Mechanism of lipolysis induced by electrical stimulation of the hypothalamus in the rabbit

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Abstract Electrical stimulation of the ventro-medial hypothalamus of the rabbit elevated plasma glycerol concentration. This elevation was suppressed by treatments of the animals with hexamethonium and propranolol, or by adrenalectomy, but was not suppressed by treatments with atropine and phentolamine. These results would indicate that the effect of electrical stimulation of ventro-medial hypothalamus is mainly mediated by catecholamine(s) liberated from the adrenal medulla and acts on β-receptor of adipose tissue.

Supplementary key words ventro-medial hypothalamus · plasma glycerol concentration · hexamethonium · propranolol · adrenalectomy.

It has been well established that various hormones stimulate lipolysis in fat cells, and that hormone-sensitivity of fat cells from various species varies (1). Lipolysis in rabbit adipose tissue is induced by catecholamines (2–4), adrenocorticotropic hormones (4, 5), α- and β-melanocyte-stimulating hormones (5), arginine-vasopressin (5), and Fraction H (5–7); thyrotropin (5, 8), oxytocin (8), and prolactin (8) have no lipolytic effect. These adipokinetic hormones can be divided into three classes; pituitary peptide hormones, catecholamines liberated from adrenal medulla, and norepinephrine directly released from postganglionic sympathetic nerve endings.

Less well known, however, is how peptide hormones of the pituitary gland and catecholamines from the sympathetic nervous system participate in physiological (in vivo) lipolysis in the rabbit and other animals. Desbals, Desbals, and Agid (9) have reported that adrenocorticotropic hormone plays a more important role than catecholamines in the rabbit. On the other hand, Schoenbaum, Steiner, and Sellers (10) have described that norepinephrine released from systemic sympathetic nerve endings is of primary importance in lipolysis due to cold acclimatization. The importance of the adrenal medulla, however, cannot be excluded, because Abramson, Arky, and Woeber (11) have reported that in man the adrenal medulla is important for the lipolysis that follows hypoglycemia due to insulin.

The purpose of this paper is to elucidate the mechanism of lipolysis induced by electrical stimulation of the ventro-medial hypothalamus (VMH) of the rabbit, and to determine which of three classes of hormones mentioned above is involved.

MATERIALS AND METHODS

Animals

Adult male albino rabbits, weighing 3.0 ± 0.1 kg, were maintained on Clea laboratory chow, a commercial laboratory chow, which they ate from 9 AM to 1 PM. The laboratory chow was composed of protein (17.3%), fat (2.8%), carbohydrate (49.2%), cellulose (13.9%), water (7.5%), and trace amounts of minerals and vitamins. The various experiments were performed at 3 PM.

Implantation of the electrode into hypothalamus

Unanesthetized animals were restrained in a box. The skull was exposed by incision, and a bipolar insulated platinum electrode with a bared tip was inserted into the ventro-medial or lateral hypothalamus through a small hole drilled in the skull. A Kurotsu-Shimizu apparatus was used for implantation of the electrode (12). The coordinates used were on the coronal suture, 0.6 mm lateral to the midline (sagittal suture), and 16.0 mm below the surface of the skull for VMH; and 1.0 mm posterior to the coronal suture, 2.0 mm lateral to the midline, and 15.5 mm below the surface of the skull for lateral hypothalamus (LH). During the insertion of the electrode, electrical current as described below was given to the hypo-

Abbreviations: VMH, ventro-medial hypothalamus; LH, lateral hypothalamus; FFA, free fatty acid.

thalamus through the electrode. When the rabbit exhibited sympathetic and parasympathetic responses for VMH and LH stimulations, respectively, the electrode was connected with a small plug, which was then anchored firmly and permanently to the skull by screws and dental cement.

Electrical stimulation of VMH and LH

One week after implantation of the electrode, the rabbit was restrained in a box at 1 PM. The hypothalamus was then stimulated electrically from 3 PM either for 1 min (single stimulation), or intermittently for 60 min (intermittent stimulation). For the latter stimulation, an interval timer was connected to allow repeated application of stimuli for 20-sec periods, once every 3 min. Electrical stimuli were supplied by an electronic stimulator through an isolation unit. The stimulation parameters were 0.3 mA, 0.3 msec duration, and 100 Hz (for VMH) or 50 Hz (for LH). Stimulation of VMH elicited sympathetic responses of the animals, such as exophthalmos, mydriasis and constriction of ear vessels. On the other hand, stimulation of LH produced parasympathetic responses, showing enophthalmos and vasodilatation of ear vessels.

Confirmation of the position of inserted electrode

At the end of the experiments, the animals were killed under anesthesia and the brain was excised to ascertain the position of the electrode. The brain was fixed with buffered formalin, and serial sections (20 μm) were made and stained with cresyl violet to identify the site of the electrode tip microscopically. Data obtained from animals in which the position of the electrode was incorrect were omitted.

Adrenalectomy

VMH-implanted rabbits were anesthetized with sodium pentobarbital (25 mg/kg, i.v.) and both adrenals were extirpated from the dorsal approach. After the operation, the animals were given saline instead of drinking water. The adrenalectomized animals were maintained for 4 days with or for 2 days without cortisone administration, respectively; VMH-stimulations were then carried out. The completeness of the adrenalectomy was checked at autopsy. If any adrenal remained, results obtained with such animals were omitted. Sham-adrenalectomy was performed in control rabbits and VMH-stimulation of sham-operated animals was carried out 2 days after the operation. The body weights of both adrenalectomized and sham-operated animals were reduced by 400–500 g in 2–4 days after the operation.

Administration of pharmacological agents

Hexamethonium as a ganglionic blocker (10 mg/kg or 20 mg/kg) was injected into the ear vein 60 min before VMH-stimulation. Propranolol as a β-receptor blocking agent (15 mg/kg) was injected intraperitoneally 15 min before VMH-stimulation. Inasmuch as phentolamine as an α-receptor blocking agent acts for only a short duration in vivo, and has extensive hypotensive effects in large doses, it was infused into the ear vein at the rate of 50 μg/kg per min from 30 min before VMH-stimulation to the end of the stimulation. Atropine (10 mg/kg) was administered intraperitoneally 15 min before VMH-stimulation.

Cortisone acetate (40 mg/kg per day), emulsified ultrasonically in saline, was administered subcutaneously at 9 AM for 4 days to one group of adrenalectomized animals. To estimate the effect of glucocorticoid per se on the plasma glycerol concentration, hydrocortisone-21-sodium succinate (hydrocortisone hemisuccinate) (15 mg/kg) in 0.3 ml of saline was injected intravenously to another group of adrenalectomized animals.

Assays

Approximately 1.2 ml of blood from each rabbit was collected from the ear vein with a heparinized syringe. The blood was transferred into a test tube containing a trace amount of solid heparin, which did not have any effect on the assays. The plasma was obtained by centrifugation at 600 *g* for 10 min. Plasma concentrations of glycerol, free fatty acid (FFA) and glucose were determined by the methods of Wieland (13), Falholt, Lund, and Falholt (14), and Saifer and Gerstenfeld (15), respectively.

Enzymes and chemicals

Glycerophosphate dehydrogenase (Cat. No. 15135) and glycerokinase (Cat. No. 15746) were purchased from Boehringer Mannheim Company (New York, N.Y.). Glucostat (Cat. No. 7451) for the glucose assay was from Worthington Biochemical Corporation (Freehold, N.J.). L-Epinephrine bitartrate, L-norepinephrine bitartrate, hexamethonium bromide, atropine sulfate, DL-propranolol hydrochloride, cortisone acetate and hydrocortisone-21-sodium succinate were purchased from Sigma Chemical Company (St. Louis, Mo.). Phentolamine hydrochloride was a generous gift from the Ciba Geigy Pharmaceutical Company. All other chemicals were of analytical grade.

Calculation

Increments of plasma glucose, glycerol, and FFA concentrations were analyzed statistically by Student's *t* test.

RESULTS

The effect of electrical stimulation of hypothalamus on concentrations of plasma glycerol, FFA, and glucose are shown in **Figs. 1** and **2.** On single stimulation of VMH (Fig. 1-A), plasma glycerol concentration reached a maximum at 20 min, and then declined toward the initial level. The maximum level at 20 min was about 1.8-fold higher than the initial concentration. The plasma glucose level also increased on single stimulation of VMH and a peak was observed at 10 min (Fig. 1-C). The peak of plasma glucose level was observed 10 min earlier than that of plasma glycerol. The lag time between peaks of plasma glycerol and glucose concentrations suggests that lipolytic response to VMH-stimulation is slower than the glycogenolytic response and that each response is caused by a different mechanism.

Plasma FFA concentration would also be expected to increase as a result of VMH-stimulation, as FFA results from hydrolysis of triglyceride. However, plasma FFA concentration was not elevated by either single or intermittent stimulations of VMH as shown in Figs. 1-B and 2-B, respectively. The lack of increase of plasma FFA on VMH-stimulation may be due to acceleration of their utilization or inhibition of their release from adipose tissue into the circulation.

Plasma glycerol and glucose concentrations on intermittent stimulation of VMH increased in proportion to the stimulation time (Figs. 2-A and 2-C), and their levels at 60 min were several times higher than their initial levels. Such elevations of plasma glycerol and glucose levels seem to be a characteristic feature of VMH-stimulation; single and intermittent electrical stimulations of LH, another hypothalamic area adjacent to VMH, had no effect on plasma glycerol, FFA, or glucose concentrations as shown in Figs. 1 and 2.

The next problem to be clarified is how the information of VMH-stimulation is communicated to adipose tissue. Two possible routes may be considered as the transmission pathway of VMH-stimulation. One is a route concerned with VMH, the pituitary gland, and peptide hormones, and the other is a route including VMH, the autonomic nervous system, and catecholamines. The possibility of the latter pathway was examined by using a ganglionic blocker. When the animals were pretreated with intravenously administered hexamethonium (10 mg/kg), the elevation of plasma glycerol level after VMH-stimulation was suppressed by 30–70% as compared with that of untreated animals (**Fig. 3**). Administration of 20 mg/kg of hexamethonium had an even stronger suppressive

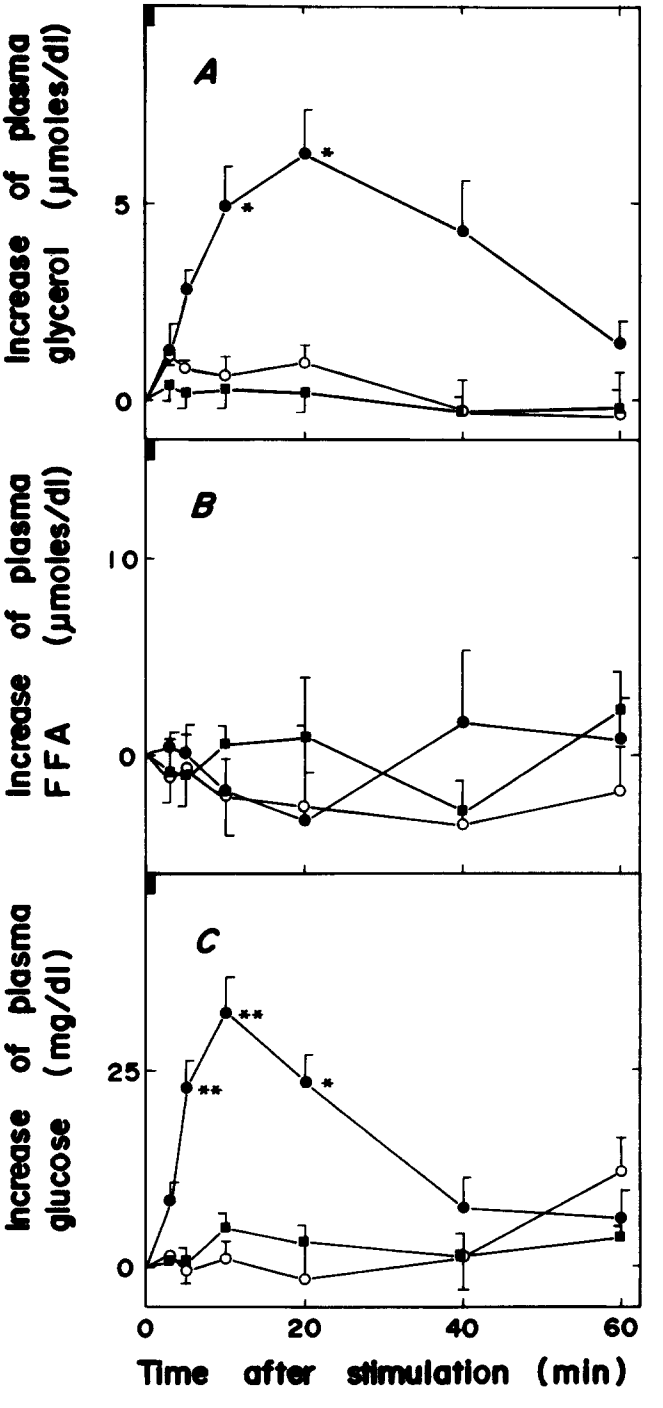

Fig. 1. Effects of single electrical stimulation of VMH and LH on plasma glycerol, FFA, and glucose concentrations. Each sample of plasma was obtained after a single stimulation (indicated at the top of each figure) of VMH (●) or LH (■). The concentrations in control animals without electrical stimulation of their hypothalamus (○) are also given. Fig. 1-A, plasma glycerol concentration; Fig. 1-B, plasma FFA concentration; Fig. 1-C, plasma glucose concentration. Mean initial concentrations of plasma glycerol, FFA, and glucose of five animals in each experiment were 7.3 ± 1.1 μmoles/dl, 16.6 ± 3.9 μmoles/dl, and 119.0 ± 7.6 mg/dl, respectively. Statistical analyses were performed on experimental data of VMH-or LH-stimulated versus control animals. **, $P < 0.05$; *, $0.05 < P < 0.1$. Vertical lines represent standard errors.

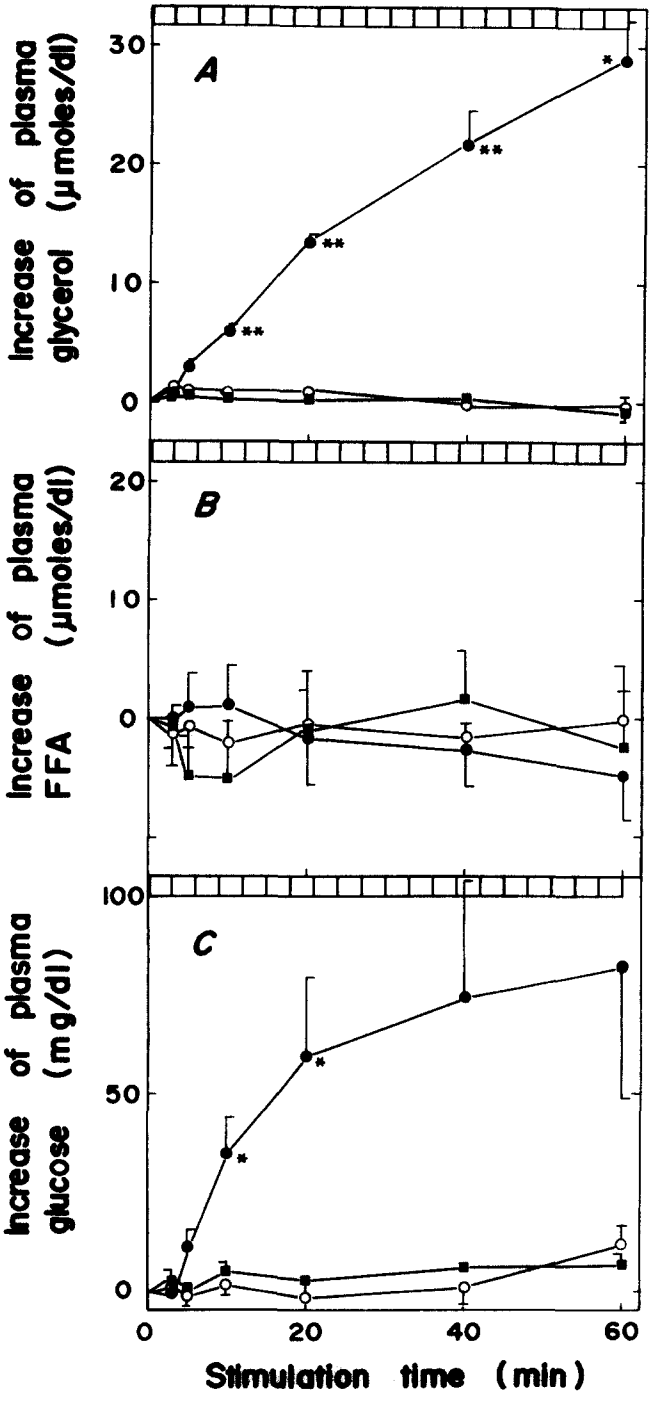

Fig. 2. Effects of intermittent electrical stimulation of VMH and LH on plasma glycerol, FFA, and glucose concentrations. Each plasma was obtained during intermittent stimulation (indicated at the top of each figure) of VMH (●) or LH (■). The concentrations of control animals (○) are also given. Fig. 2-A, plasma glycerol concentration; Fig. 2-B, plasma FFA concentration; Fig. 2-C, plasma glucose concentration. Mean initial concentrations of plasma glycerol, FFA, and glucose of four animals in each experiment were 9.9 ± 0.8 μmoles/dl, 14.0 ± 4.2 μmoles/dl, and 104.9 ± 7.6 mg/dl, respectively. Statistical analyses were performed on experimental data of VMH- or LH-stimulated versus control animals. **, $P < 0.05$; *, $0.05 < P < 0.1$. Vertical lines represent standard errors.

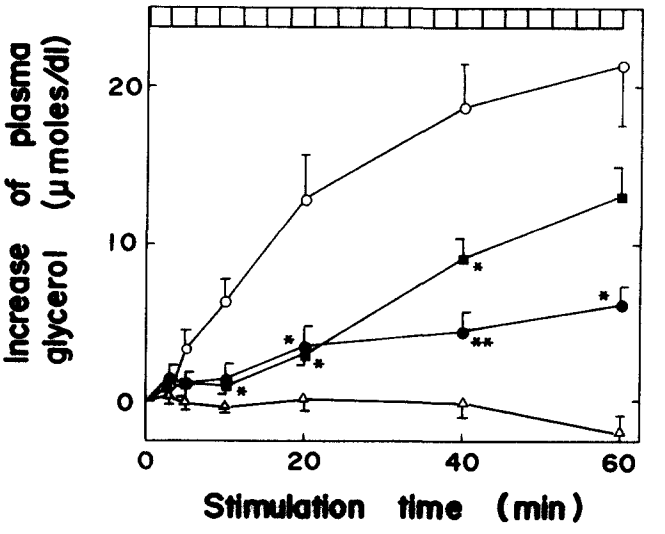

Fig. 3. Effect of hexamethonium on elevation of plasma glycerol concentration induced by intermittent stimulation of VMH. Intermittent stimulation (indicated at the top of the figure) of VMH of control animals (○) and animals pretreated with 10 mg/kg (■) or 20 mg/kg (●) of hexamethonium were performed. The effect of 20 mg/kg of hexamethonium alone on plasma glycerol concentration (△) is also shown. Mean initial plasma glycerol concentrations of control VMH-stimulated, hexamethonium (10 mg/kg)-pretreated and VMH-stimulated, hexamethonium (20 mg/kg)-pretreated and VMH-stimulated, and control hexamethonium (20 mg/kg)-pretreated animals were 8.4 ± 1.1, 6.7 ± 1.2, 10.5 ± 1.1, and 6.4 ± 0.5 μmoles/dl, respectively. There were five experimental animals in each group. Statistical analyses were performed on experimental data of hexamethonium-pretreated and VMH-stimulated versus control VMH-stimulated animals. **, $P < 0.05$; *, $0.05 < P < 0.1$. Vertical lines represent standard errors.

effect on the increase of plasma glycerol level by VMH-stimulation, and exhibited a 60–70% suppression of the control level. Therefore, it may be concluded from Fig. 3 that the information of VMH-stimulation is delivered to the autonomic nervous system via ganglia, where postganglionic neurons of sympathetic and parasympathetic nervous systems originate.

It is generally accepted that acetylcholine and catecholamines are released from postganglionic parasympathetic and sympathetic nerve endings and that their effects on the target tissues are blocked by atropine and propranolol (or phentolamine), respectively. Therefore, anti-cholinergic and anti-adrenergic drugs were used to determine which autonomic nerve participates in the lipolysis induced by VMH-stimulation (**Fig. 4**). When atropine was administered to control animals without electrical stimulation of VMH, the concentration of plasma glycerol was not changed. Intermittent stimulation of VMH of animals pretreated with atropine resulted in an elevation of plasma glycerol concentration. This concentration was slightly higher than that of control VMH-stimulated animals but the difference was not statistically signifi-

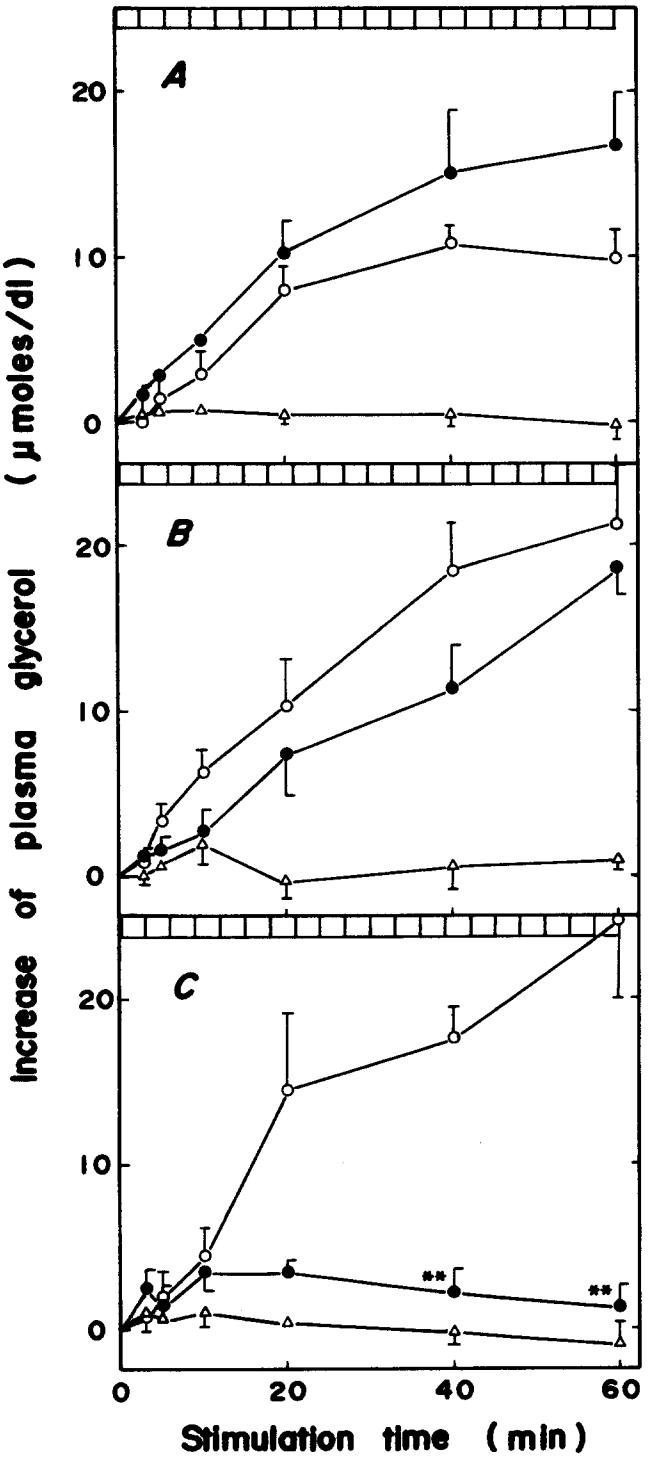

Fig. 4. Effects of atropine, phentolamine, and propranolol on elevation of plasma glycerol concentration induced by intermittent stimulation of VMH. Intermittent electrical stimulations (indicated at the top of each figure) of VMH of control animals (○) and animals treated with drugs (●) were carried out. The changes of plasma glycerol concentration of animals that were treated with indicated drugs but were not stimulated electrically (△), are also given. Statistical analyses were performed on experimental data of animals treated with each drug and stimulated electrically versus control VMH-stimulated animals. **, $P < 0.05$. Vertical lines represent standard errors. Fig. 4-A, treatment with atropine (10 mg/kg, i.p.). Mean initial plasma glycerol concentrations of animals treated with and without atropine were 8.3 ± 1.1, and 7.7 ± 0.6 μmoles/dl, respectively. There were three experimental animals in each group. Fig. 4-B, treatment with phentolamine (50 μg/kg per min, i.v.). Mean initial plasma glycerol concentrations of animals treated with and without phentolamine were 5.7 ± 0.9 and 6.4 ± 0.5 μmoles/dl, respectively. There were four animals in each group. Fig. 4-C, treatment with propranolol (15 mg/kg). Mean initial plasma glycerol concentrations of animals treated with and without propranolol were 9.9 ± 1.0 and 12.5 ± 3.1 μmoles/dl, respectively. There were four animals in each group.

cant (Fig. 4-A). Therefore, atropine does not prevent the increase in plasma glycerol concentration as a result of VMH-stimulation and parasympathetic nerves seem not to be concerned with hypothalamic lipolysis.

When phentolamine was infused before and during VMH-stimulation (Fig. 4-B), the plasma glycerol level increased as in control VMH-stimulated animals. However, pretreatment of the animals with propranolol remarkably inhibited the elevation of plasma glycerol level by VMH-stimulation as shown in Fig. 4-C. The results of Fig. 4 would be summarized as follows: the information of VMH-stimulation is exclusively transmitted through the sympathetic nervous system to the β-receptor site of fat cells.

The sympathetic nervous system has two pathways through which its effect is mediated to adipose tissue. One is direct sympathetic innervation of adipose tissue and the other is humoral control of adipose tissue by the adrenal medulla.

The next experiment was performed to estimate the role of adrenals in the lipolysis induced by VMH-stimulation as shown in **Fig. 5.** On intermittent VMH-stimulation of sham-adrenalectomized animals, there was an appreciable increase in plasma glycerol concentration that was only slightly less than in control VMH-stimulated animals (statistically insignificant). On the other hand, there was only a slight increase in concentration of plasma glycerol in both adrenalectomized, and adrenalectomized and cortisone-pretreated animals during intermittent VMH-stimulation. The increment in plasma glycerol concentration in adrenalectomized animals as a result of VMH-stimulation was only 20% of that in the stimulated control animals, and was not increased by pretreatment of adrenalectomized animals with cortisone. Moreover, intravenous injection of cortisol (15 mg/kg) to adrenalectomized animals did not elevate the plasma glycerol level even after 60 min as shown in **Table 1.** The results in Fig. 5 and Table 1 indicate that the information of VMH-stimulation is mediated to adipose tissue via the adrenals, and that glucocorticoid from the adrenal cortex does not have a permissive effect on factors released upon VMH-stimulation,

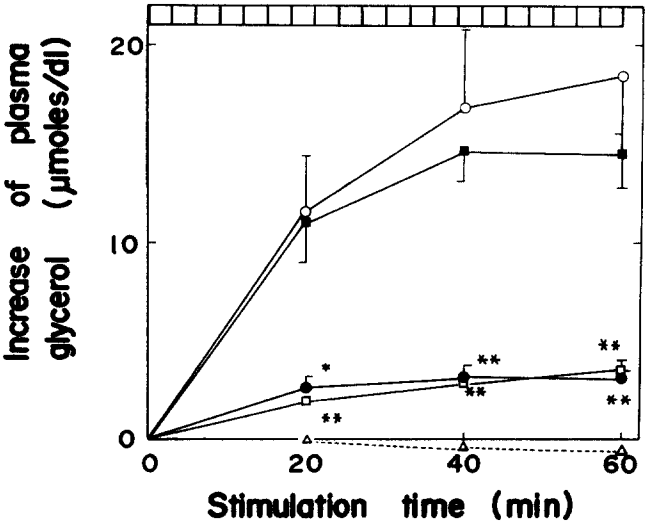

Fig. 5. Effect of adrenalectomy on elevation of plasma glycerol concentration induced by intermittent VMH-stimulation. Intermittent electrical stimulations (indicated at the top of the figure) of VMH of control (○), sham-operated (■), adrenalectomized only (●), and adrenalectomized and cortisone (40 mg/kg per day, 4 days)-pretreated (□) animals were carried out. The increment of plasma glycerol level of adrenalectomized and cortisone-pretreated animals without VMH-stimulation (△) is also shown. Mean initial plasma glycerol concentrations of control, sham-operated, adrenalectomized only, and adrenalectomized and cortisone-pretreated animals were 9.0 ± 1.1, 7.3 ± 0.6, 5.1 ± 0.8, and 7.1 ± 0.6 μmoles/dl, respectively. There were five animals in each group. Statistical analyses were carried out on experimental data of sham-operated, and adrenalectomized versus control animals. **, $P < 0.05$; *, $0.05 < P < 0.1$.

or any direct lipolytic effect on adipose tissues. The most probable mediators are catecholamines from the adrenal medulla.

DISCUSSION

The present paper indicates that the effect of VMH-stimulation in the rabbit is mainly transmitted to adipose tissue through the sympathetic nervous system including the adrenal medulla. As it is known that the adrenal medulla of the adult rabbit contains exclusively epinephrine (16), the catecholamine that finally acts on adipose tissue would be epinephrine. Wadstroem (2), and Autor and Lynn (4) have reported that administration of epinephrine can induce lipolysis in the rabbit; on the other hand, Desbals et al. (9) have reported that exogenous epinephrine does not elevate plasma FFA concentration of the rabbit, because adipose tissue of the rabbit is not able to respond substantially to catecholamines. However, we have shown (17) that exogenous epinephrine causes an increase in plasma glycerol concentration with only a minimal increase of plasma FFA concentration, while administration of norepinephrine and adrenocorticotropic hormone increases both plasma glycerol and FFA concentrations concomitantly. Therefore, the plasma FFA level in the rabbit seems to be an inappropriate indicator to estimate the extent of in vivo lipolysis by epinephrine, and contrary to the report of Desbals et al. (9), the adipose tissue of the rabbit seems to be sensitive to epinephrine. Moreover, the fact that epinephrine administration and VMH-stimulation each resulted in an elevation of plasma glycerol level without a concomitant elevation of plasma FFA level is suggestive of participation of epinephrine in the lipolysis due to VMH-stimulation.

The conditions that activate the sympathetic nervous system, such as cold acclimatization and VMH-stimulation, would be expected to promote the liberation of catecholamines from sympathetic nerve endings and the adrenal medulla. The lipolysis due to cold acclimatization is reported to be regulated by norepinephrine directly released from systemic sympathetic nerve endings, and epinephrine seems not to have primary significance in such lipolysis (10). Moreover, it has been reported that administration of 2-deoxyglucose induces the lipolysis in the dog (18) and the rat (19) by activation of neuronal lipolytic centers of the cervical portion of spinal cord and hypothalamus. However, Teixeira, Antunes-Rodrigues, and Migliorini (19) have shown that in the rat the adrenal medulla does not participate in lipolysis resulting from 2-deoxyglucose administration.

On the other hand, it has been reported that chemical (20) and electrical (21) stimulations of the hypothalamus regulate the secretion of catecholamines from the adrenal medulla and that the adrenal medulla of the human plays an important role in the lipolysis that follows insulin hypoglycemia (11). Furthermore, the lipolysis induced by VMH-stimulation of the rabbit was also regulated by catechola-

TABLE 1. Effect of cortisol on plasma glycerol concentration of adrenalectomized rabbits

Time After Injection	Plasma Glycerol Concentration	
	Cortisol	Saline
min	*μmoles/dl*	
0	5.1 ± 1.0	6.0 ± 0.8
20	6.4 ± 0.9	6.5 ± 0.7
40	4.1 ± 1.0	5.2 ± 0.7
60	4.1 ± 1.0	5.0 ± 1.0

Adrenalectomized rabbits were employed for the experiment two days after the operation. Hydrocortisone-21-sodium succinate (15 mg/kg) in 0.3 ml saline or vehicle only was injected into an ear vein at 0 min; blood was obtained from the contralateral ear vein at 20, 40, and 60 min after the injection. There were five animals in each group. Each value represents mean ± standard error.

mine(s), perhaps epinephrine, liberated from the adrenal medulla as shown in this paper. The conclusion of this report is mainly derived from the experimental evidence that elevation of plasma glycerol level on VMH-stimulation was abolished by adrenalectomy. If the lipolysis of VMH-stimulation of the rabbit were induced by norepinephrine liberated from sympathetic nerve endings, adrenalectomy should have almost no effect on the elevation of plasma glycerol concentration by VMH-stimulation, a single stimulation of VMH should cause more rapid increase of plasma glycerol level as in the case of plasma glucose, which is regulated by postganglionic sympathetic nerve (22, 23), and plasma FFA concentration should increase concomitantly with plasma glycerol level as reported by Hagen and Hagen (3). These phenomena did not occur in the present study. However, a small elevation of plasma glycerol was observed shortly after VMH-stimulation of the adrenalectomized animals (Fig. 5). This increase might have been due to direct sympathetic nerve regulation of lipolysis. Hence, the possibility of participation of norepinephrine cannot be completely ruled out.

Adrenalectomy described in this report resulted in the removal of both adrenal medulla and cortex. Since Jeanrenaud and Renold (24), and Fain, Scow, and Chernick (25) have reported that cortisol has a lipolytic effect on rat adipose tissue, there is the possibility that the suppression of plasma glycerol elevation in VMH-stimulated adrenalectomized animals might have been caused by the absence of glucocorticoid from adrenal cortex. However, cortisol injection did not elevate the plasma glycerol level (Table 1), and Autor and Lynn (4) have described that glucocorticoid is not effective in producing lipolysis in vitro in rabbit adipose tissue. These results indicate that glucocorticoid per se has no lipolytic effect. Moreover, it is known that glucocorticoid has a permissive effect on lipolysis by epinephrine in the rat (26, 27). If lipolytic factors other than epinephrine, liberated upon VMH-stimulation of adrenalectomized rabbits, could not induce lipolysis due to deficiency of glucocorticoid from adrenal cortex, VMH-stimulation of adrenalectomized animals receiving glucocorticoid administration should show the same glycerol increment as that of control VMH-stimulated animals. But the replacement of glucocorticoid had no effect on the suppression of plasma glycerol increase by adrenalectomy (Fig. 5). It is thus very likely that plasma glycerol elevation observed on VMH-stimulation would be almost derived from the lipolysis due to catecholamines liberated from the adrenal medulla.

The plasma glycerol increase upon VMH-stimulation, is considered to be derived from adipose tissues. However, another report from this laboratory (17) indicates that liver slices, as well as adipose tissue slices, also respond to epinephrine, resulting in an elevation of glycerol concentration in the incubation medium. Therefore, there is a possibility that some tissues other than adipose tissues, such as liver, also contribute in part to the plasma glycerol elevation due to VMH-stimulation.

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