Effects of catecholamines on the lipolysis of two kinds of fat cells from adult rabbit

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Abstract The administration of various catecholamines and adrenocorticotropic hormone to adult rabbit elevated plasma glycerol concentration. These catecholamines also induced the in vitro lipolysis of isolated interscapular fat cells but could not bring about the lipolysis of epididymal ones, while adrenocorticotropic hormone induced the lipolyses of both kinds of fat cells. It may be speculated from these results that catecholamines liberated endogenously in adult rabbit cannot act on all systemic adipose tissues but have lipolytic effects on a part of them.

Supplementary key words glycerol · free fatty acid · epinephrine · norepinephrine · isoproterenol · adrenocorticotropic hormone · electrical stimulation of ventro-medial hypothalamus · interscapular fat cell · epididymal fat cell

Correll (1) has reported that electrical stimulation of nerve to rabbit adipose tissue in vitro results in an elevation of free fatty acid concentration of the incubation medium. An accompanying report (2) from this laboratory has shown that electrical stimulation of rabbit ventro-medial hypothalamus (VMH) increases the plasma glycerol level through a transmission route that includes the hypothalamus, the autonomic nervous system, and the adrenal medulla. These results indicate that catecholamines liberated from sympathetic nerve endings or from the adrenal medulla act on rabbit adipose tissue to induce the lipolysis. Moreover, there are several reports that exogenous catecholamines can induce lipolysis in the rabbit (3–5).

On the other hand, Rudman, Brown, and Malkin (6) have reported that lipolysis in slices of rabbit adipose tissue is not observed even under fairly high concentrations of catecholamines in vitro. Desbals, Desbals, and Agid (7) have also reported that administration of catecholamines to the rabbit has no effect on the plasma FFA level. On the basis of these results, Rudman et al. (6) and Desbals et al. (7) concluded that adipose tissue of the rabbit is essentially insensitive to catecholamines. This conclusion is incompatible with the fact that lipolysis is observed on electrical stimulations of nerve to adipose tissue (1) and of VMH (2).

The purpose of the present paper is to investigate the in vivo and in vitro effects of catecholamines on lipolysis in the rabbit and to determine what kind of adipose tissue is concerned with lipolysis induced by these hormones.

MATERIALS AND METHODS

Animals

Adult male albino rabbits, weighing 3.1 ± 0.1 kg, were maintained on a Clea laboratory chow as described previously (2). The animals ate 9 AM to 1 PM, according to the feeding schedule, and all in vivo experiments were carried out at 3 PM. On the other hand, animals employed for the in vitro studies were killed at 9 AM. Although the animals killed in the morning were fasted and those killed in the afternoon were fed, experiments with slices and isolated fat cells obtained at 9 AM and 3 PM gave almost the same results.

VMH-Stimulation

An electrode was implanted into the VMH of the rabbit and the VMH was electrically stimulated intermittently for 60 min as reported previously (2).

Administrations of various hormones to the rabbit

Epinephrine, norepinephrine, isoproterenol, and adrenocorticotropic hormone (ACTH) were infused for 60 min at the rates of 0.2, 0.2 (or 5.0) and 0.2 μg/kg per min, and 20 mU/kg per min, respectively. Each plasma was obtained at 0, 10, 20, 30, 40, 60, 70, and 90 min after the beginning of hormone infusions.

Experiments with tissue slices

Animals were killed by intravenous injection of sodium pentobarbital, and adipose tissue from various

Abbreviations: VMH, ventro-medial hypothalamus; ACTH, adrenocorticotropic hormone; FFA, free fatty acid; DNA, deoxyribonucleic acid.

sites, liver, kidney, and skeletal muscle from the thigh were immediately excised. The tissues were immersed in saline kept at 30°C and were cut into small cubes (1–2 mm) with a razor. The tissue pieces, weighing 40–50 mg, were incubated at 37°C for 120 min in 1.0 ml of Krebs-Ringer bicarbonate buffer, containing 4% bovine serum albumin and 50 μg of epinephrine. At the end of incubation, an equal volume of 5% perchloric acid was added to the incubation medium. After the centrifugation, supernatant was neutralized and used for assays of glycerol and FFA.

Experiments with isolated fat cells

Interscapular and epididymal adipose tissues were digested by collagenase at 37°C for 60 min, according to the method of Rodbell (8). Quantities of the cells, equivalent to about 5 μg of DNA, were incubated at 37°C for 120 min in 0.56 ml of Krebs-Ringer bicarbonate or Krebs-Ringer phosphate buffer, containing 4% bovine serum albumin and various concentrations of catecholamines and ACTH. The incubations were terminated as described in the experiments with tissue slices.

Assays

Glycerol and FFA concentrations of plasma and incubation mixtures were determined by methods described previously (2). The acylglycerols in the isolated fat cell preparations were estimated by the modified method of Soloni (9). Estimation of DNA in isolated fat cells was performed according to the method of Burton (10). Calf thymus DNA was used as a standard.

Enzymes and chemicals

Collagenase of *Clostridium histolyticum* (Type IV, 160 units/mg) and bovine serum albumin were purchased from Worthington Biochemical Corporation (Freehold, N. J.) and Armour Pharmaceutical Company (Omaha, Neb.), respectively. Bovine serum albumin was treated with charcoal before use to remove contaminating FFA, according to the method of Chen (11). Norepinephrine bitartrate, L-epinephrine bitartrate, L-isoproterenol hydrochloride, and porcine ACTH (Grade II, 88 IU/mg) were the products of Sigma Chemical Company (St. Louis, Mo.). All other chemicals were of analytical grade.

RESULTS

The changes in plasma glycerol and FFA levels with time as a result of electrical stimulation of VMH or administrations of catecholamines and ACTH are given in **Fig. 1**. VMH-Stimulation for 60 min elevated plasma glycerol level in proportion to the stimulation time, while plasma FFA concentration was unchanged during the stimulation (Fig. 1-A). It has been ob-

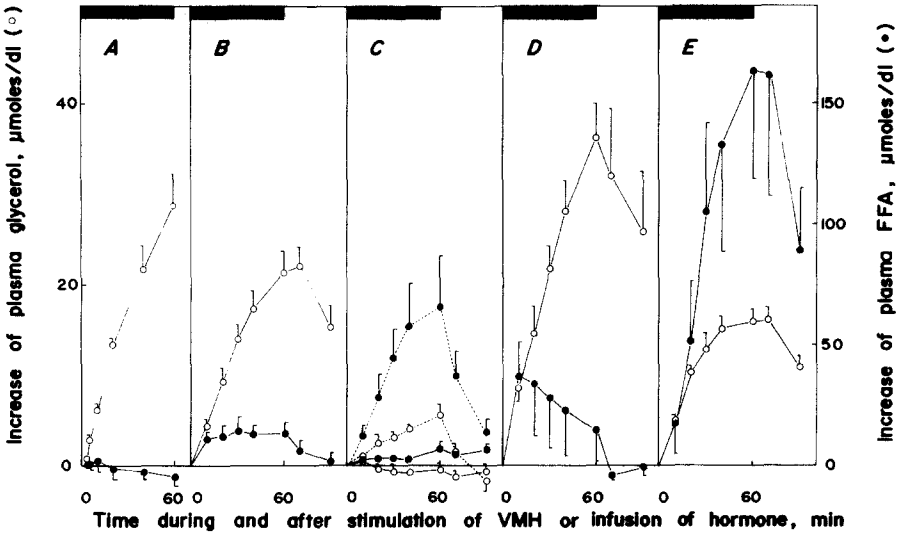

Fig. 1. Increments of plasma glycerol and FFA concentrations in the rabbit under various lipolytic conditions. Electrical stimulation of VMH and infusions of various hormones were carried out for 60 min as indicated by the black bars. Plasma glycerol (○) and FFA (●) concentrations were determined according to methods described in the text. Mean initial plasma glycerol and FFA concentrations of 30 animals employed in this experiment were 8.4 ± 0.9 (SEM), and 15.0 ± 1.6 μmoles/dl, respectively. There were five animals used in each experiment. Vertical lines represent standard errors. Fig. 1-A, electrical stimulation of VMH. Fig. 1-B, administration of epinephrine, 0.2 μg/kg per min. Fig. 1-C, administration of norepinephrine, 0.2 (solid line) or 5.0 (broken line) μg/kg per min. Fig. 1-D, administration of isoproterenol, 0.2 μg/kg per min. Fig. 1-E, administration of ACTH, 20 mU/kg per min.

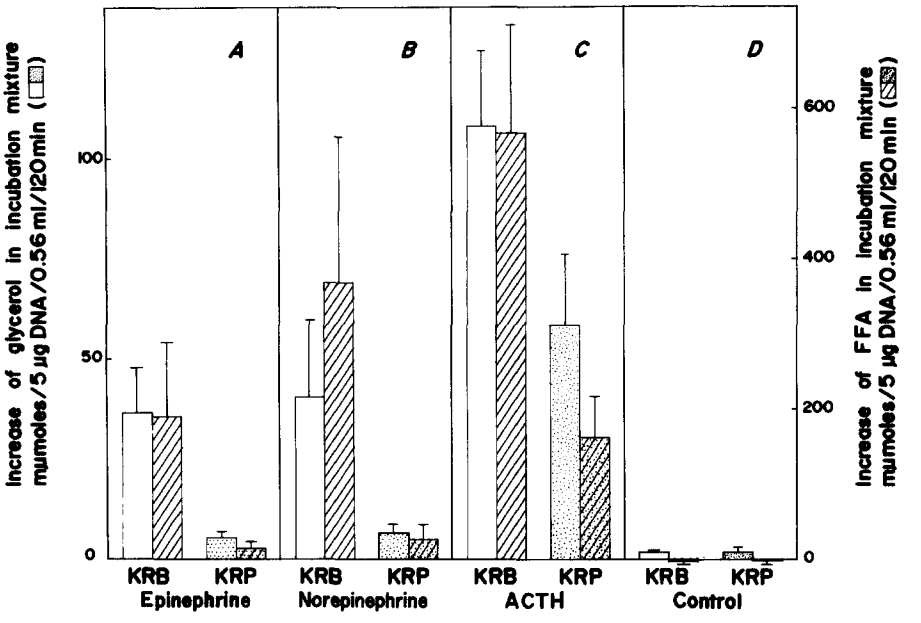

Fig. 2. Effects of Krebs-Ringer bicarbonate and phosphate buffers on the lipolytic activity of isolated interscapular fat cells. Isolated interscapular fat cells in Krebs-Ringer bicarbonate (KRB, open bar or hatched bar) and Krebs-Ringer phosphate (KRP, dotted bar or dotted and hatched bar) buffers were incubated in the presence (Fig. 2-A, 2-B, and 2-C) and absence (Fig. 2-D) of hormones. Fat cells contained 1.9 ± 0.4 mg of acylglycerols/μg of DNA. Incubation mixture was employed for analyses of glycerol (open bar or dotted bar) and FFA (hatched bar or hatched and dotted bar). Each value is the mean of three analyses; vertical lines represent standard errors. Fig. 2-A–C, effect of buffer on lipolysis induced by epinephrine, 10 μg/ml (Fig. 2-A), norepinephrine, 10 μg/ml (Fig. 2-B) and ACTH, 10 mU/ml (Fig. 2-C). Fig. 2-D, effect of buffer on lipolysis in the absence of hormone.

served that such an increase of plasma glycerol level is suppressed by adrenalectomy and that glucocorticoid does not seem to participate in this suppression (2). West has reported that the adrenal medulla of adult rabbit contains epinephrine exclusively (12). Therefore, the first candidate for a mediator liberated from adrenals on VMH-stimulation is epinephrine, although norepinephrine could not be eliminated completely as such a mediator.

Effects of infusions of catecholamines and ACTH on plasma glycerol and FFA levels

When epinephrine was infused at a rate of 0.2 μg/kg per min (Fig. 1-B), the plasma glycerol level increased in proportion to the infusion time, up to almost the same extent as on VMH-stimulation. However the plasma FFA level increased slightly in 10 min after the infusion, and was maintained at the same level. A similar phenomenon was also observed when isoproterenol was infused at a rate of 0.2 μg/kg per min (Fig. 1-D). There was an increase of the plasma FFA level after 10 min and then a decrease, but the plasma glycerol level increased linearly during the infusion. On the other hand, administrations of norepinephrine and ACTH at the rates of 5.0 μg/kg per min and 20 mU/kg per min, respectively (Fig. 1-C and 1-E), elevated both plasma glycerol and FFA levels in proportion to the infusion time, while administration of norepinephrine at a rate of 0.2 μg/kg per min had no effect on plasma glycerol and FFA levels (Fig. 1-C). The pattern of plasma glycerol and FFA levels as a result of VMH-stimulation (Fig. 1-A) seems to be similar to that administration seen when epinephrine rather than norepinephrine is administered, since the plasma FFA level was suppressed in the former case, and increased in the latter. Moreover, from the viewpoint of the effect of catecholamines on plasma glycerol elevation, isoproterenol was more potent than epinephrine, and epinephrine was more effective than norepinephrine.

Effects of incubation buffers on lipolysis of fat cells

The next problem was to determine the kind of adipose tissue responsible for the in vivo lipolysis induced by catecholamines. An experiment was carried out to study the suitable conditions for the in vitro lipolysis of rabbit adipose tissue. Isolated interscapular fat cells were incubated in two buffer systems: Krebs-Ringer bicarbonate buffer and Krebs-Ringer phosphate buffer. When fat cells in Krebs-Ringer bicarbonate buffer were incubated with epinephrine (10 μg/ml) (**Fig. 2-A**) and norepinephrine (10

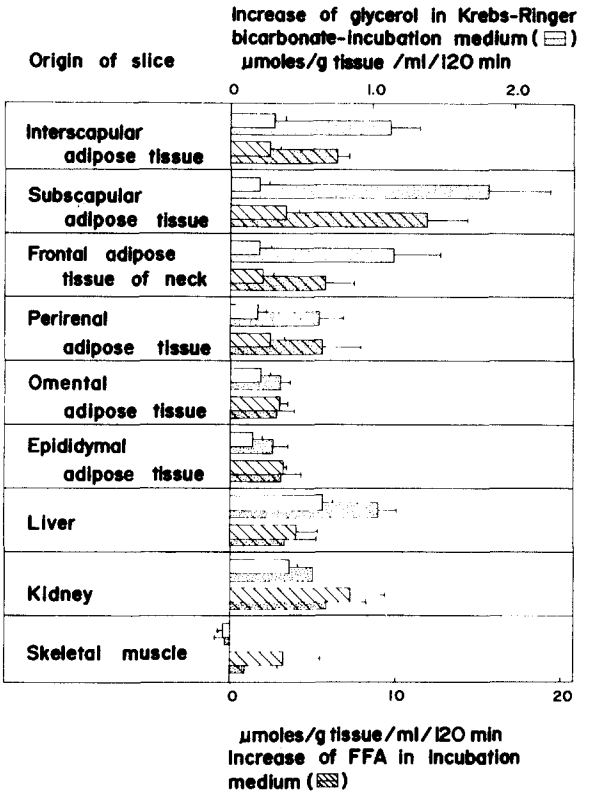

Fig. 3. The effect of epinephrine on the release of glycerol and FFA into the medium from various tissue slices. Pieces of various adipose tissues, liver, kidney and skeletal muscle (as indicated in left column) were incubated in the presence (dotted bar or dotted and hatched bar) or the absence (open bar or hatched bar) of 50 μg/ml of epinephrine. After the termination of the incubation, medium was employed for analyses of glycerol (open bar or dotted bar) and FFA (hatched bar or hatched and dotted bar). These were three experiments; vertical lines represent standard errors.

μg/ml) (Fig. 2-B), glycerol and FFA in the medium increased. However, when Krebs-Ringer phosphate buffer was used (Fig. 2-A and 2-B), catecholamines could not induce lipolysis. These results indicate that Krebs-Ringer bicarbonate buffer is suitable for the study of lipolysis in vitro. Krebs-Ringer phosphate buffer was inappropriate, because of its suppressive effect on in vitro lipolysis. Such an inhibitory effect of Krebs-Ringer phosphate buffer was not always observed; lipolysis induced by ACTH in Krebs-Ringer phosphate buffer was repressed only by 50% as compared with the lipolysis in Krebs-Ringer bicarbonate buffer (Fig. 2-C). Moreover, rat and hamster adipose tissues respond to catecholamines, even in Krebs-Ringer phosphate buffer (6). Accordingly, all experiments described below were carried out by employing Krebs-Ringer bicarbonate buffer.

Lipolytic responses of various tissues to epinephrine

The comparison of epinephrine-response of various adipose tissues, liver, kidney, and skeletal muscle was performed to detect the tissue responsible for the in vivo lipolysis by epinephrine (**Fig. 3**). When various tissues were incubated with 50 μg/ml of epinephrine, subscapular adipose tissue exhibited the highest concentrations of medium glycerol and FFA. Interscapular and perirenal adipose tissues, and frontal adipose tissue of neck were also responsive to the catecholamine.

Comparison of hormone-sensitivity of interscapular and epididymal fat cells

Dose responses of fat cells to hormones were examined by using interscapular and epididymal adipose tissues as the representatives of adipose tissues responsive and unresponsive to epinephrine, respectively (**Fig. 4**). Various catecholamines induced the lipolysis of only isolated interscapular fat cells. The minimum effective doses for lipolysis by isoproterenol, norepinephrine, and epinephrine were 0.1,

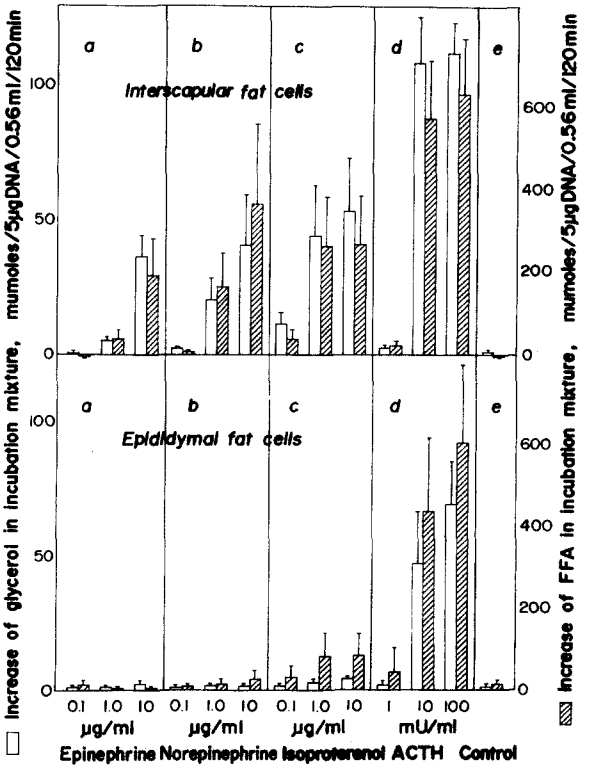

Fig. 4. Lipolytic responses of isolated interscapular and epididymal fat cells to various concentrations of hormones. Isolated interscapular (top) and epididymal (bottom) fat cells were incubated in the presence of 0.1, 1.0, and 10 μg/ml of epinephrine (Fig. 4-a), norepinephrine (Fig. 4-b), and isoproterenol (Fig. 4-c), or 1, 10, and 100 mU/ml of ACTH (Fig. 4-d), and in the absence of hormone (Fig. 4-e). Concentrations of glycerol (open bar) and FFA (hatched bar) of incubation mixture were determined. The values are means from four experiments. Vertical lines represent standard errors. Amounts of acylglycerols of isolated interscapular and epididymal fat cells were 1.7 ± 0.5 and 1.8 ± 0.3 mg/μg of DNA, respectively.

1.0, and 1.0 μg/ml, respectively. The lipolytic effect of norepinephrine at 1.0 μg/ml was several times more effective than that of the same concentration of epinephrine. On the other hand, these catecholamines could not induce the lipolysis of isolated epididymal fat cells even at a concentration of 10 μg/ml. In contrast to catecholamines, ACTH induced lipolysis in both interscapular and epididymal fat cells at concentrations of more than 10 mU/ml, and the maximum lipolysis of interscapular fat cells by ACTH was about 2-fold higher than that induced by catecholamines. The fact that isoproterenol was about 10-fold more effective than norepinephrine for the lipolysis in interscapular fat cells, and that norepinephrine was several times more effective than epinephrine, would indicate that the type of catecholamine-receptor of interscapular fat cells is a typical β_1-receptor as indicated by Fain (13). However, when catecholamine-sensitivities of in vivo (Fig. 1) and in vitro (Fig. 4) experiments were compared with each other, it was obvious that there was a different effect of norepinephrine in both experiments. That is, norepinephrine was more effective than epinephrine in vitro, but less effective in vivo.

DISCUSSION

There are two contradictory views about the catecholamine-induced lipolysis in the adult rabbit. One is the view that in vivo (7) and in vitro (6, 7) lipolysis cannot be induced by catecholamines. The other view is that intravascular injections of epinephrine (3) and norepinephrine (5) can induce the lipolysis in the adult rabbit, and that catecholamine brings about the in vitro lipolysis in perirenal (4) and interscapular (14) adipose tissues. The latter view is also supported by the present report that catecholamines are able to induce in vivo and in vitro lipolysis, with the exception of epididymal and omental adipose tissues, which are unresponsive to catecholamines. Major differences in experimental conditions between reports supporting the former view and the present paper are the method of in vivo administration of catecholamines and the sort of incubation buffer used in the in vitro experiments.

Desbals et al. (7) carried out the in vivo experiment as follows. Rabbit blood was collected at 0, 1, 2.5, 5, and 8 hr after three subcutaneous injections of 100 μg/kg of catecholamines at 0, 2.5, and 5 hr during the experiment. The FFA concentrations of blood samples after the injection were not changed as compared to the initial concentration. However, the time interval between injections of catecholamines and sampling the blood seems to be too long to estimate the FFA increase caused by catecholamines, because FFA is known to have a short half-life (15). Moreover, catecholamines injected subcutaneously take more time to reach adipose tissue than intravascularly injected hormones. The present report shows that increments of plasma glycerol and FFA induced by various hormones were reduced promptly by the termination of their infusion (Fig. 1).

Intravenous injection of epinephrine could elevate the plasma glycerol level but did not increase the plasma FFA level from 10 min after the infusion. Such an increase of plasma glycerol level without concomitant elevation of plasma FFA was also observed on VMH-stimulation (Fig. 1-A) and isoproterenol infusion (Fig. 1-D), while infusions of ACTH and norepinephrine (Fig. 1-C and 1-E) elevated both plasma glycerol and FFA levels in proportion to the infusion time. The cause of failure in the concomitant elevation of plasma glycerol and FFA upon infusions of epinephrine and isoproterenol, and VMH-stimulation, remains to be elucidated. However, two possibilities may be considered. One is that the utilization of plasma FFA released from adipose tissue is enhanced in such tissues as liver and muscle. This possibility would be eliminated for the reason that the rate of irreversible utilization of plasma FFA is directly proportional to the plasma FFA concentration (15). The other possibility is that the acceleration of re-esterification (16) or oxidation of FFA in adipose tissue inhibits the efflux of FFA from adipose tissue into the circulation. It seems to be convenient for re-esterification and oxidation of FFA that brown adipose tissue, such as interscapular adipose tissue of newborn rabbit, contains high amounts of glycerokinase (17) and cytochromes (18).

Catecholamines infused intravenously or perhaps liberated from adrenal medulla by VMH-stimulation could elevate the plasma glycerol level as shown in Fig. 1. If the elevation of plasma glycerol is the result of enhanced lipolysis of the stored fat depot, there must be the adipose tissues responsive to catecholamines. However, Rudman et al. (6) have described that such a high concentration of catecholamines as 100 μg/ml cannot increase FFA concentration in Krebs-Ringer phosphate medium that contains pieces of rabbit perirenal adipose tissue. Their report is not reconciled with the in vivo lipolysis by catecholamines shown in Fig. 1. To resolve this problem, the effects of Krebs-Ringer phosphate and bicarbonate buffers on the lipolysis of interscapular fat cells by catecholamines were compared with each other as shown in Fig. 2. It is obvious that the former buffer inhibits catecholamine-induced lipolysis; however, the

cause of this phenomenon is not known. By employing Krebs-Ringer bicarbonate buffer as incubation medium, many adipose tissues (and liver) could respond to 50 μg/ml of epinephrine (Fig. 3). The ratio of glycerol/FFA was markedly higher than the expected 1:3. This suggests that some parts of lipolysis by epinephrine depend on the partial hydrolysis of stored acylglycerols. In contrast to these epinephrine-responsive adipose tissues, a high concentration of epinephrine could not induce lipolysis in epididymal and omental adipose tissues.

In addition to epinephrine, various catecholamines could also act on interscapular fat cells, but they could not induce lipolysis of epididymal fat cells (Fig. 4). Inasmuch as ACTH could induce lipolysis in these two kinds of fat cells and both types of cells contained almost the same amount of fat per cell (Fig. 4, legend), the different lipolytic responses to catecholamines seem to be attributable to the presence or absence of a catecholamine-sensitive receptor.

It is known that subscapular and interscapular adipose tissues, and adipose tissue of frontal neck in newborn and young rabbits are classified as brown fat, while epididymal, omental, and perirenal adipose tissues are white fat. These brown adipose tissues of the young are replaced with white fat in the adult (14). Therefore, one may summarize that catecholamines administered exogenously or liberated endogenously in the adult rabbit act on the adipose tissues that were brown fat during the newborn period and a part of the white adipose tissue, such as perirenal, and that they do not induce lipolysis in other white adipose tissues, such as epididymal and omental. The physiological lipolysis of brown adipose tissue is generally considered to be under the control of norepinephrine released from systemic sympathetic nerve endings, especially on cold acclimatization (19). However, another study from this laboratory (2) indicates that VMH-stimulation of the rabbit can induce lipolysis as a result of catecholamine secretion from the adrenal medulla. This indicates that the lipolysis upon VMH-stimulation is brought about by catecholamines from sites other than the sympathetic nerve. It is not yet determined why the lipolysis of cold acclimatization and of VMH-stimulation are regulated differently. The possible cause of this difference may be derived from the differences in the type of stimulation or the animals employed.

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