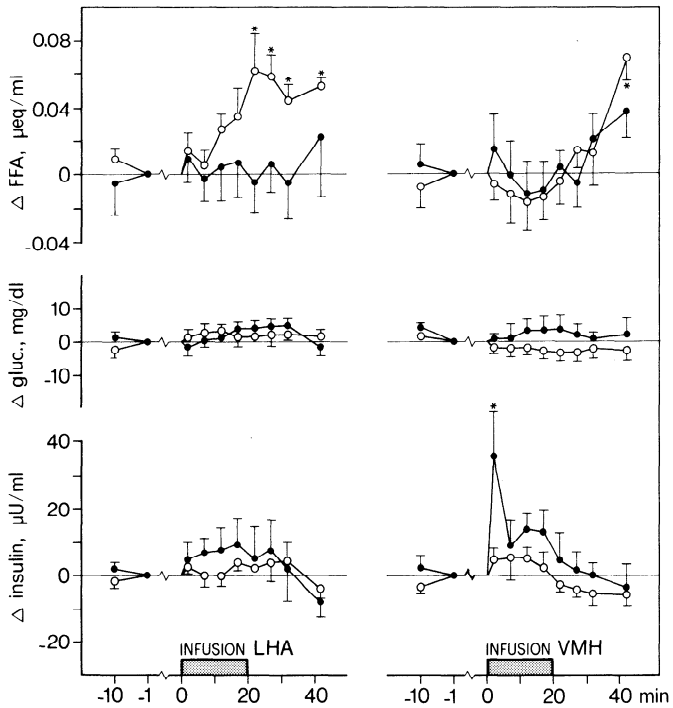

FIG. 4. Effects of acetylcholine (ACh) infusion (25 ng·min^{-1}·cannula at a rate of 0.25 μl/min during 20 min)(●——●) and diluted solvent infusion (at a rate of 0.25 μl/min)(○——○) in LHA (*left panel*) and in VMH (*right panel*) on plasma FFA, blood glucose, and plasma insulin. See Fig. 1. for further explanation.

The same animals as used in *Experiments 1* and *2* received bilaterally an infusion of 25 ng ACh/min for 20 min.

Results. The data from this experiment are presented in Table 1 and Fig. 4. Infusion of ACh into the LHA had no influence on FFA levels. Although FFA were lower during ACh infusion than during diluted solvent infusion, the difference did not attain statistical significance. Neither insulin nor glucose differed from basal levels, nor did they differ from values obtained during infusion of diluted solvent into the LHA. ACh infusion into the VMH did not elicit increases in FFA levels and the FFA profile was very similar to that observed during diluted solvent infusion. Also, glucose and insulin levels showed only minor changes compared with diluted solvent infusion, except for *time-point 2* min, where insulin increased significantly.

DISCUSSION

The most conspicuous results of this study are *1*) the decrease of FFA, which is accompanied by a simultaneous increase of insulin during noradrenergic stimulation of the LHA; *2*) the elevation of FFA, glucose, and insulin during noradrenergic stimulation of the VMH; *3*) the absence of an effect of adrenergic stimulation on FFA levels but with elevations of glucose and insulin; *4*) the increase of FFA and insulin during adrenergic stimulation of the VMH; and *5*) the absence of an effect of cholinergic stimulation of the LHA and VMH except for a small increase of insulin during stimulation of the VMH.

It is clear from this study that the effects of administration of NE and E into the hypothalamus are the opposite of that observed following peripheral administration. Accordingly, the observed effects of central administration of NE and E cannot be accounted for by leakage of NE and E from the brain into the periphery. Peripheral infusion of NE leads to a sharp increase of plasma FFA, whereas glucose does not change and insulin is only slightly suppressed. The picture is different during E infusion: both FFA and glucose levels increase strongly and there is a simultaneous suppression of insulin.

After termination of E infusion, insulin shows a sharp increase while FFA and glucose decrease. It is generally assumed that lipolysis occurring in adipocytes and the concomitant increase of FFA is caused by a β_1-adrenergic mechanism where α_2-adrenoreceptor stimulation suppresses lipolysis (21). Likewise, insulin release from the B-cell of the islets of Langerhans is stimulated by a β-adrenoreceptor mechanism (15) and counteracted by an α_2-adrenoreceptor mechanism (14). It has to be taken into consideration that both NE and E are potent α- and β-receptor agonists. In our study we found an increase of FFA during both NE and E infusions in the periphery. After termination of infusion, FFA continued to increase in the experiment giving NE, whereas FFA decreased in the experiment giving E. Also, E infusion suppressed insulin more than NE infusion (see Fig. 3). These observations indicate that the dose of E (50 ng/min) administered in this study has a stronger α_2-adrenoreceptor effect than the same dose of NE. Apart from this more powerful α_2-adrenoreceptor effect of E, which prevents FFA from increasing sharply, the hyperglycemia produced by E infusion may also have counteracted the mobilization of FFA, i.e., hyperglycemia increases the reesterification of FFA within the adipocyte (8), although it must be admitted in this case that insulin is not suppressed enough to counteract this reesterification. The previously reported escape of insulin inhibition, which is caused by NE and E infusion, due to concomitant hyperglycemia is absent (10). This lack of escape can probably be attributed to the short period of E administration (20 min). The decrease of FFA after termination of E infusion can be explained by the fact that the release of inhibition of the B-cell leads to an immediate increase of insulin because of the high glucose levels. The combined high glucose and insulin levels result in an immediate suppression of lipolysis and a stimulation of lipogenesis so that a decrease of FFA ensues.

A completely different picture is obtained after infusion of either NE or E into the hypothalamus. Available data regarding plasma FFA changes after noreadrenergic manipulation of the CNS in rats suggest that administration of NE in the lateral ventricle induces an increase in plasma FFA (2). Barbosa and Migliorini (2) injected rather high concentrations of NE into the lateral ventricles (4.225 ng–67.600 ng) and withdrew blood by cutting the tip of the tail. In previous studies we found that noradrenergic stimulation of the VMH and LHA elicits glucagon (24) and insulin release (25), respectively. This

release of glucagon and insulin was mediated by the sympathetic and parasympathetic nervous system (24, 25). Because white adipose tissue receives sympathetic fibers which stimulate adipocytes either directly or indirectly via local release of NE (3), it might be surmised that the LHA and VMH areas, which are responsible for the observed neural regulation of the function of the islets of Langerhans, are also involved in the regulation of FFA from the adipocytes. Indeed the presented data show that noradrenergic stimulation of the VMH and LHA elicits an increase and decrease respectively of FFA, thus indicating that these areas of the CNS are responsible for the observed phenomena. Only increases of FFA have been previously described after CNS manipulation with neurotransmitters. The increase of FFA after noradrenergic stimulation of the VMH can probably not be accounted for by a concomitant increase of glucagon, which has been reported in an earlier study (24). This increase is small and affects only glycogenolysis in the liver rather than lipolysis (16).

Another point merits attention here. Noradrenergic stimulation of the LHA causes a decrease of FFA, no change of glucose, and an increase of insulin, whereas adrenergic stimulation of the LHA elicits an increase of glucose and insulin and no change of FFA. A possible explanation accounting for the different effects of noradrenergic and adrenergic stimulation of the LHA on FFA and glucose levels might be as follows. The LHA contains three separate neuronal circuits involved in the regulation of blood glucose, plasma FFA, and insulin. These separate neuronal circuits contain different sets of receptors. Most probable, β_2-receptors are present in the circuit responsible for glucose release because only E elicits the response. In this respect it has to be borne in mind that NE has a markedly reduced potency at the β_2-adrenoreceptor, whereas the potency of E and NE on α_1-, α_2-, and β_1-adrenoreceptors are more or less equal. An α_1-, α_2-, or β_1-adrenoreceptor present in the circuit involved in insulin release may account for the release of insulin. The observation that NE infusion into the LHA suppresses plasma FFA, whereas E infusion is without effect, cannot be reconciled easily with the just mentioned potency of NE and E on α_1-, α_2-, β_1-, or β_2-adrenoreceptors. Further investigation is needed to clarify the nature of the receptor involved.

Regarding the VMH, it is also likely that three separate neural circuits are responsible for the observed effects on plasma FFA-insulin and blood-glucose concentrations. Regarding the circuits involved in insulin and FFA release, again stimulation of α_1-, α_2-, or β_1-adrenoreceptors might account for the observed increases. With respect to FFA, a β_1-receptor mechanism is most probably because Barbosa and Migliorini (2) found an inhibition of FFA release after NE injection into the lateral brain ventricle only if, before NE administration, the β-blocker propranolol was injected into the cerebrospinal fluid. The observation that noradrenergic stimulation of the VMH causes an increase in blood glucose and that adrenergic stimulation is without effect cannot be clarified with the current concept of α_1-, α_2-, β_1-, and β_2-adrenoreceptor stimulation by NE and E.

We find a similar release of insulin with the used dosage of NE and E (25 ng/min) irrespective of administration either in the LHA or VMH. In previous work we reported that infusion of 12.5 ng NE/min into the LHA elicits insulin release (24, 25), whereas infusion of 12.5 ng NE/min into the VMH was without effect on insulin (23). The finding that infusion of a higher dosage of NE into the VMH elicits insulin release whereas a lower dosage is without effect can be attributed to the spreading of infused NE. It is possible that the neural circuit involved in insulin release, which is definitely present in the LHA and terminates on the dorsal motor nucleus of the vagus (13), passes the VMH on a short distance.

Compared with data in the literature, our basal levels of FFA are low (~0.15 μeq/ml plasma; see Table 1). However, we sampled just at the beginning of the daylight period, a time at which lipogenesis, which is maximum during nighttime, is still high and FFA levels are low (11). Moreover, the subjects were young lean animals, which have lower insulin levels and a better glucose tolerance than older obese ones. Their adipocytes can take up glucose more easily, and this in turn inhibits lipolysis (26). Finally, we used methods of blood sampling that did not disturb the rats and therefore minimized sympathetic arousal that might otherwise elicit lipolysis and increases of FFA (cf. Fig. 3).

Attention has to be paid to the effects of infusion of diluted solvent in the LHA and VMH. In contrast to its effects on glucose and insulin, FFA levels increased slightly during infusion of diluted solvent. This was more pronounced during infusion into the LHA than into VMH (cf. left and right panels of Figs. 1 and 2). A possible explanation might be that irritation of nervous tissue in LHA and VMH occurs that is caused by the flow of fluid, an inadequate ionic composition of the diluted solvent, or a deviating pH.

The most intriguing aspect of this study is the unexpected increase of all three measured substances, i.e., insulin, glucose, and FFA, during noradrenergic and adrenergic stimulation of the VMH. The decrease of FFA during noradrenergic stimulation of the LHA might be explained by the simultaneous increase of insulin, but this explanation is evidently inadequate because noradrenergic stimulation of the VMH elicits increases of all three parameters. The most plausible explanation is that VMH can stimulate lipolysis and also increases FFA independently of the levels of glucose and insulin. Very recent data in the literature show, first, that the LHA can manipulate insulin levels without changing glucose and glucagon (25) and, second, that the VMH can stimulate glycogenolysis and glycogenesis with concomitant changes in glucose (18) independent of changes in either insulin or glucagon. These observations have very important implications regarding regulation of fuel availability (i.e., glucose and FFA) to body cells. The apparent close correlation between glucose and FFA and their regulating hormones in the blood circulation (e.g., as occurs during eating when glucose and insulin increase and FFA decrease) can be completely disrupted by interference of the CNS. This interference might be very

important during arousal of the body, e.g., physical activity, stress, and fasting or replenishment of its stores. Indications in literature suggest that the VMH must play an important role in this regulation because lesioning of the VMH interferes with fat mobilization during both fasting and exercise (4). The question arises as to which pathways are involved from the VMH and LHA to adipocytes. Both the autonomic nervous system and humoral factors might be responsible. The idea that the VMH can arouse the sympathetic system and the LHA the parasympathetic system (18) are in agreement with the decrease of FFA during noradrenergic stimulation of the LHA and the increase of FFA during adrenergic and noradrenergic stimulation of the VMH described in this study. However, humoral factors cannot be excluded because the pituitary, which can release powerful lipolytic factors such as lipotropines, is under the constant influence of the hypothalamus. Further investigations are needed to unravel the roles of the autonomic nervous system and hypothalamically induced humoral factors in the regulation of plasma FFA.

Finally, it has to be taken into account that the VMH and LHA are strongly involved in the regulation of food intake. From this study and many others (for a very recent review see Ref. 27) it is clear that these areas of the hypothalamus regulate fuel substrate availability (glucose and FFA) in response to the ever changing metabolic needs of the cells of the body. Over the long run utilization of fuel substrates by the body must be matched accurately with food intake otherwise the remarkable stability of body weight cannot be explained. Apparently the VMH and LHA are active in this process, however, the way in which they achieve their coordinated functioning is unclear at present.

The authors thank Professor Dr. B. Bohus and Dr. J. H. Strubbe for critical reading of the manuscript, J. Westerhoff for his technical assistance, and C. Bloem for typing the manuscript.

This investigation was supported in part by the Foundation for Medical Research (FUNGO), which is subsidized by the Dutch Organization for the Advancement of Pure Research (ZWO).

Received 18 January 1984; accepted in final form 11 July 1984.

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