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Decrease in Cold Tolerance of Aged Rats Caused by the Enhanced Endogenous Adenosine Activity

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WANG, L. C. H., Z. L. JIN AND T. F. LEE. *Decrease in cold tolerance of aged rats caused by the enhanced endogenous adenosine activity.* PHARMACOL BIOCHEM BEHAV 43(1) 117–123, 1992.—During severe cold exposure, old rats (24–28 months) were less capable of maintaining their body temperature compared to young rats (3–6 months) due to lower rate of heat production. Single injection of adenosine deaminase (AD) (converts adenosine to inosine) significantly increased thermogenesis in both young and old rats. However, doubling the dose of AD was required for optimal thermogenic response in old rats. In contrast, the similar enhancements in both thermogenesis and cold tolerance were observed in both young and old rats receiving the same optimal doses of specific adenosine receptor antagonists. These results lead to the suggestion that the lower capability of aged rats to withstand cold exposure could be due to an increase in adenosine stimulation because of the decreased endogenous AD activity rather than an increase in adenosine receptor sensitivity. This notion is further supported by the finding that the AD activity in the neck muscle, a key site for shivering thermogenesis, was significantly lower in old rats as compared to their younger counterparts before and after cold exposure.

Adenosine Aging Thermogenesis Cold resistance Adenosine receptors Adenosine deaminase

IT has been well established that the mortality rate is higher in the elderly after cold exposure when compared to younger subjects [for review, see (5,22)]. It is currently unknown what specific age-dependent changes are responsible for the observed deterioration in thermoregulatory competence with aging, but a reduction in heat production coupled with a reduced ability to minimize heat loss are major possibilities.

It has been known for some time that most cells are able to release adenosine following stimulation (e.g. adrenergic or local hypoxia) and that within a given tissue adenosine functions as a local hormone or messenger [for review, see (8,25)]. This purine nucleoside has been shown to be a potent antilipolytic agent both in vitro in adipocytes (12,15) and in vivo in perfused subcutaneous adipose tissue (28). In addition, adenosine has also been shown to reduce the sensitivity of insulin-stimulated glucose utilization in the soleus muscle (4). Therefore, the combined effects of adenosine could result in a decrease of both substrate mobilization and utilization, leading to reduced muscle performance. In the cold, this could precipitate the onset of hypothermia due to reduced shivering thermogenesis. This possibility is supported by our previous findings that pretreatment with either specific A_1 adenosine receptor antagonists (19) or adenosine deaminase (AD) (31) significantly enhanced thermogenesis and improved cold resistance in young rats.

Recently, a three- to fivefold increase in 5'-nucleotidase activity, generally considered involved in the production of adenosine (14), has been observed in the adipocytes from old rats and this increased adenosine production was associated with the attenuation of insulin-stimulated glucose uptake (3). Further, the inhibitory effects of adenosine on lipolysis has been shown to increase with aging (15). It is possible that enhanced inhibitory effects of adenosine may be involved in blunting the thermogenic responses to cold stimulation in older animals. This notion is indirectly supported by our previous finding that the cold tolerance of the old rat can be improved by acute pretreatment with theophylline (20). Because theophylline has a dual effect in both adenosine receptor antagonism and inhibition of phosphodiesterase activity (26), the precise cellular and molecular mechanisms via which its effect is manifested remain unknown. The present study was, therefore, undertaken to provide more direct evidence on the possibility that the endogenous adenosine activity in the aged group may be overactivated, which in turn may reduce the maximum thermogenic capacity under cold exposure.

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METHOD

All experimental protocols used in the present study received prior approval of the University of Alberta Animal Care Committee following the guideline of Canadian Council on Animal Care. Two groups of adult, male Sprague-Dawley rats, 3–6 and 26–30 months old, were used. They were housed individually in polycarbonate cages with wood shaving bedding at 22 ± 1°C in a walk-in environmental chamber under a 12 L : 12 D photoperiod. Water was made available at all times. Because both thermal conductance (2) and thermogenesis (13) have been shown to be affected by body size, food (Rodent Blox, consisting of 24% protein, 4% fat, 65% carbohydrate, 4.5% fiber and vitamins; Wayne Lab. Animal Diets, Chicago, IL) was rationed daily after the body weight had reached about 400 g to eliminate the possible variation in results due to different body sizes between the age groups. The night before the experiment, however, food was made available ad lib to ensure maximal thermogenesis (29).

Cold Exposure

The protocol for acute cold exposure was similar to that described earlier (29). Briefly, the animal was removed from its home cage, placed in a metal metabolism chamber, and exposed to −10°C under helium-oxygen (79% He:21% O_2) for 120 min. Helium-oxygen (1.5 l/min. STP) was used to facilitate heat loss. Exhaust gas from the metabolic chamber was divided into two streams; one stream was for oxygen measurement by an oxygen analyzer (Applied Electrochemistry Model S-3, Ametek, Pittsburgh, PA) following drying by Drierite (W. A. Hammond Drierite Co., Xenia, OH) and CO_2 removal by Ascarite (Thomas Scientific, Swedesboro, NJ). The second stream was only dried for the measurement of CO_2 by an Applied Electrochemistry CD-2 analyzer. Oxygen consumption and CO_2 production were recorded simultaneously and continuously and integrated by an on-line computerized data acquisition system. Heat production (HP) was calculated from oxygen consumption and respiratory quotient using Kleiber's equation (29). The integrated HP for each 15-min period was used as the rate of HP. The sum of HP from seven consecutive 15-min periods (min 16–120) constituted the total HP and the highest rate of any one 15-min period was used as the maximum HP. The colonic temperature (6 cm from anus) (Tb) was measured with a thermocouple thermometer (BAT-12, Bailey Instruments, Saddlebrook, NJ) immediately before vehicle or tested compound injection and immediately after cold exposure. The change in Tb was used as an index for cold tolerance. Either vehicle or tested compound was administered IP in a volume of 1 ml/kg 15 min prior to cold exposure. To avoid habituation and possible acclimation, at least 2 weeks was allowed between successive cold exposures and vehicle/drug treatments were randomized in each animal in a self-control design.

Biochemical Assays

Rats were killed by decapitation at different time periods after cold exposure (0, 60, and 120 min) and various tissue samples (the interscapular brown adipose tissues and the neck muscles around the shoulder) were removed rapidly with tongs precooled in liquid nitrogen. The tissue samples were homogenized in 1 N perchloric acid and the homogenate was then centrifuged to remove precipitated protein. The adenosine concentrations in the plasma, fat pads, and muscles were assayed by high-performance liquid chromatography (HPLC) as described by Jackson and Ohnishi (16). Owing to the short half-life of adenosine (24), the absolute level of adenosine may not be positively correlated to the physiological responses. Another approach was to examine the activity of adenosine metabolizing enzyme, AD, in the muscles from animals of different ages. After removing from the rat, the neck muscle was homogenized in 0.1 M Tris-buffer (pH 7.4) and then centrifuged in a refrigerated centrifuge, and the supernatant was used for enzyme assays. Activity of AD was determined by the HPLC method as described by Abd-Elfattah and Wechsler (1).

Results are expressed as the mean ± SEM. For in vivo studies, statistical analysis was by either the unpaired *t*-test for comparison between different age groups or Wilcoxon's signed ranks test for comparisons of treatment effect in individuals of the same group. Unpaired *t*-test was used for all biochemical comparisons between same and different age groups. Significance was set at $p < 0.05$ unless otherwise stated.

RESULTS

Effects of AD and Adenosine Receptor Antagonists on Cold Tolerance

Before either saline or AD treatment, old rats had an initial mean T_b of 37.2 ± 0.23°C ($n = 8$), not significantly different from that observed in control young rats (37.5 ± 0.22°C, $n = 8$). Figure 1 shows the change of HP during cold exposure in young and old rats after saline or AD treatment. After saline administration, the HP of both young and old rats increased rapidly during the first 30 min after cold exposure and continued to increase gradually until the maximum level was reached with the next 15–30 min. However, the maximum HP could not be sustained in the aged group and it declined continuously throughout the remainder of the cold exposure (Fig. 1b), resulting in deep hypothermia (28.9 ± 0.58°C). A similar pattern was observed in old rats after pretreatment with AD (200 U/kg) except the level of thermogenesis was significantly elevated. As a result, the cold tolerance after AD treatment was improved, as indicated by the higher final T_b (31.6 ± 0.62°C). Further increasing the dose of AD up to 400 U/kg failed to elicit higher rate of HP, and the final T_b was about the same as that observed in controls. In young rats (Fig. 1a), more sustained HP was observed after saline injection and consequently the final T_b (31.4 ± 0.61°C) was higher than that of the aged group. Similar to old rats, the HP was significantly enhanced after AD at 100 U/kg but not at 200 U/kg.

Table 1 summarizes the group data for all parameters. Both the total and maximum HP of control young rats were significantly higher than those observed in the elderly; the decrease in T_b after cold exposure was thus significantly less in the young group. In comparison to saline control, both the total and maximal thermogenesis were significantly enhanced (13.2 and 13.4% above control values, respectively) after young rats received 100 U/kg AD. The reduction of T_b was also significantly less after this treatment. Upon increasing the dose to 200 U/kg, maximal and total HP and the change in T_b were approximately the same as observed in the control condition. Similar thermogenic responses to systemic pretreatment with AD was also observed in rats; however, the significant increases in both HP (10.6 and 17.7% above the control values, respectively) and cold tolerance were only observed after receiving the AD at 200 U/kg.

To further examine whether the decrease in thermogenic

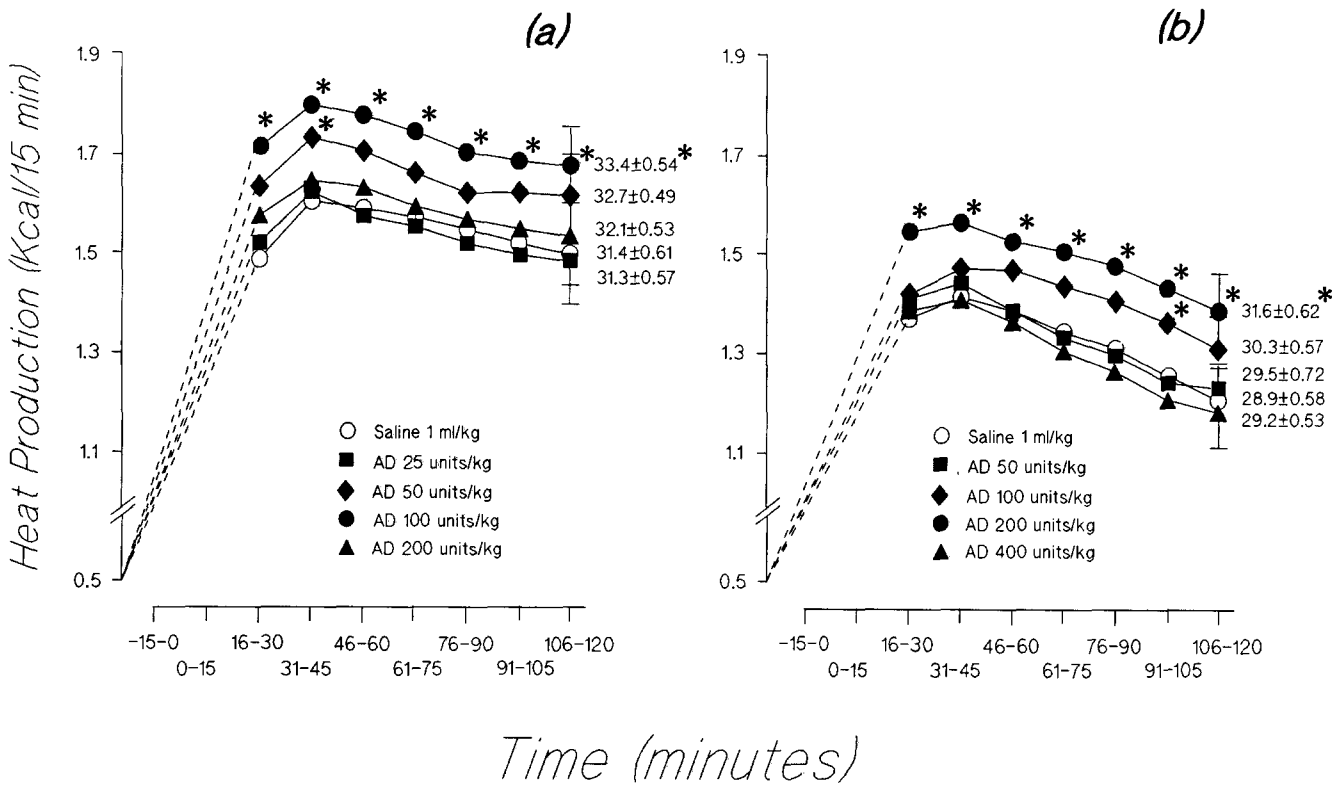

FIG. 1. Time course of thermogenic responses to HeO_2 at $-10°C$ after pretreatment with either saline or various doses of adenosine deaminase (AD) in young (a) and old (b) rats. Each point represents the mean total HP in 15 min from eight rats. For clarity, the SE of mean HP, indicated by vertical bars, is shown only for the last HP values. Numbers beside individual curves indicate final body temperature (°C) (mean ± SE) after each treatment. *Significantly different from corresponding saline control ($p < 0.05$, Wilcoxon's signed ranks test).

TABLE 1

EFFECTS OF IP INJECTION OF AD ON MAXIMAL HP, TOTAL HP, AND CHANGE IN FINAL T_b ON YOUNG (3-6 MONTHS) AND OLD (24-28 MONTHS) RATS EXPOSED TO COLD

Dosage	Maximal HP (kcal/15 min)	Total HP (kcal/105 min)	T_b Changes (°C)
Young rats (n = 8)			
Saline 1 ml/kg	1.67 ± 0.06	11.23 ± 0.52	−5.69 ± 0.71
AD 25 U/kg	1.64 ± 0.07	10.83 ± 0.48	−5.62 ± 0.68
AD 50 U/kg	1.82 ± 0.05*	12.17 ± 0.39	−4.72 ± 0.55
AD 100 U/kg	1.89 ± 0.06*	12.73 ± 0.50*	−3.65 ± 0.56*
AD 200 U/kg	1.75 ± 0.06	11.67 ± 0.42	−5.03 ± 0.39
Old rats (n = 8)			
Saline 1 ml/kg	1.41 ± 0.06†	9.24 ± 0.56†	−8.40 ± 0.39†
AD 50 U/kg	1.38 ± 0.07†	9.03 ± 0.47†	−8.06 ± 0.68†
AD 100 U/kg	1.47 ± 0.06†	9.48 ± 0.48†	−7.84 ± 0.56†
AD 200 U/kg	1.56 ± 0.07*†	10.88 ± 0.42*	−6.52 ± 0.34*†
AD 400 U/kg	1.36 ± 0.08	8.76 ± 0.47	−8.14 ± 0.39

*Significantly different from same age control treatment, $p < 0.05$.
†Significantly different from young rat group receiving same dose of AD, $p < 0.05$.

TABLE 2

EFFECTS OF IP INJECTION OF CPT ON MAXIMAL HP, TOTAL HP, AND CHANGE IN FINAL T_b ON YOUNG (3-6 MONTHS) AND OLD (24-28 MONTHS) RATS EXPOSED TO COLD

Dosage	Maximal HP (kcal/15 min)	Total HP (kcal/105 min)	T_b Changes (°C)
Young rats (n = 8)			
Saline 1 ml/kg	1.53 ± 0.04	10.64 ± 0.42	−6.26 ± 0.66
CPT 0.0002 mg/kg	1.58 ± 0.06*	11.13 ± 0.52*	−5.82 ± 0.71*
CPT 0.001 mg/kg	1.77 ± 0.05†	11.83 ± 0.45*†	−4.28 ± 0.43*†
CPT 0.005 mg/kg	1.69 ± 0.03†	11.35 ± 0.46*†	−4.93 ± 0.44*†
Old rats (n = 8)			
Saline 1 ml/kg	1.39 ± 0.04	8.84 ± 0.37	−9.30 ± 0.61
CPT 0.0002 mg/kg	1.36 ± 0.07*	9.13 ± 0.28*	−8.88 ± 0.58*
CPT 0.001 mg/kg	1.51 ± 0.04*†	9.89 ± 0.32*†	−7.96 ± 0.46*†
CPT 0.005 mg/kg	1.49 ± 0.03†	9.71 ± 0.36*†	−8.13 ± 0.52*†

*Significantly different from same age control treatment, $p < 0.05$.
†Significantly different from young rat group receiving same dose of AD, $p < 0.05$.

response of the aged rat under cold exposure is due to an increase in adenosine receptor sensitivity, cyclopentyltheophylline (CPT) and D-Lys-XAC, the most potent and effective adenosine receptor antagonists, respectively, on thermogenesis (20), were used and the results are summarized in Tables 2 and 3. As observed previously with other adenosine antagonists (30,31), pretreating the animal with CPT and D-Lys-XAC caused an inverted-U shaped dose-response curve in HP and final T_b changes. Significant increases in both the HP and cold tolerance in young rats were recorded after pretreatment with 0.001–0.005 mg/kg CPT and 1.25–2.5 mg/kg D-Lys-XAC, respectively. Similar thermogenic patterns were observed in old rats after pretreating them with either CPT or D-Lys-XAC at the same optimal doses as those used in young rats.

Changes in Adenosine Concentrations and AD Activity After Cold Exposure

Table 4 summarizes the changes in adenosine concentrations in the neck muscle and the brown fats from both young and old rats exposed to extreme cold. During cold exposure, there were no significant changes in brown fat adenosine concentration in both young and old rats. Before cold exposure, the adenosine level in the neck muscle from old rats is slightly higher than that of young rats ($p < 0.1$). A steady increase in adenosine concentration was observed in the neck muscle from both young and old rats after exposure to the cold; however, the differences failed to achieve any statistical significance ($p < 0.15$ and 0.2 for young and old rats, respectively, when comparing time 0 and 120 min). As adenosine is metabo-

TABLE 3

EFFECTS OF IP INJECTION OF D-Lys-XAC ON MAXIMAL HP, TOTAL HP, AND CHANGE IN FINAL T_b ON YOUNG (3-6 MONTHS) AND OLD (24-28 MONTHS) RATS EXPOSED TO COLD

Dosage	Maximal HP (kcal/15 min)	Total HP (kcal/105 min)	T_b Changes (°C)
Young rats (n = 8)			
Saline 1 ml/kg	1.64 ± 0.04	10.83 ± 0.37	−5.97 ± 0.71
Lys-XAC 0.625 mg/kg	1.76 ± 0.07*	11.90 ± 0.49*	−5.67 ± 0.68*
Lys-XAC 1.25 mg/kg	1.88 ± 0.04†	12.63 ± 0.33*†	−3.67 ± 0.56*†
Lys-XAC 2.50 mg/kg	1.79 ± 0.03†	12.17 ± 0.45*†	−4.43 ± 0.62*†
Old rats (n = 8)			
Saline 1 ml/kg	1.37 ± 0.04	9.54 ± 0.38	−8.26 ± 0.69
Lys-XAC 0.625 mg/kg	1.39 ± 0.07*	10.13 ± 0.52*	−8.32 ± 0.67*
Lys-XAC 1.25 mg/kg	1.57 ± 0.04†	10.69 ± 0.35*†	−6.34 ± 0.56*†
Lys-XAC 2.50 mg/kg	1.51 ± 0.03†	10.50 ± 0.46*†	−6.58 ± 0.60*†

*Significantly different from same age control treatment, $p < 0.05$.
†Significantly different from young rat group receiving same dose of AD, $p < 0.05$.

TABLE 4

CHANGES IN ADENOSINE CONTENTS IN THE NECK MUSCLE AND THE BROWN FATS FROM YOUNG AND OLD RATS EXPOSED TO COLD

	Time After Cold Exposure (min)	Young Rats (n = 8)	Old Rats (n = 6)
Neck muscle (nmol/g tissue)	0	24.4 ± 6.51	36.5 ± 5.13
	60	30.1 ± 6.19	41.6 ± 8.25
	120	34.1 ± 4.75	45.9 ± 4.97
Brown fats (nmol/g tissue)	0	50.6 ± 7.83	49.9 ± 7.92
	60	58.8 ± 6.04	47.0 ± 6.67
	120	57.6 ± 5.07	52.9 ± 7.83

lized rapidly, the absolute level of adenosine may not be positively correlated to the physiological responses. We, therefore, also examined the change in the activity of adenosine metabolizing enzyme, AD. Because no significant change in AD activities with age was observed in our preliminary study on the brown fat tissue, only the change in AD activity in the neck muscle of different aged rats was systematically examined and the results are shown in Table 5.The activity of AD of young rats was significantly higher than that of old rats before cold exposure. The AD activities of both young and old rats increased with time after cold exposure and were significantly higher than the baseline values in both age groups.

DISCUSSION

It is well known that the minimum thermal conductance and maximum thermogenesis are dependent upon the body size of the animal (2,13). Variation in body size will thus affect the thermoregulatory responses of the animal under cold exposure. To eliminate the possible variation resulted by the difference in body size, the body weights of animals used in the present study were monitored by food rationing. Our present results on thermoregulatory responses are similar to those observed previously in rats under ad lib feeding (18,23). This indicates that the decrease in thermogenic capacity of old rats observed in the present study is not affected by our food rationing regimen.

Although opinions vary as to what specific age-depended changes are responsible for the observed deterioration in thermoregulatory competence with aging, alteration in endogenous adenosine activity may be a possibility. Previously, we demonstrated that endogenously released adenosine can attenuate the thermogenic capacity of young rats in severe cold (30,31). Further, the inhibitory effects of adenosine on lipolysis (15) and insulin-stimulated glucose transport (3) in isolated fat cells have been shown to increase with age. Therefore, it is quite possible that an enhanced inhibitory effect of endogenous adenosine may set the upper limit of aerobic capacity in aging mammals and seriously impair their ability to withstand cold exposure.

In an inaugural attempt, AD, the enzyme that converts adenosine to inosine and thereby eliminates adenosine's effects, was chosen to test the possible changes in endogenous adenosine activity with aging. At 100 U/kg IP, a single injection of AD significantly enhanced both total and maximal thermogenesis and significantly improved cold resistance of young rats. This indicates that the normal thermogenic capacity of the animal can be suppressed by endogenous released adenosine during cold stimulation. At higher doses, however, AD caused a return of thermogenesis to the control level. This may be due to the dual effect of adenosine: Via the A_2 receptor, adenosine may increase regional blood flow [for review, see (6)] and therefore oxygen and substrate supply to the shivering muscle. Reducing this beneficial effect by AD could lead to reduced thermogenesis. As shown in Table 1, pretreating old rats with AD elicited a similar thermogenic stimulation to those observed in young rats. However, aging did result in a right shift of the dose–response curve relating HP and AD, resulting in a doubling of the dose required for optimal thermogenic response in aged rats. This indicates that an increase in the release of endogenous adenosine or adenosine receptor responsiveness could occur with aging, requiring greater amounts of AD to nullify the inhibitory action of adenosine on thermogenesis.

A conformational change in adenosine receptors has been demonstrated in the cerebral cortex and hippocampus of 24-month-old rats (7). Further, it has been shown that the enhanced efficacy of adenosine in inhibiting lipolysis in old rats was associated with an increase in the number of adenosine receptor per cell (15). Therefore, it is possible that the impairment in thermogenesis in the aged rat is resulted by the change in adenosine receptor responsiveness. To test this, CPT and D-Lys-XAC, selective A_1 adenosine receptor antagonists that previously have been shown to be the most potent and effective, respectively, in enhancing thermogenesis (19) were used. Both CPT and D-Lys-XAC caused a dose-related increase in HP and final T_b in both young and old rats. In contrast to

TABLE 5

CHANGES IN AD ACTIVITIES (nmol ADENOSINE/g/min) IN THE NECK MUSCLE FROM YOUNG AND OLD RATS EXPOSED TO COLD

Time After Cold Exposure (min)	Young Rats (n = 8)	Old Rats (n = 6)
0	202.3 ± 9.9	147.6 ± 7.6*
60	236.5 ± 13.9	186.9 ± 14.2*
120	253.6 ± 15.3†	196.4 ± 10.7*†

*Significantly different from young rat controls at the same time point ($p < 0.05$, t-test).

†Significantly different from the same age controls at 0 min ($p < 0.02$, t-test).

that observed with AD, the optimal doses of these antagonists in eliciting maximum beneficial effect for cold tolerance were identical in young and old rats. At higher doses, both CPT and D-Lys-XAC failed to elicit further enhancement of thermogenesis. This is consistent with our previous observation of an inverted-U shape of the dose-response curve using other adenosine receptor antagonists (30,31). Recently, it has been demonstrated that the agonist and antagonist may bind preferentially to different conformations of the adenosine A_1 receptors (21,27); the antagonist radioligand appears to specifically photoincorporate into the receptor with about 10 times higher efficiency than does the agonist (21). The inverted-U shape of the dose-response curve may be attributed to the fact that after the optimal dose the antagonist acts as a partial agonist that binds to the agonist conformation receptor site to reduce the thermogenic capacity. Regardless of the precise mechanisms responsible for the inverted-U shaped dose-response curve, a change in activity of the adenosine receptors does not appear to be the direct cause for the reduced thermogenesis observed during senescence. This is evidenced by the fact that the optimal dosage of adenosine antagonists used in eliciting the thermogenic effect is about the same in both young and old rats. However, possible age-dependent differences remain on binding of adenosine agonist and antagonist to different conformations of the adenosine receptor. Further studies are required to evaluate this possibility.

Since an age-related increase in adenosine release has been demonstrated in isolated perfused rat's heart (9) and human fibroblast cultures (10), the other explanation for the reduced cold tolerance in old rats could be due to an overproduction of endogenous adenosine after stimulation. To seek direct evidence in support of this, changes of adenosine concentration in two main thermogenic sites (neck muscle and brown fat for shivering and nonshivering thermogenesis, respectively) were examined during cold exposure in both young and old rats. No significant change in adenosine concentration was observed in the brown fat between the age groups. The failure to observe any change in the brown fat may be because that nonshivering thermogenesis constitutes only a minor portion of HP during cold exposure in 22°C-acclimated rats (11,20). Although not significantly different from each other, higher adenosine concentration was observed in the neck muscle of old rats than that of young rats before cold exposure. Gradual increase in adenosine concentration was observed in neck muscles from both young and old rats during cold exposure, indicating increased ATP utilization. It is well known that adenosine has a very short half-life (24); the absolute level of adenosine may not be positively correlated to the physiological responses. To correlate the change in tissue adenosine concentration and its physiological influence, the activity of the adenosine deactivating enzyme, AD, in the neck muscle was also investigated. The activity of AD from young rats was significantly higher than that of old rats before cold exposure. The AD activities from both young and old rats increased with time after cold exposure and were significantly higher than the baseline values in both age groups. As the change in AD activity is positively correlated with the change in adenosine concentration, these results indicate that decreased AD activity with aging could result in an increase in local adenosine concentration during cold exposure. Since neck muscle participates in shivering thermogenesis (17), any inhibitory effect of adenosine on this muscle will lead to a reduced thermogenic capacity. Since we did not measure the enzyme activities governing adenosine synthesis (5'-nucleotidase or S-adenosylhomocysteine hydrolase) in the present study, the possibility of an increased cellular adenosine production with aging under normal or cold-stimulated conditions cannot be ruled out. Nevertheless, the reduced capability to withstand cold in old rats can at least be partially explained by their decreased AD activity under both normal and cold-stimulated conditions (Table 5), resulting in greater local adenosine concentration than that of young rats.

The inability of the elderly to cope with cold stress and the resultant hypothermia have received much attention in both the lay and scientific literature [for review, see (5,22)]. From results of the present study, it is possible that the deficiency of aged rats to withstand cold is due to the presence of higher concentration of endogenous adenosine. Further, pretreating old rats with specific adenosine receptor antagonists can effectively enhance their thermogenic capacity and cold tolerance. Our findings have suggested a practical means for improving tolerance to cold in the elderly.

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