CHARACTERISTICS OF \(\beta\)-ADRENERGIC RECEPTORS IN BROWN ADIPOCYTES OF TEMPERATURE-ACCLIMATED RATS

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ABSTRACT

Characteristics of \(\beta\)-adrenergic receptors in brown adipocytes of temperature-acclimated rats were investigated by the use of the adrenergic \(\beta\)-antagonist (−)-[\(^3\text{H}\)]dihydroalprenolol (DHA). DHA binding to brown adipocytes was rapid, reversible, and saturable, displaying stereospecificity to \(\beta\)-adrenergic agonists as well as antagonists. According to the potency order of \(\beta\)-adrenergic (−)-agonists in a competition study, the adrenergic receptors of brown adipocytes proved to be the \(\beta_1\)-subtype. Both acute cold exposure and cold acclimation significantly decreased the number of DHA binding sites. Acute heat exposure did not affect the number of DHA binding sites, while heat acclimation significantly decreased it. In these experiments, the equilibrium dissociation constant of DHA was not influenced. These results indicate that \(\beta\)-adrenergic receptors of brown adipocytes respond to thermal stimuli with changes in number of binding sites, but not in affinity. Changes in \(\beta\)-adrenergic receptors of cold-acclimated as well as cold-exposed brown adipocytes could not explain enhanced thermogenic response of brown fat to norepinephrine in cold acclimation, but such changes would appear to be adaptive to increased secretion of norepinephrine through down-regulation. A decreased number of \(\beta\)-adrenergic receptors, probably caused by hypothyroidism due to heat acclimation, could explain the depressed thermogenic response of brown fat to norepinephrine in heat acclimation.

KEY WORDS \(\beta\)-adrenergic receptor / brown adipocyte / cold acclimation / heat acclimation / norepinephrine / temperature acclimation

Enhanced and depressed thermogenic responses to norepinephrine are the most characteristic signs in cold- and heat-acclimated rats, respectively (13, 19). Brown adipose tissue solely contributes to non-shivering thermogenesis in cold-acclimatized mammals, neonates, and hibernators during exposure to cold and during arousal from hibernation (23). Brown adipose tissue may be responsible for the major part of the systemic thermogenic response to norepinephrine (10) and cold (11) in the cold-acclimated rat. Recent data from our laboratory showed that the thermogenic response of isolated rat brown adipocytes to norepinephrine is increased in cold acclimation, and decreased in heat acclimation (16). However, the biochemical mechanism(s) involved in the modified thermogenic responses of these target cells to norepinephrine in temperature acclimation has not been elucidated. There is substantial evidence to show that norepinephrine elicits heat production in brown adipose tissue via \(\beta\)-adrenergic receptors in the cell membrane (2, 12). Moreover, several hormonal factors, including norepinephrine itself, have been shown to regulate the cell responses to norepinephrine by modifying the adrenergic receptors (17). Therefore, the modified thermogenic response...
of brown adipocytes to norepinephrine in temperature acclimation might be explained by the changes in the number and/or affinity of β-adrenergic receptors of these target cells. In this study, the radioactive β-adrenergic antagonist (−)[3H]dihydroalprenolol (DHA) was used to analyze β-adrenergic receptors in the rat brown adipocytes and the effect of temperature acclimation on β-adrenergic receptors of brown adipocytes was investigated.

MATERIALS AND METHODS
Male rats of the Wistar strain weighing about 180 g were used throughout the experiments. The rats were kept under artificial illumination from 7:00 to 19:00 and given commercial rat chow (Oriental MF, Oriental Yeast Co.) and tap water ad libitum. They were acclimated for 4 to 5 weeks to 25°C at 50% relative humidity (warm-acclimated control rats), 5°C (cold-acclimated rats) and 33°C at 40-45% of relative humidity (heat-acclimated rats). Cold- and heat-acclimated rats were transferred to the control temperature of 25°C 18 hr before excising brown adipose tissue in order to observe the stable adaptive changes. Some warm-acclimated control rats were exposed to 5°C (cold-exposed rats) or 33°C (heat-exposed rats) for 24 hr.

Adipocytes of the interscapular brown adipose tissue were isolated by modifications of the method of Rodbell (20). The rats were killed by decapitation between 9:00 and 10:00 a.m. Pooled brown fat excised from three rats was extensively minced with scissors and placed in a plastic bottle with 3 ml Krebs-Ringer phosphate buffer (pH 7.4) per g wet weight, containing half the recommended concentration of CaCl2, 5 mM glucose, and 4% bovine serum albumin (Armour Co., Fraction V, dialyzed for 48 hr through 0.5 μm cellulose membrane against phosphate buffer) and 3.3 mg/ml of crude collagenase (Worthington Biochem. Co.). The bottle was bubbled with 95% O2-5% CO2 and placed in a shaking water bath (37°C, 150 strokes per min). To prevent possible hypoxia during the digestion, the bottle was removed from the bath every 5 min, shaken vigorously, regassed and replaced in the bath. The time of incubation varied between 30 and 45 min according to the state of tissue digestion. The digested tissue was filtered through one layer of cheesecloth and centrifuged for 5 min at 50 g. The infranatant was removed by aspiration with a lure-lock type of syringe. The supernatant layer of cells was then washed twice with 4 ml of buffer kept at 37°C. The final suspension was prepared with fresh buffer to 4 to 8 ml. The cells were counted in Bürker-Türk chamber.

Isolated brown adipocytes (1–3 × 10^5 cells) were incubated with 0.625 to 10 nM (−)[3H]-dihydroalprenolol (DHA) (42–59 Ci/mmol, Radiochemical Centre, Amersham Co.) in a total volume of 0.5 ml of 2% albumin Krebs-Ringer phosphate buffer containing 100 μM phenolamine to minimize nonspecific binding (4), with shaking at 120 strokes per min at 37°C for 8 min. Incubation was terminated by adding 3 ml of ice-cold incubation buffer to the incubation mixture and the suspension was rapidly filtered through a single Whatman GFC glass filter by vacuum aspiration. The filter was dried by gentle heating (30 min at 80°C) and added to a Tritontoluene based scintillation cocktail, the radioactivity of which was counted in a Packard liquid scintillation spectrometer. In each experiment, total and nonspecific binding to brown adipocytes was determined by measuring the amount of radioactivity retained on the filter in the absence and in the presence of 100 μM (−)-isoproterenol, respectively. Specific binding was determined by subtracting the nonspecific binding from the total binding.

The agents used were as follows: (+)-isoproterenol bitartrate (a gift from Winthrop Lab.); (−)-stereoisomers of norepinephrine, epinephrine and isoproterenol as bitartrate forms, and (±)-propranolol hydrochloride (Sigma Chemical Co.); (±)-propranolol hydrochloride and (−)-propranolol hydrochloride (gifts from ICI Pharma Co.); phenolamine hydrochloride (Ciba-Gelgy Co.). Metabisulfite (0.2 mM) was added to the stock solutions of adrenergic agonists and antagonists to retard oxidation.

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

RESULTS
Characteristics of Specific DHA Binding to Brown Adipocytes
DHA binding to brown adipocytes from warm-acclimated control rats in the absence of (−)-isoproterenol (total binding) rapidly reached equilibrium at 2 min and was stable up to 10 min (Fig. 1(A)). At equilibrium, the addition of 100 μM (−)-isoproterenol caused a rapid dissociation of bound DHA from brown adipocytes, reaching maximal dissociation within 6 min (Fig. 1(B)). DHA binding sensitive to 100 μM (−)-isoproterenol was evaluated as
specific DHA binding to β-adrenergic receptors. One μM (−)-isoproterenol caused half maximal inhibition of specific DHA binding to brown adipocytes, while 11.2 μM (+)-isoproterenol was required to produce the same inhibition of specific DHA binding (Fig. 2(A) and Table 1). Similar stereospecificity of DHA binding to brown adipocytes was observed in the competition study employing the β-adrenergic antagonists (+)- and (−)-propranolol; half maximal inhibition of specific DHA binding occurred at 0.028 μM with (−)-propranolol, and at 2.51 μM with (+)-propranolol (Fig. 2(B) and Table 1). The β-adrenergic agonists (−)-norepinephrine and (−)-epinephrine also competed with DHA for DHA binding sites. The order of potency of agonists was as follows: (−)-isoproterenol > (−)-norepinephrine > (−)-epinephrine (Fig. 3 and Table 1). The dissociation constant of DHA for specific DHA binding sites and the maximal specific DHA binding were determined from the binding experiment performed at thermodynamic equilibrium (Fig. 4(A)). The Scatchard plot of specific DHA binding exhibited almost lineal saturation, indicating there is only one class of DHA binding

Fig. 1 Time course of DHA (5 nM) binding to brown adipocytes (A) and dissociation of DHA from brown adipocytes (B). In Figs. 1 and 2, each point and vertical line show mean ± standard error of 3 experiments performed in duplicate.

Fig. 2 Inhibition of specific DHA (5 nM) binding to brown adipocytes by (+)- and (−)-stereoisomers of isoproterenol (A) and propranolol (B).
Table 1  Equilibrium Dissociation Constant of Adrenergic Agonists and Antagonists for Specific DHA Binding Sites in Brown Adipocytes

<table>
<thead>
<tr>
<th>Competitor</th>
<th>EC₅₀</th>
<th>K'D</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Propranolol</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>(-)-Isoproterenol</td>
<td>1,000</td>
<td>520</td>
</tr>
<tr>
<td>(+)-Propranolol</td>
<td>2,510</td>
<td>1,300</td>
</tr>
<tr>
<td>(-)-Norepinephrine</td>
<td>3,550</td>
<td>1,900</td>
</tr>
<tr>
<td>(+)-Isoproterenol</td>
<td>1,1200</td>
<td>5,900</td>
</tr>
<tr>
<td>(-)-Epinephrine</td>
<td>1,4100</td>
<td>7,400</td>
</tr>
</tbody>
</table>

Equilibrium dissociation constants were calculated according to Cheng and Prusoff (5) from the equation: 
K'D = EC₅₀/[1 + S/KD], where EC₅₀ is the concentration of the competitor yielding 50% inhibition of specific DHA binding, S is DHA concentration in the binding assay (5 nM), and KD is the equilibrium dissociation constant of DHA for specific DHA binding sites (5.5 nM).

Fig. 3 Inhibition of specific DHA (5 nM) binding to brown adipocytes by (-)-stereoisomers of catecholamines. In Figs. 3 and 4, each point and vertical line show mean ± standard error of 4 experiments performed in duplicate.

Fig. 4 Equilibrium specific DHA binding to brown adipocytes at various concentrations of DHA (A) and its Scatchard plot (B).

Effects of Cold Exposure and Cold Acclimation on the Number and Affinity of DHA Binding Sites

Specific DHA binding to brown adipocytes from cold-exposed rats was not different from that in the corresponding controls at all concentrations of DHA used, while specific DHA binding to brown adipocytes from heat-acclimated rats tended to decrease at low concentrations of DHA (0.625–1.25 nM) and was significantly lower than that from controls at 10 nM DHA (Fig. 5(A)). Therefore, the number of specific DHA binding sites per unit cell estimated from Scatchard plots (Fig. 5(B)) was decreased significantly in both cold-exposed and cold-acclimated rats as compared with that in controls. However, the affinity of specific DHA binding sites for DHA was not affected by either cold exposure or cold acclimation (Table 2).
Table 2  Equilibrium Dissociation Constant of DHA for Specific DHA Binding Sites and Number of Specific DHA Binding Sites in Brown Adipocytes

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>BAT wet wt (mg)</th>
<th>Kd of DHA (nM)</th>
<th>Number of DHA binding sites (pmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm-acclimated controls(15)</td>
<td>377±8</td>
<td>420±31</td>
<td>3.9±0.3*</td>
<td>0.141±0.013*</td>
</tr>
<tr>
<td>Cold-exposed rats(24)</td>
<td>370±6</td>
<td>371±19</td>
<td>4.2±0.6</td>
<td>0.084±0.011</td>
</tr>
<tr>
<td>P vs. controls</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Cold-acclimated rats(24)</td>
<td>326±6</td>
<td>806±34</td>
<td>3.0±0.4</td>
<td>0.096±0.011</td>
</tr>
<tr>
<td>P vs. controls</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>II.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm-acclimated controls(18)</td>
<td>385±9</td>
<td>421±26</td>
<td>3.5±0.6</td>
<td>0.115±0.010</td>
</tr>
<tr>
<td>Heat-exposed rats(18)</td>
<td>365±8</td>
<td>414±22</td>
<td>2.5±0.3</td>
<td>0.100±0.012</td>
</tr>
<tr>
<td>P vs. controls</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heat-acclimated rats(18)</td>
<td>282±6</td>
<td>269±10</td>
<td>2.7±0.4</td>
<td>0.073±0.007</td>
</tr>
<tr>
<td>P vs. controls</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Equilibrium dissociation constant of DHA for specific DHA binding sites and number of specific DHA binding sites were determined by the Scatchard plots. Numbers in the parentheses indicate the number of animals. *Values are mean±standard error of 4 to 6 experiments. NS: not significant.

er than that from controls at high concentrations of DHA (2.5-10 nM) (Fig. 6(A)). Therefore, the number of specific DHA binding sites per unit cell estimated from Scatchard plots (Fig. 6(B)) in heat-exposed rats was not different from the value of controls, while heat acclimation decreased significantly the number of specific DHA binding sites. The affinity of specific DHA binding sites for DHA was not affected by either heat exposure or heat acclimation (Table 2).

DISCUSSION

We have previously reported the decreased number of β-adrenergic receptors in white adipocyte membrane due to cold acclimation in rats (14, 15). A similar finding has been reported in brown adipose tissue homogenate of cold-acclimated rats by Bukowiecki et al. (3). However, the presence of various types of cells has been described in brown adipose tissue (23), and the brown adipocytes have been shown to account for only about 25% of the total cells in the interscapular brown adipose tissue (9). Accordingly, we considered that further study of β-adrenergic receptors specifically in brown adipocytes should be performed before a final conclusion could be drawn about the significance of brown fat β-adrenergic receptors in temperature acclimation.

In the present study, we demonstrated β-adrenergic receptors in isolated brown adipocytes with the aid of DHA; DHA binding to these sites was rapid, reversible and saturable, displayed stereospecificity to β-adrenergic agonists as well as antagonists, and demonstrated a potency order of β-adrenergic (−)-agonists in competition study. These results identify β-adrenergic receptors in brown adipocytes of the β₁-subtype, and agree with the results from the respiratory responses observed with isolated rat brown adipocytes (2). The equilibrium dissociation constant for the interaction of (−)-norepinephrine with specific DHA binding sites (Kd=1.9 μM) (Table 1), estimated according to the equation of Cheng and Prusoff (5), agrees with a half maximally effective concentration of this hormone in stimulating cyclic AMP production (1). The characteristics of specific DHA binding sites of isolated brown adipocytes observed here were essentially the same as those of brown adipose tissue homogenate (3), or those of isolated hamster brown adipocytes (25).

Both acute cold exposure and cold acclimation decreased the number of β-adrenergic receptors in brown adipocytes. These results from cold-exposed and cold-acclimated rats are similar to those reported in brown adipose tissue homogenate of rats during cold acclimation (3). It has been found that the increased occupancy of β-adrenergic receptors by β-agonists leads to decrease in the receptor number and the receptor-coupled adenylate cyclase in several tissues (17). Plasma catecholamine levels of rats increase after cold exposure and remain
Fig. 5 Specific DHA binding to brown adipocytes from control, cold-exposed, and cold-acclimated rats (A) and their Scatchard plots (B). Each point and vertical line show mean ± standard error of 4 to 5 experiments performed in duplicate. Significant difference from controls: +P<0.05, ++P<0.001.

Fig. 6 Specific DHA binding to brown adipocytes from control, heat-exposed, and heat-acclimated rats (A) and their Scatchard plots (B). Each point and vertical line show mean ± standard error of 6 experiments performed in duplicate. Symbols + and ++, as in Fig. 5.

early period of cold exposure and decreased gradually to the control level, and thereafter the increased thyroidal activity is not observed throughout cold acclimation (24). We also observed the decreased number of β-adrenergic receptors in white adipocytes of cold-acclimated rats (15). It is thus deemed that the decreased number of β-adrenergic receptors of target cells in response to norepinephrine is generally associated with the process of cold acclimation. If this is the case, increased metabolic responses to norepinephrine during cold acclimation could not be explained at the receptor level. Brown adipose tissue, however, undergoes remarkable hypertrophy and hyperplasia, and the mitochondria of this tissue become larger and have increased oxidative enzyme activity during cold

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acclimation (26). Moreover, it has been recently reported that cold acclimation increases the amount of the purine nucleotide sensitive proton conductance pathway in association with thermogenic capacity of brown adipose tissue mitochondria (7). Our results and these findings suggest that the increased thermogenic response of brown adipose tissue (8) and brown adipocytes (16) to norepinephrine in the cold-acclimated rats reflect an increased capacity for thermogenesis through hyperplasia and altered intracellular events, such as an increased mitochondrial mass and uncoupling machinery of mitochondria.

Although the activity of the sympathetic nervous system seems to be suppressed in rats during heat acclimation (18), the number of β-adrenergic receptors in brown adipocytes was decreased in the heat-acclimated rats. This finding suggests that factor(s) other than sympathetic activity might modify β-adrenergic receptors in brown adipocytes during heat acclimation. In contrast to cold exposure, thyroidal activity is reported to decrease in heat-acclimated rats (21). Since hypothyroidism is reported to decrease the number of β-adrenergic receptors in brown adipocytes (22), it is very likely that the decreased thyroidal activity might be responsible for the decreased number of β-adrenergic receptors in brown adipocytes of heat-acclimated rats. The decreased number of β-adrenergic receptors per unit cell would partially explain the decreased metabolic response of brown adipose tissue (8) and brown adipocytes (16) to norepinephrine during heat acclimation, although it is not excluded that altered intracellular events, such as a decreased oxidative capacity, could be responsible for the decreased thermogenic response of brown adipocytes to norepinephrine in heat acclimation.

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REFERENCES

5. Cheng Y. and Prusoff W. H. (1973) Relationship between the inhibition constant (K) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108


21. **ROUSSET B. and CURE M.** (1975) Variations of rat thyroid activity during exposure to high environmental temperature (34°C). Relation between hypothalamic pituitary and thyroid hor-


