Changes in Adipocyte β -Adrenergic Receptor of Cold-acclimated Rats

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Abstract The changes in the number and affinity of binding sites in the β -adrenergic receptors of rat white adipocytes after cold exposure were studied with the aid of (—)-[3 H]dihydroalprenolol. One day cold exposure did not change the number and affinity of binding sites in β -adrenergic receptors. Chronic exposure of rats to cold (5°C) for 1 and 4 weeks significantly decreased the affinity of β -adrenergic receptors without any alteration in the number of binding sites. Such changes in the binding affinity observed in cold-acclimated rats (4 weeks, 5°C) remained for 18 hr after these animals were transferred to a warm environment of 25°C. The decreased affinity of binding sites in β -adrenergic receptor induced by cold acclimation could not explain the enhanced metabolic response of cold-acclimated animals to noradrenaline.

The marked enhancement of the calorogenic response to noradrenaline is the most characteristic sign of cold acclimation in small mammals as well as in humans, and is conceded to be an essential basis in the development of nonshivering thermogenesis (JANSKÝ, 1973). However, the precise mechanism(s) involved in this phenomenon has not been thoroughly elucidated. Hormonal factors, including noradrenaline itself, have been shown recently to influence the tissue responses to noradrenaline *via* modifying the number and/or affinity of adrenergic receptors by the use of direct binding methods for studying these receptors (LEFKOWITZ *et al.*, 1976). Ample evidence has been compiled to show that the calorogenic response to noradrenaline is mainly mediated by β -adrenergic receptor (LEBLANC *et al.*, 1972; SCHÖNBAUM *et al.*, 1966). Judging from these findings, it is conceivable that a potentiated action of noradrenaline in cold-acclimated animals is exerted through the changes in the number and/or affinity of β -adrenergic receptors of target tissues. White adipose tissue is believed to be important for supplying the energy substrate, free fatty acids, for nonshivering thermogenesis. Lipolytic (HANNON and LARSON, 1962; THERRIAULT *et al.*, 1969a) and thermogenic (HIMMS-HAGEN and HAGEN, 1964) responses of this tissue to noradrenaline are reported to be markedly increased in cold acclimation. The present study was

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undertaken to know the effect of cold exposure on the β -adrenergic receptors in white adipocyte of rats and the possible participation of receptor component in the cold-induced potentiation of the tissue responses to noradrenaline.

MATERIALS AND METHODS

Male rats of the Wistar strain weighing 150–180 g were used throughout the experiments. They were kept under artificial illumination from 7:00–19:00 hr and given commercial rat chow (Oriental MF, Oriental Yeast Co., Ltd.) and tap water *ad libitum*. They were divided into two groups; one the warm control group kept at 25°C, and the other the cold-exposed group kept at 5°C. In order to match the age of the animals, they were so prepared as to be about 90 days old at the time of the binding experiments.

Isolated adipocytes were prepared by the modified method of RODBELL (1964). The rats were killed by decapitation. The distal portions of epididymal adipose tissues from 3 rats were pooled in each experiment, weighed, minced with scissors and added to a 50 ml plastic bottle with 8 ml albumin phosphate buffer (pH 7.4), containing 113 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 1.3 mM CaCl₂, 15.6 mM Na₂HPO₄, 5 mM glucose, 4% bovine serum albumin (Armour Co., Fraction V dialyzed for 48 hr through 0.5 μ m cellulose membrane filter against phosphate buffer), 1 mg/ml crude collagenase (Worthington Biochem. Co.). The suspensions were flushed with O₂:CO₂ (95:5, by vol.) for 2 min and incubated with shaking at 150 strokes per min at 37°C for 60 min. The digestion was filtered through one layer of cheesecloth. This was followed by centrifugation at 400 $\times g$ for 1 min. Cells were washed twice with 37°C albumin buffer and the centrifugation was repeated.

Crude adipocyte membranes were prepared by the method of WILLIAMS *et al.* (1976). Washed fat cells were suspended in 30 ml of the homogenization buffer (0.25 M sucrose, 1 mM EDTA, 10 mM Tris, pH 7.5 at 10 to 15°C) and homogenized by six up-and-down strokes in a glass Potter-Elvehjen homogenizer fitted with a Teflon pestle driven at 1,600 rpm. The homogenates were then centrifuged at 15,000 $\times g$ for 15 min and the pellet was washed twice in ice cold wash buffer (50 mM Tris, 10 mM MgCl₂, pH 8.0 at 4°C). The final pellet was resuspended in 2 ml of incubation buffer (50 mM Tris, 10 mM MgCl₂, pH 7.4 at 37°C), resulting in the suspension containing about 3 mg of protein/ml, which was used for binding assays.

Binding assays were performed according to the method of WILLIAMS *et al.* (1976), using radioactive antagonist, (—)-[³H]dihydroalprenolol (DHA). Crude membrane protein (*ca.* 300 μ g) was incubated with 2.5 to 80 nM DHA (32–39 Ci/mmol, Radiochemical Center, Ltd.) in a total volume of 300 μ l of incubation buffer with shaking at 150 strokes per min at 37°C for 12 min. Incubations were terminated by diluting 200 μ l incubation aliquots into 2 ml of ice-cold incubation

buffer and the suspension was rapidly filtered through a single Whatman GFC glass filter by vacuum aspiration. Filters were then washed rapidly with two 5 ml portions of cold incubation buffer. Filters were dried by gentle heating (30 min at 80°C) and added to a Triton/toluene-based scintillation cocktail, and the radioactivities of filters were counted in a Packard liquid scintillation spectrometer. In each experiment, total or nonspecific binding to protein was determined by measuring the amount of radioactivity retained on filters when incubations were performed in the absence or in the presence of a high concentration (10 μ M) of ($\pm$)-propranolol (Sigma Chemical Co.), respectively. Specific binding was determined by subtracting the nonspecific binding from the total binding counts. Protein was determined by the method of LOWRY *et al.* (1951).

The statistical significance of the results was tested by the student *t*-test.

Fig. 1. DHA binding to adipocyte membrane from control rats.

(A) $\circ$, total binding, binding in the absence of 10 μ M ($\pm$)-propranolol; $\bullet$, nonspecific binding, binding in the presence of 10 μ M ($\pm$)-propranolol. (B) specific binding as determined by the difference between total binding and nonspecific binding. Each point and vertical line shows the mean $\pm$ standard error of 17 experiments. (C) Scatchard plots of specific DHA binding. (D) Hill plots of specific DHA binding. B_{max} : specific binding at saturation as determined from the Scatchard plot (0.636 pmol/mg). B (bound): specific binding at a particular radioligand concentration. The value of the slope n_H , was determined by regression analysis ($r=0.99$). Each point shows the mean of 17 experiments.

RESULTS

Figure 1 shows the DHA binding to crude adipocyte membrane from control animals. Nonspecific binding of DHA increased lineally as the concentration of DHA increased (Fig. 1 (A)). Specific binding was 84.6–56.6% of total binding and was a saturable process (Fig. 1 (B)). Scatchard plots for specific binding displayed a departure from linearity with an upward concavity (Fig. 1 (C)) and the Hill coefficient, n_H , was 0.86, being less than unity (Fig. 1 (D)). These data are essentially similar to those of WILLIAMS *et al.* (1976) and MALBON *et al.* (1978), suggesting the possibility of multiple orders of binding sites, negatively cooperative interactions among the binding sites or both of them. The K_D derived from the Hill transformation (Fig. 1 (D)) was 16 nM, which was comparable to those obtained by WILLIAMS *et al.* (1976) and MALBON *et al.* (1978). Subsequently, in the

Fig. 2. Changes of specific DHA bindings to adipocyte membrane after cold exposure. ○, control rats (25°C); ●, cold-exposed rats (5°C). (A) 1 day cold exposure, (B) 1 week cold exposure, (C) 4 week cold exposure, (D) after 4 week cold exposure, the rats were transferred to a room at 25°C 18 hr before binding assay. Each point and vertical line shows the mean $\pm$ standard error of 4 to 5 experiments. +, $P < 0.02$ vs. control; ++, $P < 0.01$ vs. control; +++, $P < 0.001$ vs. control.

present study, the number of specific DHA binding sites ($B_{\max}$) was determined from the Scatchard plots and the dissociation constant of DHA (K_D) was determined from the Hill plots according to MALBON *et al.* (1978).

Figure 2 shows the time course of changes of specific DHA binding to crude adipocyte membranes from control and cold-exposed rats as a function of DHA concentration. Table 1 summarizes the protein content of plasma membrane, the number of β -adrenergic receptor binding sites ($B_{\max}$) and the dissociation constant of DHA (K_D) which were calculated from each binding experiment. Protein content of plasma membrane per gram wet weight significantly increased after cold exposure. However, total membrane protein per tissue did not change after cold exposure, since wet weight of adipose tissue significantly decreased after such treatment. Specific binding of DHA to adipocyte membrane obtained from 1-day-cold-exposed rats was not different from that obtained from corresponding control at any concentration of DHA (Fig. 2 (A)). Specific binding of DHA to adipocyte membrane obtained from 1-week-cold-exposed rats tended to decrease at low concentrations and to increase at high concentrations of DHA as compared with the control (Fig. 2 (B)). These changes caused significant decrease in the binding affinity of DHA ($1/K_D$) in 1-week-cold-exposed animals (Table 1). Specific

Table 1. Effect of cold exposure on the number and affinity of binding sites in the adipocyte β -adrenergic receptors.

	Plasma membrane protein content (mg/g wet wt)	Number of (-)- ³ H-DHA binding site (pmol/mg protein)	K_D of (-)- ³ H-DHA (nM)
A			
Warm control (4)	0.90 $\pm$ 0.08*	0.842 $\pm$ 0.026	27.4 $\pm$ 1.1
Cold 1 day (4)	0.92 $\pm$ 0.01	0.713 $\pm$ 0.070	23.3 $\pm$ 4.0
<i>P</i> vs. control	NS	NS	NS
B			
Warm control (5)	0.72 $\pm$ 0.06	0.596 $\pm$ 0.063	15.3 $\pm$ 1.8
Cold 1 week (5)	1.01 $\pm$ 0.04	0.769 $\pm$ 0.060	25.3 $\pm$ 2.9
<i>P</i> vs. control	<0.002	NS	<0.05
C			
Warm control (4)	0.80 $\pm$ 0.03	0.626 $\pm$ 0.024	16.3 $\pm$ 0.9
Cold 4 weeks (4)	1.18 $\pm$ 0.05	0.709 $\pm$ 0.078	32.4 $\pm$ 1.3
<i>P</i> vs. control	<0.001	NS	<0.001
D			
Warm control (4)	0.63 $\pm$ 0.02	0.658 $\pm$ 0.090	11.9 $\pm$ 1.9
Cold 4 weeks +18 hr at 25°C (4)	0.86 $\pm$ 0.08	0.758 $\pm$ 0.101	26.0 $\pm$ 3.5
<i>P</i> vs. control	<0.05	NS	<0.02

Number of specific binding sites was determined from the Scatchard plots and binding affinity from the Hill plots as shown in Fig. 1. Numbers in the parentheses indicate the number of experiments.

*: Mean $\pm$ standard error. NS: not significant.

binding in the 4-week-cold-exposed rats at low concentrations of DHA significantly decreased, while those at high concentrations were not different between control and cold-exposed rats (Fig. 2 (C)), resulting in the increased K_D with the same binding capacity compared to control (Table 1). The results in the 4-week-cold-exposed rats transferred to warm environment at 25°C for 18 hr prior to binding assay were similar to that in the 4-week-cold-exposed rats (Fig. 2 (D), Table 1).

DISCUSSION

The changes observed as to the property of β -receptor in adipocyte membrane after cold exposure seem to be leisurely and adaptive, in consideration of a prompt action of noradrenaline to adipose tissue, since one day cold exposure did not change the affinity of the receptor to DHA and such changes induced by cold acclimation remained unaltered for 18 hr after the rats were transferred to a warm environment of 25°C (Fig. 2 (A), (D)).

The effect of cold acclimation on calorogenic action of noradrenaline has been the subjects of many investigators (JANSKÝ, 1973), who unanimously described the potentiation of noradrenaline action in cold-acclimated mammals. However, the mechanism of the enhanced responsiveness to noradrenaline is still not known. PORTET *et al.* (1971) suggested from the chronic noradrenaline infusion experiments that an increased noradrenaline sensitivity in cold acclimation could be due to an increased affinity in binding sites of receptors. The present study investigated the same line of problem directly by means of the specific receptor assay with a potent β -adrenergic antagonist, DHA, which has been recently developed (LEFKOWITZ *et al.*, 1976), using the white adipocytes. The result of this study could not provide an explanation on the involvement of noradrenaline receptors in the cold-induced enhancement of responsiveness to noradrenaline, however, it has become evident that cold acclimation leads to a decrease in the affinity of binding sites of β -adrenergic receptors, at least, of white adipocytes. Previously, MUIRHEAD and HIMMS-HAGEN (1971, 1974) scrutinized the changes in the noradrenaline-sensitive adenylyl cyclase of brown adipose tissue and skeletal muscle in cold acclimation. Although β -adrenergic receptor is known to be associated with adenylyl cyclase, this noradrenaline responsive membrane structure did not show any significant alterations in either brown adipose tissue or skeletal muscle of cold-acclimated rats. FOSTER *et al.* (1977) also studied the changes of the concentration of plasma cyclic-AMP of warm- and cold-acclimated rats during noradrenaline infusion to see whether the enhancement of calorogenic response to noradrenaline observed in cold-acclimated rats could be attributable to the effect of adenylyl cyclase-cyclic-AMP system. However, they did not find the difference between warm- and cold-acclimated rats in ability to produce heat by nonshivering thermogenesis coinciding with the changes in the cyclic-AMP. Recently, BUKOWIECKI *et al.* (1978) reported in abstract form the reduced β -receptors per cell

of cold acclimated rat brown adipose tissue, although three fold increase of total number of receptor sites was found due to cold-induced hyperplasia of this tissue. However, they did not observe any changes in the receptor affinity. In the present study, the total number of receptor sites in white adipose tissue seems not to change after cold exposure, since both the total membrane protein and receptor sites per mg membrane protein did not change after cold exposure. Furthermore, it should be pointed that since cold acclimation could increase the total number of adipocytes in white adipose tissue (THERRIAULT *et al.*, 1969a), the number of β -receptor per cell may be reduced after cold exposure. This assumption would be solved by observing the changes in the receptors in intact adipocytes. Accordingly, direct or indirect findings as to the changes in properties of β -adrenergic receptors induced by cold acclimation could not explain the enhanced effect of noradrenaline on lipolysis (HANNON and LARSON, 1962; THERRIAULT *et al.*, 1969a) and thermogenesis (HIMMS-HAGEN and HAGEN, 1964) due to cold acclimation.

Further, THERRIAULT *et al.* (1969b) and BERTIN and PORTET (1976) observed that cold acclimation induced an increased sensitivity of white fat cells to the cyclic-AMP accumulation due to noradrenaline. The change of cyclic-AMP accumulation was reported to be different from that of binding affinity of β -receptor in the effect of catecholamine on the cerebral β -adrenergic receptor system (NAHORSKY, 1977) and in the effect of thyroid hormone on adipocyte β -adrenergic receptor system (MALBON *et al.*, 1978). One possible explanation of the dissociation between the decreased binding affinity observed here and the increased cyclic-AMP accumulation in other studies might be that cold acclimation affects the efficiency in coupling between the receptor and adenylyl cyclase, or the destruction rate of cyclic-AMP by phosphodiesterase, or mitochondrial function (HIMMS-HAGEN *et al.*, 1975).

It has been recently demonstrated in a number of systems that the occupancy of receptor sites leads to alterations in the receptor number; an increased occupation decreases the number of receptors, while a decreased occupation increases the number of receptors (GLAUBIGER and LEFKOWITZ, 1977; HARDEN *et al.*, 1977; NAHORSKY, 1977). During the process of cold acclimation, an increased secretion of noradrenaline has been well known (LEDUC, 1961). However, it is interesting to note that chronically increased occupation of β -adrenergic receptor by noradrenaline in cold acclimation would appear specifically to decrease the affinity of noradrenaline to its receptor, but not the number of receptor. In this connection it is interesting to note the finding that the affinity of insulin receptor increased in adipocytes of the diabetic and fasted animals (WIERINGA and KRANS, 1978).

Further studies should be performed before a final conclusion could be drawn, such studies as the analysis of α - and β -receptors of skeletal muscle and brown fat cells, both of which are known to be the sites for nonshivering thermogenesis in cold acclimation (JANSKÝ, 1973). At any rate, the finding obtained in the present study, the decrease in the affinity of β -adrenergic receptor, should be

deemed to be of physiological significance. The decrease in the receptor affinity probably secure an appropriate availability of noradrenaline secreted copiously in cold acclimation to the tissues, preventing the havoc in the body.

REFERENCES

- BERTIN, R. and PORTET, R. (1976) Effects of lipolytic and antilipolytic drugs on metabolism of adenosine 3':5'-monophosphate in brown adipose tissue of cold acclimated rats. *Eur. J. Biochem.*, **69**: 177-183.
- BUKOWIECKI, L., FOLLEA, N., and VALLIÈRES, J. (1978) Changes of β -adrenergic receptor number in rat brown adipose tissue during cold-acclimation. *Fed. Proc.*, **37**: 249.
- FOSTER, D. O., FRYDMAN, M. L., and USHER, J. R. (1977) Nonshivering thermogenesis in the rat. I. The relation between drug-induced changes in thermogenesis and changes in the concentration of plasma cyclic AMP. *Can. J. Physiol. Pharmacol.*, **55**: 52-64.
- GLAUBIGER, G. and LEFKOWITZ, R. J. (1977) Elevated beta-adrenergic receptor number after chronic propranolol treatment. *Biochem. Biophys. Res. Commun.*, **78**: 720-725.
- HANNON, J. P. and LARSON, A. M. (1962) Fatty acid metabolism during norepinephrine-induced thermogenesis in the cold-acclimatized rat. *Am. J. Physiol.*, **203**: 1055-1061.
- HARDEN, T. K., WOLFE, B. B., SPORN, J. R., POULOS, B. K., and MOLINOFF, P. B. (1977) Effects of 6-hydroxydopamine on the development of the beta adrenergic receptor/adenylate cyclase system in rat cerebral cortex. *J. Pharmacol. Exp. Ther.*, **203**: 132-143.
- HIMMS-HAGEN, J., BEHRENS, W., MUIRHEAD, M., and HBOUS, A. (1975) Adaptive changes in the calorogenic effect of catecholamines: Role of changes in the adenylyl cyclase system and of changes in mitochondria. *Mol. Cell. Biochem.*, **6**: 15-31.
- HIMMS-HAGEN, J. and HAGEN, P. B. (1964) Actions of adrenaline and noradrenaline on metabolic systems. In: *Actions of Hormones on Molecular Processes*, ed. by LITWACK, G. and KRITCHEVSKY, P., John Wiley and Sons, Inc., New York-London-Sydney, pp. 268-319.
- JANSKÝ, L. (1973) Non-shivering thermogenesis and its thermoregulatory significance. *Biol. Rev.*, **48**: 85-132.
- LEBLANC, J., VALLIÈRES, J., and VACHON, C. (1972) Beta-receptor sensitization by repeated injections of isoproterenol and by cold adaptation. *Am. J. Physiol.*, **222**: 1043-1046.
- LEDUC, J. (1961) Catecholamine production and release in exposure and acclimation to cold. *Acta Physiol. Scand.*, (Suppl. 183), **43**: 1-101.
- LEFKOWITZ, R. J., LIMBIRD, L. E., MUKHERJEE, C., and CARON, M. G. (1976) The β -adrenergic receptor and adenylyl cyclase. *Biochim. Biophys. Acta*, **457**: 1-39.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. (1951) Protein measurements with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.
- MALBON, C. C., MORENO, F. J., CABELLI, R. J., and FAIN, J. N. (1978) Fat cell adenylyl cyclase and β -adrenergic receptors in altered thyroid states. *J. Biol. Chem.*, **253**: 671-678.
- MUIRHEAD, M. and HIMMS-HAGEN, J. (1971) Changes in the amount and properties of adenylyl cyclase in brown adipose tissue during acclimation of rats to cold. *Can. J. Biochem.*, **49**: 802-810.
- MUIRHEAD, M. and HIMMS-HAGEN, J. (1974) Changes in the adenylyl cyclase system of skeletal muscle of cold-acclimated rats. *Can. J. Biochem.*, **52**: 176-180.
- NAHORSKY, S. R. (1977) Altered responsiveness of cerebral beta adrenoceptors assessed by adenosine cyclic 3',5'-monophosphate formation and [3 H]propranolol binding. *Mol. Pharmacol.*, **13**: 679-689.
- PORTET, R., BERTIN, R., LAURY, M. C., and CHEVILLARD, L. (1971) Calorogenic effects of catecholamines in rats adapted to various temperatures. In: *Nonshivering thermogenesis*, ed.

- by JANSKÝ, L., Academia, Prague, pp. 57-71.
- RODBELL, M. (1964) Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.*, **239**: 375-380.
- SCHÖNBAUM, E., JOHNSON, G. E., SELLERS, E. A., and GILL, M. J. (1966) Adrenergic β -receptors and non-shivering thermogenesis. *Nature*, **210**: 426.
- THERRIAULT, D. G., HUBBARD, R. W., and MELLIN, D. B. (1969a) Endocrine control of fat mobilization in the isolated fat cells of cold-exposed rats. *Lipids*, **4**: 413-420.
- THERRIAULT, D. G., MORNINGSTAR, J. F., Jr., and WINTERS, V. G. (1969b) Adenyl cyclase activity in isolated fat cells from cold exposed rats. *Life Sci.*, **8**: Part II, 1353-1358.
- WIERINGA, T. and KRANS, H. M. J. (1978) Reduced glucose transport and increased binding of insulin in adipocytes from diabetic and fasted rats. *Biochim. Biophys. Acta*, **538**: 563-570.
- WILLIAMS, L. T., JARETT, L., and LEFKOWITZ, R. J. (1976) Adipocyte β -adrenergic receptors. *J. Biol. Chem.*, **251**: 3096-3104.