Possible Role of Polyamines in the Function of Brown Adipose Tissue

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Abstract In order to ascertain possible involvement of polyamines in the physiological regulation of brown fat function, effect of temperature acclimation on the polyamine contents of this tissue and effects of polyamines on the noradrenaline-induced thermogenesis of isolated brown adipocytes were investigated in rats. Daily urinary excretion of polyamines measured collectively for spermidine and spermine per body weight was decreased in heat acclimation and increased in cold acclimation. Polyamine concentrations per fresh weight of brown fat showed extremely low values compared with those of other tissues previously reported. Putrescine and spermidine contents per fat-free dry matter of brown fat were decreased in cold acclimation, but were not affected in heat acclimation. Spermidine and spermine inhibited the noradrenaline-induced thermogenesis of brown adipocytes dose-dependently. These results suggest that polyamines regulate the heat production of brown adipose tissue in an inhibitory way and cold acclimation potentiates heat production of this tissue by reducing polyamine levels.

Polyamines are widely distributed in animal tissues and have been implicated in many cellular processes. In particular, spermidine and its precursor, putrescine, are intimately associated with the rate of nucleic acid and protein synthesis during growth (Tabor and Tabor, 1976). Recent studies indicate that polyamines also affect the metabolic aspects of cellular activities (Chaffee et al., 1978, 1979; Clo et al., 1979; Kitada et al., 1979; Lockwood and East, 1974). For example, changes in polyamine concentration could cause very significant effects on mitochondrial respiration during ADP-ATP conversion, suggesting that polyamines have regulatory effects on cellular respiration (Chaffee et al., 1978, 1979).

Cold-acclimated small mammals such as rats show an enhanced non-shivering thermogenesis in the cold, where heat production is mediated mainly by noradre-
naline released from the sympathetic nerve terminals. The capacity for this efficient thermogenesis is closely related to the presence of brown adipose tissue. The sole function of this tissue is heat production and the tissue is found in many mammals, particularly in the cold-acclimated animals, hibernators and neonates (ROTHWELL and STOCK, 1979; SMITH and HORWITZ, 1969). However, the biochemical mechanism of non-shivering thermogenesis in this tissue has not been thoroughly elucidated.

Hereupon, it is interesting to study a possible involvement of polyamines in the physiological function of brown adipose tissue. This report concerns the urinary excretion of polyamines, polyamine contents of brown fat and the effects of polyamines on thermogenesis of isolated brown adipocytes in heat-acclimated as well as cold-acclimated rats.

MATERIALS AND METHODS

All studies were performed on the male rats of Wistar strain, weighing about 200 g. The animals were given free access to tap water and standard rat biscuit (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) with a daily photoperiod of 12 hr from 7:00 to 19:00. Control rats were placed at 25°C, 50% relative humidity, cold-acclimated rats at 5°C and heat-acclimated rats at 33°C, 45–50% relative humidity. After acclimation to each temperature for 4 to 5 weeks they were used for the experiments. The rats were kept individually in metabolic cages and had been accustomed to them for 2 days before the urine samples were collected. Six N HCl was added to the collecting vessel to prevent formation and/or destruction of amines by bacteria during the sampling period of 2 days. After rinsing the collecting equipment with distilled water, the washing was filtered through Toyo Filter Paper No. 1. Polyamines in the urine were measured colorimetrically for the combined polyamines (spermidine and spermine) with polyamine-test reagent kit (Code 279-35101, Wako Pure Chemical Industries, Ltd., Tokyo). The fat-free dry matter was determined by extracting total lipids by the method of FOLCH et al. (1951).

Polyamines in the brown fat were determined according to the method by INOUE and MIZUTANI (1973). Briefly, the tissue homogenate prepared with 2% perchloric acid was applied to a column of Dowex-50. Polyamines were finally eluted with 6 N HCl and the eluate was evaporated. The residue was dissolved in 0.05 N HCl. After separation by paper electrophoresis, each fraction of polyamines developed with ninhydrin reagent was measured colorimetrically.

Isolation and measurement of heat production of brown adipocytes were performed as previously described (KUROSHIMA and YAHATA, 1979). Brown adipocytes were isolated by the modified method of HITTLEMAN et al. (1974). Heat production of adipocytes was measured by a twin-type conduction microcalorimeter in the gas phase of 95% O₂, 5% CO₂ at 37°C (RCM-2F, Rhesca Co., Ltd., Tokyo) (FUJITA et al., 1976). The sample glass vessel contained 2 com-
partments; one contained cell suspension (4.4 ml) plus polyamine solution (0.1 ml) diluted with phosphate buffer or phosphate buffer only, and the other noradrenaline solution (0.5 ml). Noradrenaline solution was prepared with phosphate buffer to give a final concentration of 1 μg/ml, which exerted the maximum response on the brown adipocytes (Kuroshima and Yahata, 1979). The reference vessel also consisted of 2 compartments; one contained 4.4 ml of cell suspension plus 0.1 ml polyamine solution and the other the buffer solution only.

RESULTS

**Urinary excretion of polyamines in temperature acclimation**

As shown in Table 1, the daily urinary excretion of polyamines measured collectively for spermidine and spermine was significantly reduced in the heat-acclimated rats, while it was not affected in the cold-acclimated rats. The increment in the body weight was small in both cold- and heat-acclimated rats as reported previously (Kuroshima et al., 1978). The daily amount of polyamines excreted in the urine per 100 g body weight increased significantly in the cold-acclimated rats and was reduced in the heat-acclimated rats.

**Polyamine contents in brown adipose tissue**

Table 2 summarizes the polyamine contents of interscapular brown adipose tissue in the temperature-acclimated rats. Polyamine concentrations per fresh weight of the brown adipose tissue are comparable to those of the white adipose tissue, showing the lowest value among other tissues studied previously (Janne et al., 1964). As in other tissues, the concentrations of spermidine and spermine in the brown adipose tissue were similar and the putrescine level was lower than those of other polyamines (Inoue and Mizutani, 1973; Janne et al., 1964; Tabor and Tabor, 1976). The weight of brown adipose tissue increased in the cold-acclimated rats and decreased in the heat-acclimated rats. Consequently, the polyamine contents in whole tissue were significantly higher in the cold-acclimated rats than in the controls, while polyamine levels except for putrescine decreased in the heat-acclimated rats. The fraction of fat-free dry matter, which is considered to represent the active mass of the tissue, was significantly augmented with cold acclimation and lessened with heat acclimation (Table 2). Spermidine and putrescine concentrations per unit fat-free dry matter were found to be reduced significantly in the cold-acclimated brown adipose tissue. This finding indicates that the polyamine level in the active component of brown adipose tissue decreased in cold acclimation. Putrescine, spermidine and spermine levels per unit fat-free dry matter tended to increase in the heat-acclimated rats, but they are not significantly different from those in the controls, due to the great variation of the values.

**Effect of polyamines on noradrenaline-induced thermogenesis of brown adipocytes**

Noradrenaline induced a marked thermogenic response from the isolated
### Table 1. Urinary excretion of polyamines in temperature-acclimated rats.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Urine volume (ml/day)</th>
<th>Daily excretion of polyamines (µmol/day/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Experimental</td>
<td></td>
</tr>
<tr>
<td>Controls (8)</td>
<td>193 ± 3.5</td>
<td>352 ± 15.7</td>
<td>5.8 ± 0.72</td>
</tr>
<tr>
<td>Cold-acclimated rats (6)</td>
<td>192 ± 3.4</td>
<td>284 ± 7.0 b)</td>
<td>14.4 ± 1.52 c)</td>
</tr>
<tr>
<td>Heat-acclimated rats (12)</td>
<td>193 ± 4.3</td>
<td>262 ± 6.7 b)</td>
<td>3.6 ± 0.92</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1.417 ± 0.1180</td>
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<td>0.404 ± 0.0308</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of animals. Polyamines = spermine + spermidine. Mean ± standard error of the mean. P vs. controls: a) < 0.05; b) < 0.01; c) < 0.001.

### Table 2. Polyamine contents in brown adipose tissue (BAT).

<table>
<thead>
<tr>
<th>BAT weight (mg/100 g)</th>
<th>Chemical composition of BAT (%)*</th>
<th>Putrescine (nmol)</th>
<th>Spermidine (nmol)</th>
<th>Spermine (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ffdm</td>
<td>Lipid</td>
<td>Water</td>
<td>ffdm</td>
</tr>
<tr>
<td>Controls (12)</td>
<td>82 ± 4.1</td>
<td>7.86 ± 0.34</td>
<td>59.21 ± 2.932</td>
<td>33.03 ± 2.843</td>
</tr>
<tr>
<td>Cold-acclimated rats (12)</td>
<td>247 ± 14.1 c)</td>
<td>12.83 ± 1.761 a)</td>
<td>39.47 ± 1.933 e)</td>
<td>47.70 ± 1.655 e)</td>
</tr>
<tr>
<td>Heat-acclimated rats (12)</td>
<td>61 ± 3.1 c)</td>
<td>6.08 ± 0.426 b)</td>
<td>65.34 ± 2.539</td>
<td>28.58 ± 2.179</td>
</tr>
</tbody>
</table>

* These values were obtained from BAT of animals different from those used for the measurements of BAT polyamine contents. But they were the same both in their ages and the means of temperature acclimation. Each value is the mean of 7–8 samples.

** These figures were calculated indirectly by the use of per cent of ffdm. Other legends same as in Table 1.
brown adipocytes (Kuroshima and Yahata, 1979). Spermine as well as spermidine significantly suppressed this noradrenaline-induced thermogenesis in a dose-dependent manner, as shown in Fig. 1.

**DISCUSSION**

The results showed the elevated urinary excretion of polyamines in the cold-acclimated rats and the reduced excretion in the heat-acclimated rats. It is well known that cold acclimation causes the increases in the weights of gut and liver (Emery et al., 1939; Héroux and Gridgeman, 1958), where high concentrations
of polyamines are found (JANNE et al., 1964). Therefore, it is likely that the increased excretion of polyamines would have resulted from the increased mass of tissues containing large amounts of polyamines and/or increased formation of polyamines. Changes in polyamine synthesis are followed by corresponding changes in urinary excretion during pregnancy (ANDERSSON et al., 1978; ROJANSKY et al., 1979). On the other hand, heat acclimation results in suppressed thyroidal function (COLLINS and WEINER, 1968), lessened growth (JOUANNETEAU and PÈRES, 1979; KUROSHIMA et al., 1978) and reduced liver size (CHAYOTH et al., 1977; JOUANNETEAU and PÈRES, 1979). Ablation of thyroid gland decreases the hepatic polyamine concentration (HARIK, 1979). Thus, it is inferred that the overall formation of polyamines is decreased in the heat-acclimated rats, resulting in the reduced excretion of urinary polyamines.

Cold acclimation has been shown to decrease the concentrations of putrescine and spermidine, but not spermine, in fat-free dry matter of brown adipose tissue. In the heat-acclimated animals, all the polyamine levels tended to increase, though the changes were not statistically significant (Table 2). Regarding these findings, it is noted that an inhibition of ornithine decarboxylase, which is a rate-limiting enzyme in the biosynthesis of polyamine, results in lowering of putrescine and spermidine levels, but not that of spermine in several rat tissues (DANZIN et al., 1979). Therefore, it is conceivable that cold acclimation inhibits the activity of polyamine synthesizing enzyme, decreasing the polyamine levels in the brown adipose tissue. In the present study, spermidine as well as spermine suppressed the thermogenesis in the isolated brown adipocytes in response to noradrenaline, which is the main agent regulating the function of this tissue. Polyamine concentrations used in order to affect the noradrenaline-induced thermogenesis of brown adipocytes were much higher than those in brown adipose tissue. Accordingly, the physiological validity of the present finding appears amenable to further study. But in the experiment in vitro a physiologically appropriate concentration of active agent might not be simply determined by its endogenous average level, since it might be subjected to rapid destruction during the incubation period or it might not be distributed evenly in the situation in vivo. From these results, we should like to reason that polyamines take part in the regulation of thermogenic function of brown adipose tissue in an inhibitory manner, although the mechanism involved remains obscure.

The polyamines decrease the activities of adenylate cyclase and cyclic AMP-dependent protein kinase, and the cellular cyclic AMP level (CLÔ et al., 1979; WRIGHT et al., 1978). It has been also shown that the polyamines activate cellular phosphodiesterase, causing the decrease of cyclic AMP levels (CLÔ et al., 1979). The function of brown adipose tissue is mainly regulated by noradrenaline released from the sympathetic nerve terminals. Noradrenaline stimulates lipolysis in this tissue by increasing cyclic AMP levels through activation of adenylate cyclase and causes heat production via the highly exothermic process of mito-
Polyamines and Brown Adipose Tissue

Accordingly, the decreased level of polyamines in the cold-acclimated brown adipose tissue may induce enhanced lipolysis in this tissue, leading to enhanced thermogenesis by means of modification of the adenylate cyclase-cyclic AMP system. In this context, the report by Begin-Heick et al. (1979) should be referred to, which states that catecholamine-stimulated adenylate cyclase levels are markedly increased in the membrane-rich fraction of brown adipose tissue of cold-acclimated rats. On the other hand, a reduction was reported in the enzyme activity in the homogenates of brown adipose tissue in the cold-acclimated rats (Muirhead and Himms-Hagen, 1971; Skala et al., 1972). The discrepancy between these results might have resulted from the differences in the assay procedures employed or in the preparations of tissues for the assay. In interpretation of the results, due precaution must be taken as to which tissue preparations are used, membrane-rich fractions, homogenates or adipocytes of brown adipose tissue. The brown adipocytes account for only about 25% of the cells in the total brown adipose tissue of rat (Fain et al., 1967). Pertaining to this point, it is noted that the phosphodiesterase activity per mg protein was increased in total tissue homogenates but decreased in the brown adipocytes of cold-acclimated rats (Bertin and Portet, 1975).

The polyamines are reported to mimic the antilipolytic action of insulin, possibly suppressing the cellular cyclic AMP levels (Lockwood and East, 1974). Both white and brown adipose tissues react to insulin as well as noradrenaline in a similar fashion (Fain et al., 1967; Krishna et al., 1970). The polyamines, like insulin, may exert antilipolytic action on the brown adipose tissue and suppress the heat production of this tissue.

Moreover, the polyamines may play a role in the mitochondrial function of brown adipose tissue. Chaffee et al. (1979) demonstrated that the polyamines would increase the respiratory control ratio and suppress the activity of the respiratory cycle in rat liver mitochondria. They also showed that the liver mitochondria from the heat-acclimated animals have a significantly greater elevation of respiratory control ratio than those from the control in the presence of polyamines (Chaffee et al., 1978). Recently, a unique uncoupling mechanism in the form of a high-conductance of H+ in the inner membrane of mitochondria without obligatory ATP synthesis has been proposed as being responsible for the remarkably high thermogenic capacity of brown adipose tissue (Heaton et al., 1978). It might be argued that the polyamines effect a role as a factor tightening the respiratory control of this tissue, enhancing thermogenesis with a decrease in their levels.

The mechanism by which the polyamine levels are altered in the cold-acclimated animals remains to be identified. Polyamine synthesis in various tissues is under the control of several hormones (Harik, 1979; Scalabrino et al., 1979). Further consideration should be given to evaluating the endocrine regula-
tion of polyamine metabolism.

Although the results of the present investigation suggest that the polyamines regulate the heat production of brown adipose tissue in an inhibitory way, further work is needed to ascertain if the polyamines are indeed related physiologically to the function of brown adipose tissue.

REFERENCES


