Effect of electrical stimulation of the hypothalamus on plasma free fatty acid concentration in cats

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Abstract The effect of electrical stimulation of various hypothalamic regions on levels of plasma free fatty acids, glucose, triglycerides, and cholesterol was studied in fasted cats. Appreciable changes were observed in plasma free fatty acids and glucose but not in plasma triglycerides or cholesterol. These changes appeared to be dependent upon small differences in the placement of electrodes and could not be related to a distinct hypothalamic locus. The results indicate that there is a dissociation between hypothalamic neurons that may affect plasma glucose concentration and those that may affect the plasma free fatty acids. It is suggested that the hypothalamus of the cat contains neurons that may influence autonomic discharge to adipose tissue and thus affect the plasma free fatty acid level and other neurons that may influence autonomic discharge to the liver and thus affect glucose output into the circulation. The distribution of both types of neurons is not limited to a distinct region of the hypothalamus in cats.

Supplementary key words fat mobilization

The role of nervous and endocrine mechanisms in the regulation of the storage and mobilization of fat has been extensively studied (1–6). It is also known that the hypothalamus controls many autonomic (7) and endocrine functions (8). However, relatively few experiments bearing directly on the influence of the hypothalamus on fat mobilization have been reported. It has been shown that rabbits (9) and dogs (10) may respond with an appreciable increase of plasma FFA concentration as a result of stimulation of various regions of the brain stem. Other evidence that electrical stimulation of the hypothalamus may influence the metabolism of various lipids has also been presented (11–14), but these investigations did not include plasma analyses of FFA and are not directly related to fat mobilization.

This report deals with experiments that were designed to investigate the possibility that the hypothalamus can exert an influence on the mobilization of fat. A survey of the cat's hypothalamus had been carried out in an attempt to locate regions the stimulation of which would result in changes in the plasma FFA concentration. The concentrations of plasma glucose, TG, and cholesterol were also measured in order to investigate a possible relationship between induced change in plasma FFA and changes in the concentration of these compounds. Blood pressure, respiration, heart rate, and EEG were monitored in each experiment for the purpose of studying the relationship between possible changes in these variables and changes in the plasma content of the various compounds after hypothalamic stimulation. The effects of stimulus intensity and duration on the responses obtained were also investigated.

METHODS

Animals

All experiments were carried out on male cats, 2–4 kg in weight, that were fasted for 24 hr prior to the operation. The animals were anesthetized with ether, and then sodium pentobarbital was administered through a polyethylene catheter in the femoral vein as required to maintain surgical anesthesia. Both femoral arteries were catheterized with polyethylene tubes (Clay Adams PE-90). One

Abbreviations: FFA, free fatty acids; TG, triglycerides; EEG, electroencephalogram.

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of these catheters was used for the measurement of blood pressure and the other was used for the withdrawal of blood samples. The trachea was cannulated and the animal's head was then fixed in a stereotaxic instrument. The skull was exposed and small brass screws were placed in the parietal bone for the recording of the EEG. Holes were drilled in the skull where necessary for the stereotaxic placement of electrodes. Blood pressure, respiration, and the EEG were recorded with a Grass model 5 polygraph. A pressure transducer (Statham p-23 AC) was used for the measurement of blood pressure. Respiration was recorded with a thermocouple.

Serial blood samples from immobilized cats were obtained after the animal was injected with Flaxedil (gallamine triethiodide). Operated areas were swabbed with 2% lignocaine hydrochloride solution, and animals were artificially ventilated with room air at frequencies and volumes that were estimated from the literature (15).

All experiments began about 2 hr after the end of the operation, by which time the animals appeared to have reached a steady state judging from a stable blood pressure, heart rate, respiration rate, and a uniform EEG.

Blood sampling

In all the experiments, arterial blood samples were taken from the cannula in the femoral artery. When TG was estimated, blockage of the cannula was prevented by the use of a nylon stylet, which was approximately 1 mm longer than the catheter. This stylet was removed before sampling, and the first 0.5 ml of blood was discarded as dead space. The required volume was then withdrawn into a heparinized syringe. When plasma TG was not determined, blockage of the cannula was prevented by the use of a diluted heparin solution. Preliminary observations have shown that the presence of heparin did not change appreciably the concentration of FFA in the plasma (this may be explained by a low activity of lipoprotein lipase in the serum of fasted cats). The blood samples were centrifuged at 2000 rpm for 10 min at 4°C. Aliquots of plasma were removed as required for the determinations of plasma glucose, FFA, cholesterol, and TG.

Determination of compounds in plasma

Free fatty acids. Plasma FFA concentration was determined by titration or by colorimetry. Titration was done after extraction of the plasma according to Dole (16) and washing of the heptane phase with water. A 2-ml aliquot of the upper heptane phase was then transferred to a clean centrifuge tube, and 1 ml of isopropanol was added with a drop of thymol blue, which served as indicator. Nitrogen gas was then passed through a CO_2 trap into the tube for mixing. Sodium methoxide, 0.1 N in methanol, was used for titration.[1] In nine experiments, colorimetric determinations were also performed according to Duncombe (17) as modified by Stoner and Matthews (18). FFA values obtained by colorimetric determination were 10–15% lower than FFA values determined by titration in the same blood sample; nevertheless, the percentage change observed by both methods after hypothalamic stimulation did not differ appreciably. In both cases, standard curves were prepared with palmitic acid for each experiment. The standard curves were accurate to within ± 2%, and recoveries from plasma were 96–102%.

Triglycerides. This analysis was done by colorimetric determination of glycerol (19) after hydrolysis of the TG in a sample of Dole's extract.

Cholesterol. The total plasma cholesterol was determined colorimetrically (20).

Glucose. The enzymatic method modified for the determination of glucose in small amounts of plasma (21) was used.

Electrical stimulation

The apparatus for electrical stimulation was arranged in the accepted manner (22). A Tektronix 160A power supply was used together with Tektronix 161 and 162 pulse generators. The output from the pulse generator was led to an isolation transformer and from there passed through a resistance of 1000 ohms. The voltage across the resistor was monitored with an oscilloscope, and constant current was employed in all experiments.

Bipolar concentric electrodes, insulated throughout their length except for 0.5–1 mm at the tip, were prepared from stainless steel tubes (I.D. 0.38 m.m, O.D. 0.71 mm), through which was threaded an insulated copper wire.

Stereotaxis

The coordinates of the hypothalamic region to be stimulated were determined from a stereotaxic atlas of the brain stem of the cat (23), and the electrode position in the brain was verified by histological examination (24).

Analysis of data

In each experiment, the mean concentration of the measured plasma constituent in blood samples drawn during the period prior to stimulation (control period) was taken as 100%, and the standard deviation from this mean was calculated. Values representing blood samples obtained during the stimulation period or after the end of stimulation were converted to percentages of the mean

[1] Unpublished method for FFA determination developed by Dr. C. Allweis.

value for the control period. Values above two standard deviations from the mean control value were considered as representing an increase in the concentration of the measured compound that could be causally related to the stimulation with a probability of at least 95% ($P < 0.05$). Similarly, values below two standard deviations from the mean control value were considered as representing a decrease due to the effect of the hypothalamic stimulation. Values from individual animals used in the control experiments were treated similarly, and the range of distribution of control values for each time of blood sampling was obtained and expressed as percentage of the mean value that represented the control prestimulatory period. Data from stimulated animals that indicated either an increase or a decrease in the concentration of a given compound at a certain sampling time were tested against values of that compound obtained at the same sampling time from the control group. The nonparametric Mann-Whitney U test (25), which is a most useful alternative to the parametric t test, was used in order to avoid the t test's assumption that the values obtained in the experimental and control groups were drawn from a normally distributed population and that the variance was of the same order. The null hypothesis was that values indicating either an increase or a decrease in the concentration of a given compound after stimulation in individual experiments were obtained from a population that had the same distribution as values in the control group at the same time of blood sampling. An effect of the stimulation was considered significant only when the probability of its occurrence under the null hypothesis was less than 1%.

RESULTS

Plasma concentrations of FFA, TG, cholesterol, and glucose in Nembutal-anesthetized or immobilized cats

Since changes in plasma constituents are later given as percentage changes compared with their levels prior to stimulation, it seems necessary to present mean values for their concentrations during control periods. Levels of several plasma constituents in Nembutal-anesthetized cats are compared in Table 1 with the levels of the same constituents in the plasma of Flaxedil-immobilized animals. The analyses were carried out on blood samples withdrawn from animals during a period of 2–3 hr after termination of the operation, i.e., 3–4 hr after initiation of anesthesia or immobilization.

The mean values for the concentrations of FFA, TG, and glucose in the plasma of anesthetized cats were lower than the corresponding values from immobilized cats, whereas no significant difference was seen in the case of cholesterol (Table 1). It is interesting to note a similar relationship between glucose and FFA or between FFA and TG in the plasma of either anesthetized or immobilized cats. The molar ratio of the mean concentration of glucose to the mean concentration of FFA in the anesthetized cats is 14.5 compared with 14.2 for the immobilized cats. Similarly, the ratio for FFA and TG in the anesthetized cats is 2.6 as compared with 2.7 for the immobilized animals.

Effect of weak electrical stimulation on the concentrations of FFA, TG, cholesterol, and glucose in the plasma

Weak stimulation (intensity, 100–500 μa; pulse duration, 1 msec; frequency, 20/sec) was applied to 11 cats. Electrode placement in these experiments varied but was generally in the regions of the supraoptic nucleus, the ventromedial nucleus, and the mammillary bodies. Blood samples (6 ml) were removed each 60 min during a period of 240 min. The stimulation was applied before withdrawal of the third blood sample. In some experiments brief stimulation (2 min) was used and in other experiments a prolonged stimulation (60 min) was applied.

Stimulation in hypothalamic loci posterior to the frontal plane 12 (see Fig. 1) uniformly resulted in a decrease in the concentration of plasma FFA. These results were obtained in six cats after brief or prolonged stimulation and were statistically significant at the first sampling periods following stimulation (Table 2). The other five cats, stimulated in hypothalamic loci anterior to the frontal plane 12, did not show significant changes in their plasma FFA.

An increase in the plasma glucose concentration was seen in 3 out of the 11 cats. This hyperglycemia was seen following prolonged stimulation and appeared to be significant when compared with the control group (Table 2). Loci which when stimulated elicited the hyperglycemic response are shown in Fig. 1.

TABLE 1. Plasma levels of glucose, FFA, TG, and cholesterol in anesthetized or immobilized cats[a]

Group	Glucose	FFA	TG	Cholesterol
	mg/100 ml	*μmoles/ml*	*μmoles/ml*	*mg/100 ml*
Anesthetized (Nembutal)	183 ± 32 (40)	0.71 ± 0.23 (51)	0.27 ± 0.02 (12)	101 ± 29 (12)
Immobilized (Flaxedil)	305 ± 47[b] (12)	1.20 ± 0.32[b] (12)	0.44 ± 0.10[b] (12)	128 ± 14 (6)

[a] Concentrations given are averages ± SD. Numbers in parentheses are the number of animals in each group.
[b] Significantly different ($P < 0.01$) from the corresponding value for the anesthetized group.

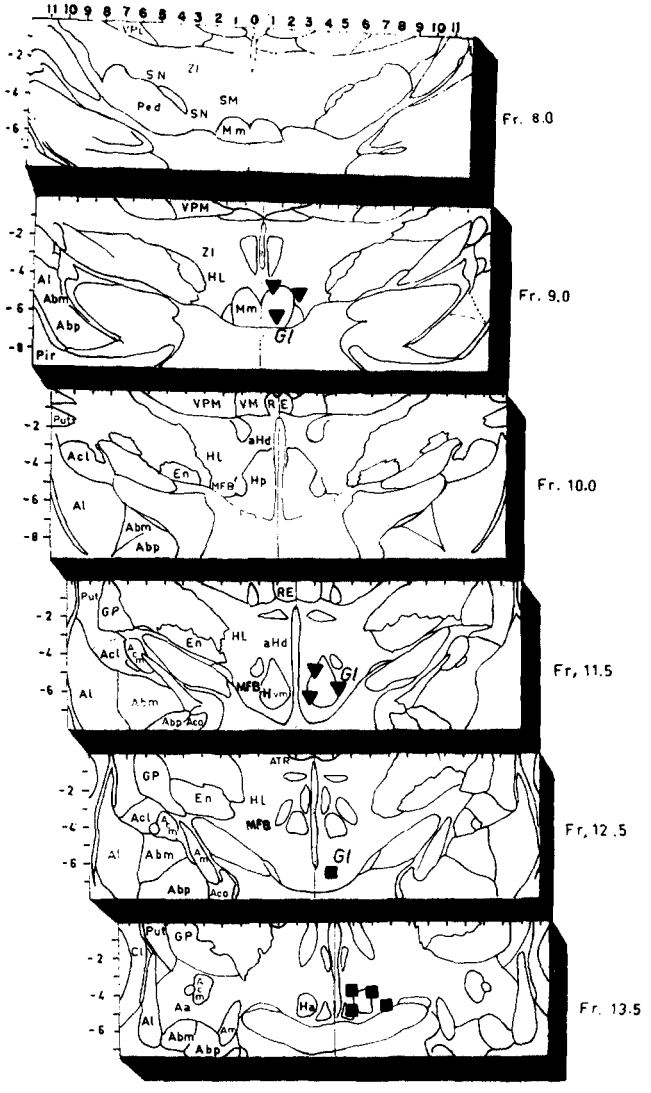

FIG. 1. Electrode positions in hypothalamus, and plasma FFA and glucose concentrations after weak electrical stimulation (100–500 μa). ■, no change in plasma FFA; ▼, plasma FFA decreased; *Gl*, plasma glucose increased.

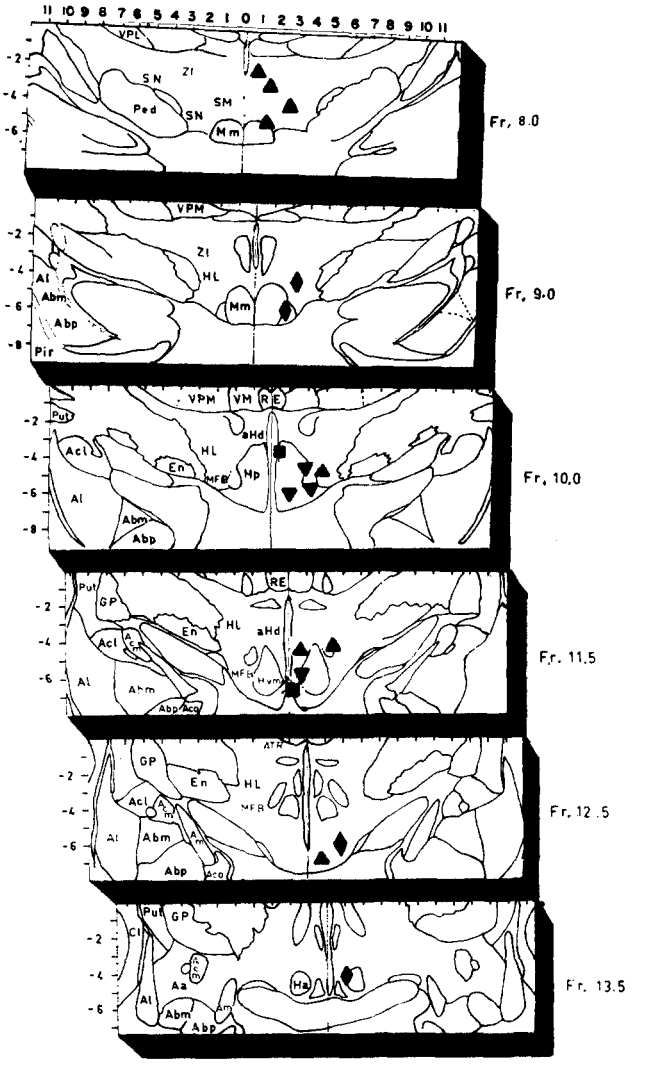

FIG. 2. Electrode positions in hypothalamus, and plasma FFA concentrations after moderate electrical stimulation (1 ma). ■, no change; ◆, rebound; ▲, increase; ▼, decrease.

No differences were observed in TG or cholesterol. The stimulation did not affect blood pressure, heart rate, or the EEG in any of these experiments.

Effect of moderate stimulation on the concentrations of FFA and glucose in the plasma

This series of experiments was designed after preliminary observations on several cats in which strong electrical stimulation (2.5 ma across the hypothalamus over a period of 5 min) resulted in either an increase or a decrease in the plasma FFA concentration while the plasma glucose level increased consistently. An increase in the systemic blood pressure had also been observed, whereas no change in the concentration of either TG or cholesterol in the plasma was found. These observations raised a question as to whether the hypothalamus of the cat contains two types of neurons that may affect the plasma FFA level in opposite directions. The following series of experiments was carried out in order to study this possibility and also to clarify whether there is a spatial separation of hypothalamic neurons influencing the concentration of plasma FFA and those influencing the plasma glucose level.

This series included 18 stimulation experiments and 8 control experiments on anesthetized cats. The experimental time was shortened to 120 min in order to avoid considerable variations in the concentrations of FFA and glucose that were observed occasionally after hr 2 in previous control experiments. Blood samples (3 ml) were removed each 15 min and the first three samples were used for the determination of control levels of FFA and glucose in the plasma. Electrical stimulation was applied over a period of 30 min starting after the with-

TABLE 2. Changes in plasma levels of FFA and glucose after weak stimulation[a]

Observation	Cat No.	Time of Blood Sampling (min): Before Stimulation (Control) 0–60	After Stimulation 120[c]	After Stimulation 180	After Stimulation 240	Stereotaxis[b] F	Stereotaxis[b] S	Stereotaxis[b] H
FFA variability in controls (n = 8)		100 ± 6	102 ± 8	101 ± 10	103 ± 16			
FFA decrease after brief stimulation (2 min)	1B	100 ± 5	80	113	114	9	1	−5
	4B	100 ± 3	76	99	101	9	3	−6
	6B	100 ± 6	73	81	93	11.5	1.5	−6
			$U = 0$	NS	NS			
			$P \leqslant 0.006$					
FFA decrease after prolonged stimulation (60 min)	2P	100 ± 3	53	56	62	9	1	−6
	3P	100 ± 5	79	72	93	11.5	2	−5
	6P	100 ± 3	65	78	95	11.5	2.5	−5
			$U = 0$	$U = 0$	NS			
			$P \leqslant 0.006$	$P \leqslant 0.006$				
Glucose variability in controls (n = 8)		100 ± 4	99 ± 6	102 ± 11	108 ± 14			
Glucose increase after prolonged stimulation	2P	100 ± 6	129	122	115	9	1	−6
	3P	100 ± 5	138	140	121	11.5	2	−5
	5P	100 ± 4	131	116	109	12.5	1	−6
			$U = 0$	NS	NS			
			$P \leqslant 0.006$					

[a] Changes are presented as percentages of the mean value observed for each animal during the prestimulatory period (indicated as 100 ± SD). Changes obtained in control animals were averaged, and the mean ± SD is presented for each time of blood sampling. For further explanation on analysis of data, see text.

[b] Coordinates of electrode placement are given according to maps in Ref. 23. F, frontal plane; S, saggital; H, horizontal.

[c] Sample taken immediately after stimulation was terminated.

drawal of the third blood sample. The stimulus used was a square pulse of 1 ma intensity; pulse duration was 1 msec and the frequency 50/sec. This stimulus is arbitrarily referred to as a moderate stimulus.

Moderate stimulation in defined hypothalamic regions elicited three types of responses in the plasma FFA level. Out of 18 cats stimulated, 4 showed a significant decrease in the plasma FFA either during or immediately after stimulation. Four other cats showed a rebound in their FFA concentration: a decrease during the stimulation period followed by an increase during the 40–60 min after the stimulation was terminated. Eight cats showed an increase in their plasma FFA concentration during the stimulation or immediately upon the end of the stimulation period. Only two of the animals did not respond to stimulation with an appreciable change of plasma FFA level. The magnitude of the response obtained in different cats is shown in Table 3. An overlapping of hypothalamic areas that are related to the observed response was indicated when the type of plasma FFA response was related to the position of the electrode in the hypothalamus (Fig. 2).

Changes in the concentration of plasma glucose were seen in nine cats (Table 3). In three cats an increase that varied in the range of 25–50% of the average concentration prior to stimulation was noted. The other six cats responded to stimulation with a decrease (20–40%) in their plasma glucose level. Regression analysis of the data from these cats showed that there was no significant linear relationship between the significant changes in plasma glucose and plasma FFA concentrations after stimulation.

Electrical stimulation under these conditions caused changes (either an increase or a decrease) in the mean blood pressure and in the heart rate in several experiments, but such changes could not be correlated with changes observed in plasma FFA during the same period.

DISCUSSION

On the basis of our results it appears that the hypothalamus of the cat contains neurons whose activation by electrical stimulation produces a decrease in the concentration of plasma FFA, and other neurons whose activation causes an increase in plasma FFA concentration. The direction and magnitude of the response to electrical stimulation will therefore be dependent upon the relative number of each type of neuron activated and the time courses of their effects.

The fact that weak stimulation in the posterior hypothalamus uniformly resulted in a decrease in plasma FFA concentration suggests that neurons capable of causing a decrease in plasma FFA are located mostly in the posterior hypothalamus. This region also appears to contain neurons capable of causing an increase in plasma FFA concentration, but their activation requires a stronger current.

Our results show that an elevation of plasma FFA could be obtained during or immediately after the stimulation of various hypothalamic areas with a current

TABLE 3. Changes in plasma levels of FFA and glucose after moderate stimulation[a]

Observation	Cat No.	Time of Blood Sampling (min): Before Stimulation (Control) 0–30	After Onset of Stimulation[c] 45	60	75	90	105	120	Stereotaxis[b] F	S	H
FFA range in controls (n = 8)		100 ± 4	102 ± 4	99 ± 5	103 ± 3	98 ±6	102 ± 8	104 ± 5			
FFA increase	1I	100 ± 6	124	132	106	107	109	105	8	0.5	−2.5
	2I	100 ± 2	114	119	99	101	94	100	8	1.5	−3
	3I	100 ± 4	108	126	132	102	98	96	8	3	−4
	4I	100 ± 2	122	118	109	98	101	102	8	1	−5
	5I	100 ± 3	114	125	101	103	99	96	10	3	−5
	6I	100 ± 5	196	221	158	125	119	108	11.5	3	−4.5
	7I	100 ± 4	112	144	141	141	122	106	11.5	1	−4.5
	8I	100 ± 5	98	153	121	99	96	103	12.5	1	−6
			$U = 5$ $P \leqslant 0.001$	$U = 2$ $P \leqslant 0.001$	NS	NS	NS	NS			
FFA decrease	1D	100 ± 4	79	77	88	96	94	98	10	1	−5
	2D	100 ± 2	71	72	65	69	70	70	10	2	−4.5
	3D	100 ± 2	80	84	89	94	102	105	10	2.5	−5
	4D	100 ± 3	70	75	89	102	104	109	11.5	1	−6
			$U = 0$ $P \leqslant 0.002$	$U = 0$ $P \leqslant 0.002$	NS	NS	NS	NS			
FFA rebound	1R	100 ± 5	73	77	88	105	138	147	9	2	−6
	2R	100 ± 3	66	60	89	99	128	145	9	2.5	−4.5
	3R	100 ± 6	75	80	148	154	131	143	12.5	2	−5.5
	4R	100 ± 3	65	70	83	90	126	148	13.5	1.5	−5
			$U = 0$ $P \leqslant 0.002$	$U = 0$ $P \leqslant 0.002$	NS	NS	$U = 0$ $P \leqslant 0.002$	$U = 0$ $P \leqslant 0.002$			
Glucose range in controls (n = 8)		100 ± 6	102 ±6	103 ±7	106 ±5	106 ±6	109 ±5	110 ±7			
Glucose increase	2I	100 ± 5	122	125	125	116	112	112	8	1.5	−3
	7I	100 ± 3	148	112	110	98	96	104	11.5	1	−4.5
	3D	100 ± 5	142	148	128	106	101	99	10	2.5	−5
			$U = 0$ $P \leqslant 0.006$	NS	NS	NS	NS	NS			
Glucose decrease	2D	100 ± 6	71	69	78	78	108	112	10	2	−4.5
	1I	100 ± 7	60	102	111	109	115	118	8	0.5	−2.5
	3I	100 ± 3	78	96	105	109	107	104	8	3	−4
	2R	100 ± 5	98	77	73	82	99	109	9	2.5	−4.5
	4D	100 ± 5	73	99	112	108	111	111	11.5	1	−6
	4R	100 ± 6	68	89	97	103	107	109	13.5	1.5	−5
			$U = 2$ $P \leqslant 0.001$	NS	NS	NS	NS	NS			

[a] Changes are presented as percentages of the mean value observed for each animal during the prestimulatory period (indicated as 100 ± SD). Changes obtained in control animals were averaged, and the mean ± SD is presented for each time of blood sampling. See text for further explanation on analysis of data.

[b] Coordinates of electrode placement are given according to maps in Ref. 23. F, frontal plane; S, saggital; H, horizontal.

[c] Stimulation was begun after withdrawal of the 30-min blood sample and was continued for 30 min. The 60-min sample was taken immediately after stimulation was terminated.

intensity of 1 ma. A concentration of neurons capable of causing this response was found in the zona incerta. Stimulation of this region has been reported to produce similar results in dogs (10). The comparatively short time before the onset of the response suggests that it was mediated by a neural rather than a humoral mechanism. Correll (9), working with rabbits, has demonstrated that the increase in plasma FFA after electrical stimulation in the brain stem disappeared after section of the spinal cord at the first cervical vertebra.

The lowering of plasma FFA after stimulation may have resulted from a decrease in the rate of FFA liberation from fat tissue, an increase in the rate of FFA uptake from the plasma, or both. The importance of insulin as the hormone responsible for inhibition of FFA liberation from fat tissue in the intact animal has been discussed (26–28). However, the decrease in plasma FFA observed in our experiments does not seem to have been caused by insulin, since this hormone also affects the plasma glucose level and we were unable to show a significant correlation between the changes observed in the concentrations of these two plasma constituents after stimulation.

It is also unlikely that hormones such as vasopressin or oxytocin, which are capable of lowering plasma FFA

(29), were responsible for the decrease in the plasma FFA in our experiments. We could demonstrate a decrease in plasma FFA after stimulation of posterior hypothalamic areas with a low current intensity but not after similar stimulation of anterior regions that included the supraoptic and paraventricular nuclei, structures that are involved in the secretion of these two hormones (30).

The possibility that an increase in the rate of FFA uptake brought about a decrease in plasma FFA level was also considered. Hypothalamic stimulation may cause vasodilation in the muscles (31) followed by enhanced blood flow and an increase in FFA uptake, as described in man during exercise (32). However, preliminary data obtained by us in experiments with radioactive palmitate were not compatible with this possibility. The decrease in plasma FFA as observed in our experiments was interpreted as being due to activation of hypothalamic neurons that are capable of inhibiting spontaneous activity in sympathetic neurons that innervate fat tissue, thus resulting in a lower rate of FFA liberation.

The cause of the delayed increase in plasma FFA level in those cats that showed a rebound of the response must, it seems, be sought in an endocrine effect. It is possible that stimulation in these cats brought about the secretion of an adipokinetic hormone from the pituitary gland. A delayed elevation of plasma FFA has been obtained in experiments in vivo after the injection of growth hormone into rabbits (33) and dogs (34, 35), but this hormone is also known for its diabetogenic properties (36). We have been able to show a delayed increase in plasma FFA after lipotropin[2] injection into cats. This effect was not consistently reproducible, but when it did occur there was no change in the concentration of plasma glucose.

Our results did not show changes in the concentrations of TG or cholesterol in the plasma after hypothalamic stimulation. Changes in the concentrations of several plasma lipids after electrical stimulation of the hypothalamus have been reported in mammals (12–14). However, these results were produced in animals that received a fat-enriched food but not in fasted ones.

The lack of correlation between the changes in FFA and glucose concentrations after stimulation suggests a spatial separation of the hypothalamic elements that influence the concentration of these two compounds in the plasma, and the possibility that separate hypothalamic elements control the innervation of liver and fat tissue requires consideration. The effect of the autonomic nervous system on glycogen metabolism in the liver has been investigated by Shimazu and Amakawa (37). These authors have shown that the activities of glycogen phosphorylase and glucose-6-phosphatase of rabbit liver increased markedly after electrical stimulation of the splanchnic nerve, an effect that was counteracted by simultaneous stimulation of the vagus. The increased activities of these enzymes would cause glycogenolysis in the liver followed by a rise in plasma glucose. Thus, activation of hypothalamic neurons that control the autonomic innervation of the liver might have caused a rapid change in plasma glucose without immediately affecting plasma FFA in our experiments. The rate of FFA liberation into the plasma appears to be influenced by neural activity of sympathetic neurons that innervate fat tissue (4, 27). FFA release into the incubation medium was enhanced in vitro after electrical stimulation of the nerve that supplies the fat pad (38). It is therefore tenable that activation of those hypothalamic neurons that control the autonomic innervation of fat tissue would cause a change in the rate of FFA liberation from this tissue without immediately affecting the concentration of glucose in the plasma.

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[2] A sample of β-lipotropin was kindly supplied by Dr. C. H. Li, Hormone Research Laboratory, University of California, Berkeley, Calif.

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