Pflügers Arch. 381, 113–117 (1979)

Pflügers Archiv
European Journal of Physiology


# Calorigenic Effects of Noradrenaline and Glucagon on White Adipocytes in Cold- and Heat-Acclimated Rats

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**Abstract.** Calorigenic actions of noradrenaline and glucagon on isolated epididymal fat cells from warm-acclimated controls, cold-acclimated and heat-acclimated rats were measured by the use of a twin-type conduction microcalorimeter. Both noradrenaline and glucagon stimulated heat production in isolated adipocytes maximally in doses of 1 μg/ml and 10μg/ml, respectively. Maximal responsiveness of adipocytes per unit cell to noradrenaline was not influenced by cold acclimation, while it was reduced by heat acclimation. Maximal response in total epididymal fat cells to noradrenaline was increased in cold acclimation and not changed in heat acclimation at increased numbers of adipocytes in both cold-acclimated and heat-acclimated animals. Maximal response per unit cell as well as per total epididymal fat cells to glucagon was increased in cold acclimation and reduced in heat acclimation.

The present results indicate that the modified responses of target adipocytes to noradrenaline and glucagon are involved in the development of temperature acclimation.

**Key words:** Thermogenesis of fat cells – Temperature acclimation – Noradrenaline – Glucagon.

## Introduction

White adipose tissue is well known to supply an energy substrate, free fatty acids (FFA), for heat production, particularly nonshivering thermogenesis, in cold acclimation [18]. The activity of this tissue is mainly regulated by noradrenaline released from the sympathetic nervous system [8]. Glucagon is also a potent lipolytic agent in the white adipose tissue [7, 12]. Both noradrenaline and glucagon have been found to be involved in cold and heat acclimation through the modification of lipid metabolism [13, 14, 20, 23]. Moreover, it is noted that not only the secretion of noradrenaline is increased, but also the responsiveness to the metabolic action of this neurohormone is enhanced in cold-acclimated animals and men [2]. In heat-acclimated animals the secretion of noradrenaline and the systemic calorigenic action of noradrenaline are lessened [19, 20]. Therefore, the responsiveness of white adipose tissue to humoral factors such as noradrenaline and glucagon might be altered by temperature acclimation. In cold-acclimated rats noradrenaline was reported to produce greater lipolytic [9] and respiratory [10] responses in white adipose tissue.

The present study aimed to investigate whether the thermogenic responses of isolated white adipocytes to noradrenaline and glucagon are changed in cold- as well as in heat-acclimated rats.

## Materials and Methods

Adult male rats of Wistar strain, weighing ca. 250 g, fed on usual rat biscuit (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) were acclimated for 4 to 5 weeds to 25° C (warm-acclimated control rats) at 50% relative humidity, 5° C (cold-acclimated rats) and 34° C (heat-acclimated rats) at 40–45% of relative humidity. Cold- and heat-acclimated rats were kept in the control temperature of 25° C for 18 h before the experiments in order to obtain the basal metabolic state as much as possible and, in addition, to observe the rather stable adaptive changes.

Isolated adipocytes were prepared by the modified method of Rodbell [22]. The rats were killed by decapitation and the whole or distal portion of both epididymal fat pads was removed, weighed, minced with scissors and put in a 50 ml plastic bottle with 4 ml Krebs-Ringer phosphate buffer (pH 7.4), containing half the recommended concentration of $CaCl_2$, 5 mM glucose, 4% bovine serum albumin (Armour Co., Fraction V, dialyzed for 48 h through 0.5 μ cellulose membrane against phosphate buffer) and 4 mg crude collagenase (Worthington Biochem. Corp.). The tissue suspension was bubbled with 95% $O_2$ – 5% $CO_2$ for 2 min and incubated in a shaking water bath at 150 strokes per min at 37° C for 60 min. The digest was filtered through one layer of cheesecloth and centrifuged at 400 g for 1 min. Cells were washed twice with the albumin buffer kept at 37° C.

0031-6768/79/0381/0113/$01.00

**Table 1.** Effects of environmental temperatures on the number and size of epididymal white adipocytes

| | Warm-acclimated controls | Cold-acclimated rats | Heat-acclimated rats |
|---|---|---|---|
| Number of animals | 5 | 5 | 5 |
| Tissue weight (g/100 g) | 1.005 ± 0.0823 | 0.760 ± 0.0790*** | 0.903 ± 0.1030 |
| Recovery of cells (%) | 46.3 ± 5.29 | 51.1 ± 4.59 | 48.1 ± 1.31 |
| Number of cells ($\times 10^6$) | 6.08 ± 0.452 | 10.12 ± 0.489* | 8.35 ± 0.619** |
| Diameter of cells (μm) | 80.4 ± 2.55 | 61.6 ± 2.66* | 67.1 ± 2.16** |

* Significantly different ($P < 0.001$) from warm-acclimated controls
** Significantly different ($P < 0.01$) from warm-acclimated controls
*** Significantly different ($P < 0.05$) from warm-acclimated controls
Values are the means ± SEM

Finally, 10 ml cell suspension was made by adding the albumin buffer kept at 37° C and bubbled with 95% $O_2$ – 5% $CO_2$ to the isolated cells.

Heat production of adipocytes was measured by a twin-type conduction microcalorimeter (RCM-2F, Rhesca Co., Ltd., Tokyo) [6]. The pyrex glass vessels used for calorimeter were siliconized. The sample vessel consisted of two compartments, one contained cell suspension (4.5 ml) and the other noradrenaline or glucagon solution (0.5 ml). Crystalline glucagon (Sigma Chemical Co.) was dissolved in 0.01 N HCl. Glucagon and noradrenaline solution (Sankyo Pharmaceutical Co., Ltd.) were diluted with Krebs-Ringer phosphate buffer to an appropriate concentration immediately before the experiments. The reference vessel also consisted of two compartments, one contained 4.5 ml of cell suspension, but the other the buffer solution only. The gas phase in the vessels was 95% $O_2$ – 5% $CO_2$, since $CO_2$ was found to be important for the continuous respiration of the tissue [21]. The temperature of the vessels was maintained at 37° C. After temperature equilibration of the vessels, which required about 2 h, the vessels were continuously rotated at 4 rpm in order to mix, aerate and agitate the contents for 60 min. The difference in the heat output between the stimulated and unstimulated cells was monitored on an electric recorder. The amount of heat generated is proportional to the chart area under the thermogram and calculated by comparing with the calibrated value for a constant heat input [6].

The diameter and number of isolated fat cell were measured on a hemocytometer. The number of fat cells in the total fat pads was calculated from the lipid content of the tissue and the cell volume [5].

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

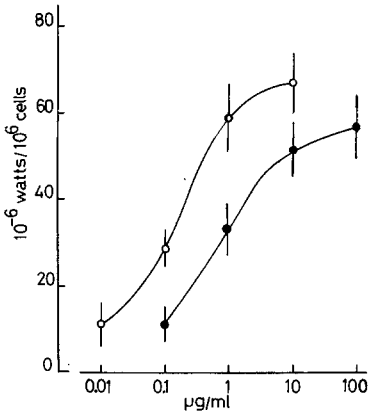


**Fig. 1.** Dose-response relation in noradrenaline (○) and glucagon (●) thermogenesis of isolated adipocytes. Each point is the mean ± SEM of 5 to 8 experiments. The isolated cells were obtained from warm-acclimated controls

## Results

*Effect of Temperature Acclimation on the Size and Number of White Adipocytes.* Table 1 shows the changes in the size and number of fat cells. The recovery rate of isolated adipocytes was not significantly different among the experimental groups, being 46.3 – 51.1%. Therefore, the number of fat cells in the total epididymal adipose tissue obtained by actual counting was listed in Table 1 without correction. Cold acclimation decreased the size and increased the number of fat cells as previously described [24], although it decreased the tissue weight per unit body weight. Heat acclimation also decreased the size and increased the number of fat cells as cold acclimation, while it did not affect the tissue weight per unit body weight.

*Dose-Response Relation for Noradrenaline and Glucagon.* As shown in Fig. 1, noradrenaline and glucagon exerted about maximum actions on heat production per unit cell at the concentrations 1 μg/ml and 10 μg/ml, respectively. These results were compatible to those reported for the actions of these agents on oxygen uptake and free fatty acid release in the sliced white adipose tissue [7,12]. Consequently, the above concentrations of noradrenaline and glucagon were used in the following experiments.

*Response to Noradrenaline* (Table 2). Thermogenic response per unit cell was not affected by cold acclimation, while it was significantly reduced by heat acclimation. However, total heat production of epididymal fat cells was significantly greater in cold-acclimated rats and not significantly different in heat-acclimated rats, since both cold and heat acclimation increased the number of fat cells in the epididymal fat

**Table 2.** Effects of noradrenaline and glucagon on heat production of isolated adipocytes from temperature-acclimated rats

| | | A: Cells from total epididymal fat pads | | B: Cells from distal thin portion of fat pads |
|---|---|---|---|---|
| | | $10^{-6}$ watts/$10^6$ cells | $10^{-6}$ watts/total tissue cells | $10^{-6}$ watts/$10^6$ cells |
| I | Noradrenaline (1 μg/ml) | | | |
| | Warm-acclimated controls | 48.3 ± 5.30 (8) | 281.4 ± 22.81 | 46.6 ± 4.24 (8) |
| | Cold-acclimated rats | 53.1 ± 4.39 (6) | 535.6 ± 50.45* | 45.7 ± 7.56 (6) |
| | Heat-acclimated rats | 32.2 ± 3.34*** (12) | 252.1 ± 16.78 | 30.0 ± 3.44** (8) |
| II | Glucagon (10 μg/ml) | | | |
| | Warm-acclimated controls | 40.5 ± 3.44 (9) | 247.4 ± 30.03 | 30.0 ± 4.18 (8) |
| | Cold-acclimated rats | 57.5 ± 5.16*** (9) | 503.2 ± 64.70** | 62.3 ± 12.14**** (6) |
| | Heat-acclimated rats | 23.4 ± 4.08** (8) | 153.3 ± 14.18*** | 15.7 ± 1.73** (8) |

* Significantly different ($P < 0.001$) from warm-acclimated controls
** Significantly different ($P < 0.01$) from warm-acclimated controls
*** Significantly different ($P < 0.02$) from warm-acclimated controls
**** Significantly different ($P < 0.05$) from warm-acclimated controls
Each value represents the mean ± SEM. Numbers in the parenthesis indicate the number of animals

pads (Table 1). The same results expressed by the response per unit cell were observed in the fat cells obtained from the thin distal portion of epididymal fat pads.

*Response to Glucagon* (Table 2). Heat production of the fat cells related either to per unit cell or to total tissue cells was enhanced by cold acclimation and reduced by heat acclimation. This is also the case in the fat cells obtained from the thin distal portion of epididymal fat pads.

## Discussion

It has been previously shown that both noradrenaline and glucagon are capable of stimulating oxygen consumption in white adipose tissue [7, 12]. The present results indicating that noradrenaline and glucagon provoke heat production in isolated white adipocytes are compatible with the above findings. The present study also confirms the previous report that cold acclimation increases the number and decreases the size of epididymal fat cells [24]. It is also noted that heat acclimation shows the same direction of the changes in the number and size of fat cells to those due to cold acclimation. The changes in the number of fat cells brought about the greater noradrenaline-induced thermogenesis of the total epididymal fat cells from cold-acclimated animals, although the tissue weight was reduced and the responsiveness per unit cell was not significantly affected. In the case of heat-acclimated fat cells with the reduced responsiveness per unit cell to noradrenaline the amount of heat generated in the total epididymal fat cells was not different from that in the warm-acclimated control fat cells. It is well evidenced that the activity of sympathetic nervous system is stimulated in cold acclimation [2, 23] and low in heat acclimation [20, 23]. Thus, it could be said that the activity of noradrenaline-white adipocyte system is enhanced in cold acclimation and depressed in heat acclimation. In contrast to our results, Himms-Hagen and Hagen [10] reported that the action of noradrenaline on the oxygen consumption per mg protein N of rat sliced epididymal adipose tissue was enhanced by cold acclimation for 4–5 weeks. Other investigators [11] reported that no increment of oxygen consumption was found in the minced epididymal fat pads from the cold-acclimated rats for 7 days by addition of noradrenaline.

Possible involvement of glucagon in temperature acclimation has been previously reported from our laboratories [13, 14]. Plasma glucagon level decreased in the heat-acclimated rats, while it increased at the early stage of cold acclimation, but it returned to the normal value at the later stage of cold acclimation. The present study indicates that the heat production in white adipocytes stimulated by glucagon either per unit cell or per total tissue cells is enhanced in the cold acclimation of 4 weeks, when the plasma glucagon level becomes similar to that in the warm-acclimated controls, suggesting that glucagon is closely associated with temperature acclimation through regulating the metabolic activities of white fat cells.

The thermogenic process in the white adipocytes has been shown to reflect the active metabolic functions such as lipolysis-fatty acid reesterification cycle, glycolysis, glucose and fatty acid oxidation in the citric acid cycle. Especially, lipolysis-reesterification cycle would

be responsible for the thermogenic response in white adipocytes [10]. It is likely that an altered response of fat cells to humoral agents in the temperature acclimation is useful for regulating storage and delivery of energy substrate, FFA, in the temperature acclimation. On the other hand, Therriault et al. [24] reported that the reesterification in the white adipose tissue is not influenced by cold acclimation. These findings appear to indicate that precise metabolic events occurring in the white adipocytes responsible for the altered responsiveness to noradrenaline and glucagon should await further study. However, from the results obtained we can say at least that not only the altered secretions of noradrenaline and glucagon, but also the modified responsiveness of target cell such as adipocyte to these humoral factors are involved in the development of temperature acclimation.

It is also noted that the mechanism underlying the altered responsiveness of adipocytes from the temperature acclimated animals remains unknown. It was reported [15] that the size of adipocyte could influence the cell responsiveness to the lipolytic agents; the lipolytic response elicited by glucagon in small cells from young rats is greater than in large cells from adult rats. Therefore, the diminished size of adipocyte in the cold-acclimated rats may be responsible for an enhanced thermogenic response to glucagon. However, it is shown that the adipocytes from cold-acclimated rats are more sensitive to a lipolytic action of noradrenaline independent of differences in cell size, although it is observed that the smaller adipocytes are usually more sensitive to a lipolytic action of noradrenaline [24]. Moreover, the present results reveal that the smaller adipocytes from the heat-acclimated rats are less sensitive to the calorigenic actions of both noradrenaline and glucagon, suggesting that other factor(s) than the cell size contributes to the altered responsiveness in the temperature acclimation.

Recently, the changes in the adrenergic [3, 17] and glucagon [16] receptors have been implicated in explaining the altered responsiveness of adipocytes induced by hormonal factors. Hyperthyroidism induced an acceleration of glucagon-stimulated production of cyclic AMP and a lipolysis in association with an increase in binding sites of glucagon to fat cells [16]. Ciaraldi and Marinetti [3] described an increase in adrenergic beta-receptors in fat cells from the hyperthyroid rats, while Malbon et al. [17] reported that both the number and the affinity of adrenergic beta-receptors were the same for fat cells obtained from control, hyperthyroid and hypothyroid rats. The latter investigators suggest that the thyroid hormones may exert their influences on the fat cells by regulating the transduction of information between hormone receptors and adenylate cyclase. It is well established that the changes in hormonal environment consisting of thyroid hormones, adrenocortical hormones, catecholamines, glucagon, etc. are seriously involved in the development of temperature acclimation [2, 4, 14]. Therefore, it is likely that the adrenergic and glucagon receptors in fat cells are modified by cold and heat acclimation, resulting in the altered responsiveness to the calorigenic actions of these humoral factors. The studies on this line are now under contemplation.

The structural changes of intracellular thermogenic units, mitochondria, may be also related to the altered responsiveness of fat cells induced by temperature acclimation [1].

*Acknowledgments.* This work was in part supported by Grant in Aid for Scientific Research No. 248116 from the Ministry of Education of Japan. We wish to express our gratitude to Dr. L. Asato and Dr. T. Sakurai, Department of Nutrition, College of Health Sciences, University of The Ryukyus, for their valuable advice about the operation of microcalorimeter.

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*Received March 26, 1979*