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ORIGINAL ARTICLE

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Thermogenesis in brown adipose tissue with age: post-receptor activation by forskolin

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Abstract β_3-Adrenergic-stimulated thermogenesis in brown adipose tissue (BAT) is diminished with age. β_3-Adrenergic receptors are positively coupled to adenylyl cyclase in BAT. To determine whether thermogenesis, in response to direct activation of adenylyl cyclase, is also impaired with age, we examined whole body oxygen consumption, mitochondrial guanosine diphosphate (GDP) binding and BAT mitochondrial uncoupling protein (UPC) mRNA levels in 4- and 24-month-old F-344 rats following forskolin administration. We also examined the forskolin-induced change in body temperature in 4-month-old rats. In some instances, the results were compared with administration of the specific β_3-adrenergic agonist, CGP-12177. Forskolin (3.5 mg/kg) increased oxygen consumption but decreased body temperature. In subsequent experiments the BAT was unilaterally denervated. In these rats, the forskolin-(1.8 mg/kg) stimulated increase in oxygen consumption was similar in young and old rats. Forskolin increased GDP binding and UCP mRNA levels in both the denervated and innervated BAT pads. The increases were equal or greater in the BAT from senescent rats. These findings, coupled with our previous report of an impaired CGP-12177-stimulated increase in GDP binding in senescent rats, suggests β_3-adrenergic-stimulated, but not post-receptor-stimulated, thermogenesis is diminished with age.

Key words Adenylyl cyclase · CGP-12177 · F-344 rats · β_3-Adrenergic agonist

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Introduction

The ability to regulate body temperature decreases with age [5, 9, 10, 18]. In rats, thermoregulation is impaired in senescent animals after cold exposure [9, 10, 18] and after infection [16]. Nonshivering thermogenesis in brown adipose tissue (BAT) is an important contributor of the heat necessary to maintain body temperature after exposure to cold [6] and to raise body temperature in response to infection [15].

Thermogenesis in BAT is mediated by norepinephrine activation of adenylyl cyclase through sympathetically innervated β_3-adrenergic receptors [2, 13]. We have reported previously that thermogenesis, as indicated by the increase in whole body oxygen consumption, body temperature and guanosine diphosphate (GDP) binding to the mitochondrial uncoupling protein (UCP) in BAT, is decreased or absent in senescent rats following stimulation with a β_3-adrenergic agonist [17]. Although the specific subtype quantification of the β_3-adrenergic receptor has not been determined in young, compared with senescent, rats, the total number of β-adrenergic receptors declines by 50% with age [14]. In addition, there is a similar decrease with age in the ability of β-adrenergic agonists to activate adenylyl cyclase [14].

These studies suggest that the β_3-adrenergic signal transduction pathway is impaired with age and may account for the attenuation of the sympathetically activated thermogenesis in BAT with senescence. The enzyme adenylyl cyclase can be directly activated by the post-receptor agent forskolin [19]. If thermogenesis in senescent rats is limited by early events in the β_3-adrenergic signal transduction pathway, i.e. receptors or stimulatory guanine nucleotide-binding proteins (G proteins), then thermogenesis stimulated by direct activation of adenylyl cyclase will be maintained with age. To test this hypothesis, we examined thermogenesis in BAT stimulated by forskolin and, in some cases,

compared this with stimulation by the β_3-adrenergic agonist CGP-12177. To this end, we assessed whole body oxygen consumption, mitochondrial GDP binding and BAT UCP mRNA levels in 4- and 24-month-old rats following drug administration.

Materials and methods

Animals

Male F-344 NNia rats of 4 and 24 months of age were obtained from Harlan Sprague-Dawley (Indianapolis, Ind, USA) under contract with the National Institute on Aging. Upon arrival, rats were examined and remained in quarantine for 1 week. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals. Rats were housed individually in micro-isolated cages and maintained on rat chow (Purina) ad libitum with a 12:12 h light-dark cycle (0600 to 1800 hours). Experiments were begun 60–90 min after the beginning of the light cycle. Ambient temperature was 26° C.

Chemicals

CGP-12177 was a gift of Ciba-Geigy (Summit, N.J., USA). CGP-12177 was prepared in pyrogen-free saline. Forskolin (Calbiochem, La Jolla, Calif., USA) was prepared in 50% dimethylsulfoxide, 25% ethanol and 25% H_2O. All other chemicals were obtained from Sigma (St. Louis, Mo., USA).

Surgical denervation

Some young and senescent rats underwent unilateral surgical denervation of the interscapular BAT under pentobarbital sodium anesthesia according to the method of Bartness et al. [1]. A transverse incision was made just anterior to the BAT, separating the BAT from the muscles of the scapulae. The BAT was raised to expose the five intercostal nerve bundles entering each pad. On one side, a section of each nerve bundle was removed with scissors. The rats were maintained on a heat pad until recovery from the anesthesia. Experiments were begun on the 4th day after unilateral denervation. Denervation was verified in selected rats by assessing norepinephrine levels in the innervated compared with the denervated BAT pads. In all tested cases the denervation was successful (norepinephrine content 1.97 ± 0.42 ng/mg BAT vs. 0.06 ± 0.04 for innervated and denervated pads respectively, $n = 4$–5).

Body temperature

Body temperature was measured using a battery-operated biotelemetry device consisting of a radio transmitter with a pulse rate that varies in direct proportion to temperature (Mini-Mitter, Sunriver, Ore, USA). Each transmitter was calibrated and implanted surgically into the peritoneal cavity and the animals allowed to recover for 1 week. Receivers were placed under each cage. Core body temperature was measured remotely every 3 min and averaged over 3-min or 15-min intervals.

Isolation of interscapular BAT mitochondria

Rats were sacrificed by cervical dislocation under anesthesia (50 mg/kg pentobarbital sodium). The circulatory system was perfused with 20 ml cold saline and the interscapular BAT excised and trimmed of visible white fat. Mitochondria for use in the GDP-binding assay were isolated as described previously [15]. The final pellet was resuspended in 100 mM sucrose, 1.2 mM ethylenediamine tetraacetic acid, K Salt, (K-EDTA) 12.2 mM choline Cl and 40 mM *N*-tris (hydroxy methyl)/methyl-2-aminomethane-sulfonic acid, K-salt (K-TES) pH 7.1. Mitochondria were used immediately in the GDP-binding assay.

GDP-binding assay

The density of binding sites was determined from a multipoint Scatchard analysis of [^{3}H]GDP binding (specific activity adjusted to 1.012 Ci/mmole, Dupont-NEN, Boston, Mass., USA) as described previously [15]. Briefly, protein (50 µg) and 0.02–3.0 µM [^{3}H]GDP were incubated both with and without 1.5 mM unlabeled GDP for 15 min at 37 °C in a total volume of 250 µl of the buffer used for final suspension with the addition of 0.1 mg/ml fatty acid-free bovine serum albumin and 2 µM rotenone. The reaction was terminated by dilution with buffer and the bound [^{3}H]GDP separated from the free by filtration over glass fiber filters (Whatman GF/B, Clifton, N.J., USA). Specific binding was determined from the difference in binding with and without 1.5 mM unlabeled GDP.

O_2 consumption

O_2 consumption was assessed on up to four rats simultaneously with an Oxyscan analyzer (OXS-4; Omnitech Electronics, Columbus, Ohio, USA) as described previously [13].

UCP mRNA

Total cellular ribonucleic acid (RNA) and UCP messenger RNA (mRNA) were determined by extraction from 100 mg minced and sonicated interscapular BAT tissue as described previously [18]. For extraction, 1 ml of RNAzol B (Biotecx, Friendswood, Tex, USA) and 100 µl chloroform were added to the sonicated interscapular BAT, and this preparation was mixed vigorously, incubated 15 min on ice, centrifuged and the aqueous phase retained. The RNA was precipitated twice with an equal volume of isopropanol, washed with 70% ethanol, suspended in a 10-mM EDTA solution and heated to 65° C for 10 min. After centrifugation, the supernatant was harvested. The integrity of the isolated RNA was verified using agarose gels (1%) stained with ethidium bromide. The RNA was quantified by spectrophotometric absorption at 260 mµ using multiple dilutions of each sample. For measurement of UCP mRNA levels, several concentrations of serially diluted RNA samples were immobilized on nylon membranes (Gene Screen, Dupont-NEN,) using a slot blot apparatus (Biorad, Richmond, Calif., USA). The membranes were baked at 80° C for 2 h. The baked membranes were prehybridized using 25 mM potassium phosphate, 750 mM NaCl, 75 mM Na citrate, 5X Denhardt's solution, 50 µg/ml denatured salmon sperm deoxy ribonucleic acid (DNA) and 50% formamide. After incubation for 14–16 h at 42° C, the membranes were hybridized with a ^{32}P random prime-labeled complementary DNA (cDNA) probe in the prehybridization buffer with the addition of 10% dextran sulfate [18]. The cDNA clone for UCP was kindly provided by Dr. Leslie Kozak, Jackson Laboratory, Bar Harbor, Maine [8] and verified by Northern analysis as previously described [18]. After hybridization for 14–16 h at 42° C, the membranes were washed and exposed to X-ray film (Kodak X-AR, Rochester, N.Y., USA) for 96 h at −70° C using intensifying screens. Optical density per microgram total cellular RNA was calculated by comparison with internal laboratory standards of interscapular BAT UCP mRNA present on each nylon membrane.

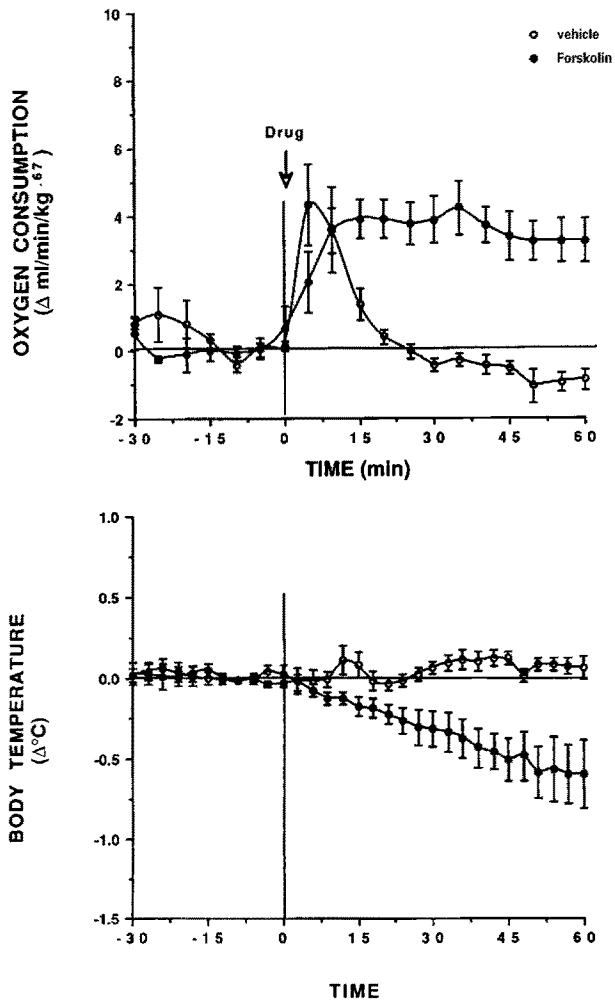
Results

Oxygen consumption and body temperature in young rats

All experiments were performed on conscious, unanesthetized rats during the light phase of the light-dark cycle. The time-course response of whole body oxygen consumption and body temperature were determined simultaneously following intraperitoneal administration of forskolin (3.5 mg/kg) or vehicle (50% dimethylsulfoxide, 25% ethanol, 25% H_2O) in young rats (Fig. 1). The process of injecting the animals with vehicle solution in itself resulted in a significant increase in oxygen consumption that peaked at 3 min but was evident for only 15 min. In contrast, forskolin resulted in a rapid increase in oxygen consumption that was sustained for greater than 1 h (Fig. 1, top). The cumulative 1-h increase (effectively the area under the curve) in oxygen consumption following forskolin administration was 204 ± 31 $(ml \cdot min^{-1} \cdot kg^{-0.67}) \cdot min$ compared with 28 ± 18 for vehicle-injected rats.

In contrast to the increase in whole body oxygen consumption, forskolin administration resulted in a decrease in body temperature (Fig. 1, bottom). The cumulative 1-h decrease was −19.6 ± 5.1° C·min compared with control rats (+ 3.2 ± 1.5° C·min; $P < 0.001$). Administration of vehicle solution resulted in no changes in body temperature.

Fig. 1 Increase over baseline in mass-independent oxygen consumption (*top*) and body temperature (*bottom*) after administration of vehicle (O) or 3.5 mg/kg forskolin (●) in surgically intact 4-month-old rats. Mean body weight was 285 ± 6g. Mean baseline values were 13.9 ± 0.4 $ml \cdot min^{-1} \cdot kg^{-0.67}$ and 37.4 ± 0.1° C for oxygen consumption and body temperature, respectively. Ambient temperature was 26° C. Data represent the mean ± SE, $n = 6$ rats per group

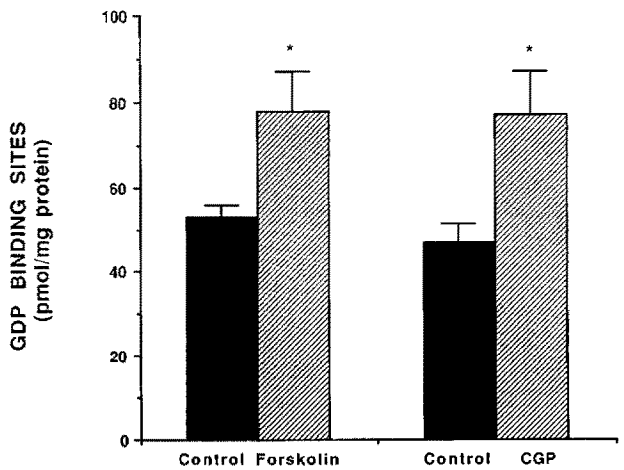


GDP binding in young rats

The decrease in body temperature following forskolin administration was qualitatively different from our previous study demonstrating an increase in body temperature following administration of the specific β_3-adrenergic agonist, CGP-12177 [17]. The experiments described above are thus inconclusive as to whether forskolin activates thermogenesis in BAT. To determine whether some of the forskolin-induced increase in oxygen consumption is due to thermogenesis in BAT, we assessed GDP binding to UCP in interscapular BAT mitochondria following forskolin administration to young rats. We have determined previously that the β_3-adrenergic agonist CGP-12177 induces an increase in oxygen consumption and body temperature in parallel with an increase in GDP binding in BAT [17].

In the present study, in separate experiments, both forskolin and CGP-12177 increased GDP binding in BAT 1 h post-injection (Fig. 2). The increases in GDP binding were similar, suggesting both drugs activate thermogenesis in BAT. Dissociation constants for GPD

Fig. 2 The density of available guanosine diphosphate (GDP) binding sites with and without administration of 3.5 mg/kg forskolin or 0.75 mg/kg CGP-12177 (*CGP*) in surgically intact 4-month-old rats. Rats were sacrificed 1 hr post-injection. Data are means ± SE, $n = 6$ rats $^*P < 0.05$ (forskolin), $P < 0.02$ (CGP-12177) for differences from respective controls by Student's *t*-test



binding were 300 ± 20 nM (control), 290 ± 17 nM (forskolin) and 211 ± 16 nM (control), 194 ± 22 nM (CGP-12177). Despite these nearly equal responses, the specific β_3-adrenergic agonist CGP-12177 increased body temperature (previous study, [17]) whereas the non-specific activator of adenylyl cyclase, forskolin, decreased body temperature (Fig. 1, bottom). This latter observation renders it difficult to determine whether the forskolin-induced increase in GDP binding is a direct effect of forskolin on BAT or an indirect effect due to the decrease in body temperature. If forskolin lowers body temperature (possibly due to action on the vascular system) then the thermoregulatory center in the hypothalamus would respond with a sympathetic discharge to the innervated BAT, resulting in an increase in GDP binding. To circumvent these compounding effects, subsequent experiments were conducted in rats in which the interscapular BAT was unilaterally denervated.

Unilateral denervation of BAT in young and senescent rats

The time course of stimulation of whole body oxygen consumption by forskolin was determined in young and old rats in which the BAT was unilaterally denervated. The older animals could not tolerate the dose of forskolin used in the previous experiments. Thus, oxygen consumption was assessed following administration of forskolin at 1.8 mg/kg. The pattern of forskolin-stimulated oxygen consumption was similar in the young and senescent rats. The peak increase was greater in the young rats, but the sustained stimulation was the same in rats of both ages (Fig. 3). Surprisingly,

Fig. 3 Increase (△) over baseline in mass-independent oxygen consumption after administration of forskolin (1.8 mg/kg) in young (○) and old (●) rats in which the interscapular brown adipose tissue (*BAT*) was unilaterally denervated. Mean body weights were 312 ± 17 g (young) and 402 ± 2 g (old). Mean baseline values were 12.6 ± 0.3 and 11.4 ± 0.5 $ml \cdot min^{-1} \cdot kg^{-0.67}$ for young and old rats respectively. Data are means ± SE, n = 3–4 rats

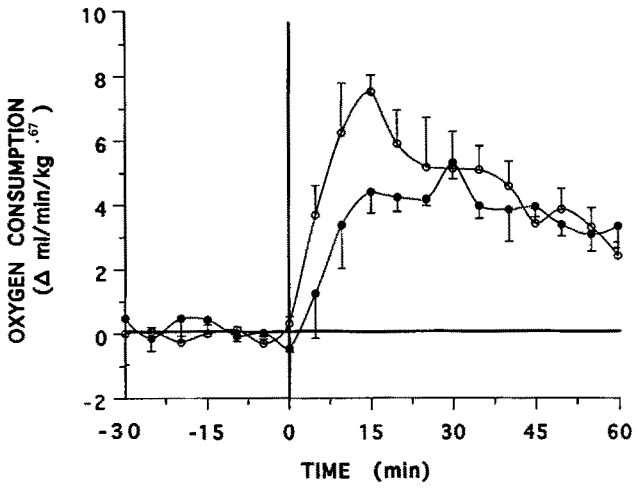


Table 1 Mitochondrial guanosine diphosphate (*GDP*) binding following forskolin administration in young and senescent rats in which the interscapular brown adipose tissue (*BAT*) was unilaterally denervated. Values are means ± SE, n = 7–10 rats in each group. Rats were sacrificed 1 h after forskolin (3.5 mg/kg in young, 1.8 mg/kg in old rats) administration

	GDP binding sites (pmol/mg protein)			
	4-month-old rats		24-month-old rats	
BAT pad	Control	Forskolin	Control	Forskolin
Innervated	51.7 ± 4.5	82.1 ± 7.9*	51.1 ± 2.1	63.8 ± 2.5*
Denervated	37.7 ± 3.5†	61.4 ± 5.8*†	39.9 ± 3.0†	81.7 ± 6.7*†

*P = 0.003 (innervated, 4 month); = 0.004 (denervated, 4 month); = 0.002 (innervated, 24 month); = 0.001 (denervated, 24 month) for differences from corresponding control by Student's t-test
†P = 0.03 (control, 4 month); = 0.01 (forskolin, 4 month); = 0.001 (control, 24 month); = 0.02 (forskolin, 24 month) for difference between innervated and denervated pads by paired t-test

this lower dose of forskolin stimulated oxygen consumption in the young rats to an extent comparable with the higher dose used in the previous experiment (Figs. 1 and 3).

To determine whether forskolin activates thermogenesis in BAT directly, GDP binding was compared in the innervated and denervated BAT pads with or without forskolin administration. In these experiments, two doses of forskolin were administered to young rats (1.8 and 3.5 mg/kg). Among control animals (no drug administration), there was no difference in the level of GDP binding in the two age groups; however, GDP binding was 37% less in the denervated BAT pads than in the innervated pads of rats of both ages (Table 1). In young rats following administration of forskolin at the lower dose of 1.8 mg/kg, there were no significance increases in GDP binding in either the innervated or denenervated BAT pads (data not shown). In contrast, following administration of the high dose in young rats, forskolin increased GDP binding in both the innervated and denervated pads. Both the absolute increase in GDP binding (30.4 vs 23.7) and the percent increase (58.8 vs 62.9) were nearly equal. Similarly, in the

Fig. 4 Northern analysis of the hybridization of the UCP complementary desoxyribonucleic acid probe with 10 μg total RNA from BAT of young (*lane 1*) or senescent (*lane 2*) rats. The *major band* corresponds to 1.5 kb, the *minor band* to 1.9 kb

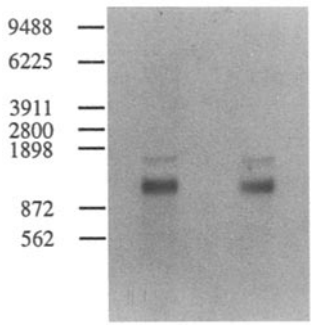


Table 2 Mitochondrial uncoupling protein messenger ribonucleic acid (*UCP mRNA*) following forskolin administration in young and old rats in which the BAT was unilaterally denervated. Values are mean ± SE, n = 9–10 rats in each group. The 4 month-old innervated control rats were arbitrarily set to 100. Rats were sacrificed 5 h after forskolin (3.5 mg/kg in young, 1.8 mg/kg in old rats) administration

	UCP mRNA (arbitrary OD units)			
	4-month-old rats		24-month-old rats	
BAT pad	Control	Forskolin	Control	Forskolin
Innervated	100 ± 7.48	222.4 ± 29.7*	51.3 ± 19.3**	68.9 ± 13.4
Denervated	37.7 ± 5.0†	78.6 ± 11.8*†	21.7 ± 4.9**	89.6 ± 21.0*

*P = 0.001 (innervated, 4 month); = 0.008 (denervated, 4 month); < 0.001 (denervated, 24 month) for differences from corresponding control by Student's t-test
†P < 0.001 (control, 4 month); = 0.002 (forskolin, 4 month) for difference between innervated and denervated pads by paired t-test
**P = 0.025 (innervated) and P = 0.035 (denervated) for difference with age by Student's t-test

senescent rats, forskolin increased GDP binding in both the innervated and denervated pads (Table 1). In this case, the increase was greater in the denervated pads.

In assessing the direct effects of forskolin on BAT thermogenesis, the data from the denervated BAT pads, which are free of endogenous stimulation, permits a less complicated interpretation. In these pads, forskolin increased GDP binding, and the increase was equal or greater in the senescent rats despite the lower dose per unit rat weight of forskolin administered to these older rats (Table 1).

UCP mRNA

UCP mRNA levels were assessed by hybridization with a full-length UCP cDNA probe [8]. Northern analysis indicates that this probe hybridizes to two mRNA species, a major band corresponding to 1.5 kb and a minor band corresponding to 1.9 kb (Fig. 4). The probe did not hybridize to any mRNA species from the cerebral cortex (data not shown). To investigate further whether forskolin activates thermogenesis in BAT directly and whether there are differences with age, we examined the induction of UCP mRNA following forskolin administration in young and old rats in which the BAT was unilaterally denervated. Among control animals, similar to the finding with GDP binding, the amount of UCP mRNA was less in the denervated compared with the innervated BAT pads (Table 2). However, in contrast to the data on GDP binding, the amount of UCP mRNA was significantly less in the senescent rats compared with the young rats in both the innervated and denervated BAT pads (Table 2). This is also in contrast to our previous findings in non-surgically manipulated rats in which the steady state level of UCP mRNA is unchanged with age [18]. In the young rats following forskolin administration, UCP mRNA was significantly increased in both the innervated and denervated pads. In the senescent rats, there was a significant increase only in the denervated pad. The magnitude of the forskolin-induced increase in the denervated pads was greater in senescent compared with the young rats (Table 2).

Discussion

In rodents, thermoregulation is impaired with age and varies according to the severity of the cold stress and the age, strain and gender of the animal [9, 10, 11, 17]. The β_3-adrenergic receptor is the principal mediator of thermogenesis in the mature adipocyte [2], and β_3-adrenergic agonist stimulation of thermogenesis, including oxygen consumption, body temperature and mitochondrial GDP binding, is impaired with age in the male F-344 rat [14]. In addition, although the specific subtype quantification of β_3-adrenergic receptors has not been determined in young compared with senescent rats, the total number of β-adrenergic receptors declines with age [14]. These observations have led us to suggest that the decline in β_3-adrenergic stimulation of BAT thermogenesis contributes to the impaired thermoregulatory capacity in older rats [17].

The β_3-adrenergic receptor is coupled to adenylyl cyclase through the stimulatory G-protein (G_s). The purpose of the present study was to determine if events proximal or distal to adenylyl cyclase activation are involved in the decline of the β_3-adrenergic-stimulated thermogenesis in BAT. To this end we examined whether several indices of thermogenesis are stimulated by forskolin in young and senescent rats. Forskolin is a diterpene that activates directly the catalytic subunit of adenylyl cyclase [19]. In young rats, forskolin stimulated whole body oxygen consumption and GDP binding in BAT mitochondria. However, despite this apparent increase in BAT thermogenesis, forskolin actually decreased body temperature. This latter observation is in agreement with previous studies showing that the nonspecific β-adrenergic agonist isoproterenol increases oxygen consumption while decreasing body temperature [3, 4]. In these studies, the hypothermia was specifically mediated by β_2-adrenergic receptors, presumably by altering peripheral blood flow and heat loss [4]. Since forskolin activates adenylyl cyclase directly, it should mimic the effects of a nonspecific β-adrenergic agonist (as well as the combined effects of all other receptors coupled to adenylyl cyclase), and thus the observed hypothermia is not unexpected. The forskolin-induced hypothermia does,

however, complicate the interpretation of the effects of forskolin on BAT thermogenesis. The hypothermia, through a mechanism involving the hypothalamus, may increase sympathetic outflow to BAT, thus stimulating thermogenesis. Because of these secondary effects resulting from hypothermia and because of the multiple sites of action for forskolin, we used rats in which the BAT was unilaterally denervated for all subsequent studies. In these rats, forskolin stimulated whole animal oxygen consumption to a similar extent in young and old, except the peak increase was greater in the young rats.

The rats with unilaterally denervated BAT also allowed comparison of the forskolin-induced increase in GDP binding between BAT pads that were exposed only to forskolin and those exposed to both forskolin and sympathetic stimulation. Our results indicated that forskolin stimulated GDP binding and induced UCP mRNA in both the innervated and denervated BAT pads. As expected, in the absence of sympathetic innervation, the basal level of GDP binding was lower in the denervated BAT pads. In the young rats, forskolin stimulated GDP binding to an equal extent in both BAT pads, whereas in the older rats the increase was greater in denervated BAT pads, possibly due to denervation-induced supersensitization. Similarly, UCP mRNA levels were lower in denervated than in innervated BAT pads, and both types of pads responded to forskolin stimulation.

The main objective of this study was to examine the direct effects of forskolin on thermogenic responses and assess any differences with age. Unfortunately, the dose of forskolin per unit rat weight that stimulated BAT GDP binding in young rats caused cardiac distress in the senescent rats. This necessitated using a lower dose per unit rat weight in the older rats, thus complicating direct comparisons between young and old rats. However, since the results demonstrated similar levels of stimulation in the older rats despite the lower dose, we can still conclude that effects of forskolin are either equal or greater in the senescent rats. This is best demonstrated in the data from denervated BAT pads. These pads are essentially isolated from endogenous stimulation. The data from the denervated pads indicate that the magnitude of both the forskolin-induced increase in GDP binding and the induction of UCP mRNA is equal or greater in BAT from the older rats. In contrast, the β_3-agonist-stimulated increase in mitochondrial GDP binding is virtually absent in older rats [17]. This indicates that β_3-adrenergic-stimulated thermogenesis in BAT is impaired with age whereas the post-receptor-stimulated thermogenesis is maintained or is even greater in the senescent rat.

Our previous study indicated that the level of UCP mRNA in the unstimulated state is not changed with age [18]. However, in the present report this was not true in the surgically manipulated rats. In both the innervated and denervated BAT pads, the UCP mRNA levels are reduced with age. This may indicate that there is a compensatory increase in the UCP synthesis in the young, innervated BAT pad that is absent in the old, innervated BAT pad. This may also explain why the forskolin-induced increase in the innervated BAT pad was greater in the young compared with the senescent rats. Nevertheless, in the denervated BAT pad, which presumably is free of endogenous stimulation, the induction of UCP mRNA by forskolin was maintained with age.

Two important thermoregulatory functions that decline with age in the male F-344 rat are the ability to maintain body temperature after exposure to the cold and the ability to raise body temperature in response to an infection. Both of these functions are believed to be mediated by β_3-adrenergic receptors. Because β_3-adrenergic-stimulated thermogenesis is impaired with age, it follows that both cold exposure and infection in senescent rats will result in reduced thermoregulatory responses and reduced thermogenesis in BAT. This is the case following an *E. coli* infection in senescent rats in which there is a diminished febrile response and decreased GDP binding to BAT mitochondrial membranes [16]. In contrast, following cold exposure in older rats, there is the expected hypothermia but the cold-induced increase in BAT GDP binding and the cold-induced induction of UCP mRNA are unchanged in senescent compared with young rats [18]. Collectively, these observations suggest that thermogenesis in BAT is either selectively enhanced by cold exposure to a greater extent in senescent rats, or is mediated by other receptors than the β_3-adrenergic receptors. The former is the more likely possibility, especially because the impaired febrile response in aged rats is associated with reduced GDP binding [16]. However, it is also possible that another receptor, such as the β_1-adrenoreceptor, also coupled to adenylyl cyclase, may mediate BAT thermogenesis in senescent rats. This receptor is tightly coupled to adenylyl cyclase in immature adipocytes and mediates important physiological functions including cell proliferation [2]. However, in the mature adipocyte, the thermogenic functions of BAT are mediated by β_3-adrenergic receptors. It is possible that, with senescence, there is reemergence of the β_1-adrenergic receptor as a mediator of BAT function. Such a role-reversal for β-adrenergic receptor subtypes is not without precedent. In the rat liver, glycogenolysis mediated by β_2-adrenergic stimulation of adenylyl cyclase is prominent in neonatal rats, declines during development to a minimum at adulthood, but then reemerges during senescence [7]. In this same tissue, there is a reciprocal relationship between β_3- and β_2-adrenergic stimulation of adenylyl cyclase with age. β_3-Adrenergic stimulation of adenylyl cyclase is absent in the neonatal liver, but increases during development concurrent with the loss of β_2-adrenergic stimulation. With senescence, β_3-adrenergic stimulation again abates [12]. In BAT, there

may also be a reciprocal relationship between β_1/β_3 physiological function. β_1-Adrenergic receptor-mediated processes may be prominent in immature and senescent tissue, whereas β_3-adrenergic receptors may dominate in mature tissue. Further work is necessary to clarify the roles of these β-adrenergic subtypes in thermogenesis at different ages.

In summary, the post-receptor activation of thermogenesis in BAT, including whole body oxygen consumption, GDP binding and induction of UCP mRNA in BAT were unchanged with age. These data suggest that biochemical components proximal to adenylyl cyclase, either the β_3-adrenergic receptor, G_s-protein, or the coupling of these proteins to adenylyl cyclase, are impaired in BAT from senescent rats and account for the reduced ability of β_3-adrenergic agonist to stimulate BAT thermogenesis in older animals.

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