DOI 10.1007/s11055-016-0283-0
Neuroscience and Behavioral Physiology, Vol. 46, No. 5, June, 2016

The Role of the Sympathoadrenal System in Adaptation to Cold

L. N. Maslov and E. A. Vychuzhanova

Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 101, No. 2, pp. 145–162, February, 2015. Original article submitted January 27, 2014. Revised version received October 13, 2014.

Noradrenaline released from sympathetic nerve endings and adrenaline secreted by the adrenal cortex have been shown to play roles in cold adaptation. The calorigenic effect of noradrenaline increases during acclimation. Catecholamines stimulate noncontractile thermogenesis in brown fat tissue, white fat tissue, and skeletal muscle. Adrenergic increases in cold tolerance are associated with increases in UCP expression in the tissues. The following signal pathway operates in mediating the calorigenic effect of catecholamines in prolonged cold exposure: catecholamines → β-adrenergic receptors → adenylate cyclase, cAMP → protein kinase A → p38 kinase → transcription factors → increased UCP expression. The following signal pathway operates on acute exposure to cold: catecholamines → β-adrenergic receptors → adenylate cyclase → cAMP → protein kinase A → hormone-sensitive lipase → free fatty acids → UCP → uncoupling of oxidative phosphorylation. The calorigenic effect of stimulation of α_1-adrenoreceptors is mediated by the following mechanism: noradrenaline → α_1-adrenergic receptors → phospholipase C → inositol-1,4,5-triphosphate → $[Ca^{2+}]_i$ → increased calorigenic effect of catecholamines.

Keywords: stress, cold, adaptation, catecholamines, adrenoreceptors.

Abbreviations

MAPK	mitogen-activated protein kinase
mRNA	messenger RNA
SAS	sympathoadrenal system
FFA	free fatty acids
cAMP	cyclic adenosine monophosphate
$[Ca^{2+}]_i$	cytoplasmic calcium ion concentration
ATF-2	activating transcription factor 2
B_{max}	receptor density
CREB	a transcription factor, cAMP response element-binding protein
K_d	dissociation constant
K_i	inhibition constant
PGC-1-α	a transcription factor, peroxisome proliferator activator receptor-γ (PPAT-γ) coactivator 1-α
UCP	uncoupling proteins

Cold adaptation occurs as a result of chronic continuous or discontinuous exposure of the body to low environmental temperature. It is now accepted that the key role in acclimation to cold is played by the sympathoadrenal and thyroid systems. In this review we aim to systematize and analyze existing publications on the role of the sympathoadrenal system (SAS) in cold adaptation.

The calorigenic effect of catecholamines. Smith and Hoijer [69] published a review in 1962 in which they analyzed the then sparse published data on the role of catecholamines in cold acclimation. Cold adaptation was found to induce adrenal hypertrophy, to increase the calorigenic effect of noradrenaline, and to increase the adrenaline and noradrenaline levels in the adrenals by factors of 3 and 4, respectively [69]. In adapted rats, noradrenaline induced a more marked increase in the serum free fatty acids (FFA) level than in nonadapted individuals [59]. Precisely the opposite situation was obtained with glucose: a more marked increase in the glucose level was obtained in response to injections of noradrenaline in nonadapted rats [59]. Hannon and Larson noted increases in the calorigenic effects of noradrenaline in adapted rats [30]. They documented an increase in noradrenaline-induced mobilization of FFA from fat tissue in cold-acclimated individuals. However, they

Research Institute of Cardiology, Tomsk, Russia;
e-mail: Maslov@cardio-tomsk.ru.

noted that noradrenaline was not the only mediator of non-contractile thermogenesis in cold-adapted animals [30]. A group of Japanese physiologists showed that infusion of noradrenaline promoted a 20% increase in FFA oxidation in intact rats and a 50% increase in adapted individuals [51]. Thus, cold adaptation not only enhances noradrenaline-induced mobilization, but also FFA oxidation. Joy showed in 1963 that cold adaptation in humans significantly increases O_2 consumption in response to noradrenaline infusions, while increases in systolic and diastolic arterial pressure after noradrenaline were more marked in nonadapted volunteers [36]. Basal oxygen consumption levels were identical in adapted and nonadapted men. Thus, adaptation to cold in humans increases the calorigenic effect of noradrenaline but decreases the sensitivity of the vascular bed to this catecholamine. Experiments on cold-adapted rats yielded similar data [20]. Infusion of noradrenaline was found to induce a more marked elevation in O_2 consumption in adapted rats than in intact individuals. The increase in arterial blood pressure after use of noradrenaline in adapted rats was transient in nature, while intact rats displayed stable hypertension. Acclimation promoted an increase in the inotropic effect of noradrenaline (due to an increase in stroke volume). The positive chronotropic effects of noradrenaline were identical in acclimated and intact rats [20]. The adaptation-related increase in noradrenaline-induced thermogenesis was confirmed in later studies [16, 19, 43]. Experiments on cold-adapted (+5°C) rats reported by an American group demonstrated a paradoxical calorigenic effect of noradrenaline [80]. When acclimated animals were placed at room temperature (+25°C) or moderate cold (+5°C), noradrenaline injections induced an increase in oxygen consumption. At –15°C, conversely, noradrenaline decreased oxygen consumption. In nonadapted individuals, decreases in O_2 consumption after noradrenaline injections were seen at +15°C, and a further reduction in temperature only exacerbated the "anticalorigenic" effect of noradrenaline [80]. Blockade of α_2-adrenoreceptors with yohimbine completely eliminated the "anticalorigenic" effect of noradrenaline. This allowed the authors to conclude that this effect of exogenous noradrenaline is linked with activation of presynaptic α_2-adrenoreceptors and a limit on the release of endogenous noradrenaline from sympathetic terminals.

However, not all investigators have been able to confirm the existence of cold-induced enhancement of the calorigenic effect of catecholamines. Thus, results obtained by Kuroshima et al. [44] indicated that the basal plasma FFA level in cold-adapted rats was lower than that in intact individuals. After noradrenaline injections, acclimated animals showed smaller increases in glucose and FFA than control rats. Similar data were reported by a Canadian group [60]. Their experiments on isolated brown fat adipocytes showed that cold acclimation significantly decreased the lipolytic effect of catecholamines. Observations of healthy cold-adapted volunteers reported by Budd et al. [12] did not show any calorigenic effect with noradrenaline. The authors of this study did not assess cold tolerance, so it remains unclear whether their volunteers developed cold resistance. It is possible that the adapting effect was insufficiently strong, such that resistance simply did not form. Similar data were obtained by Jansky et al. [34] from observations of swimmers taking winter dips. Basal metabolic rate was measured in terms of the quantity of inhaled oxygen. Intravenous infusion of the β_1-adrenoreceptor agonist dobutamine or the β_2-adrenoreceptor agonist bricanyl was found to increase basal metabolic rate in untrained volunteers but had no effect on oxygen consumption in swimmers. This is evidence of desensitization of β_1- and β_2-adrenoreceptors. However, these data do not exclude the possibility that there is an adaptational increase in the calorigenic effect of noradrenaline, as it interacts not only with β_1- and β_2-adrenoreceptors, but also with β_3-adrenoreceptors which, as has been demonstrated by several authors, play a key role in acclimation to cold [19, 79].

The reasons why some authors have observed adaptational increases in the calorigenic effect of catecholamines and others have not remains unclear.

The roles of brown fat, white fat, and skeletal muscle in the calorigenic effect of catecholamines. Studies in 1969 showed that the calorigenic effect of noradrenaline was linked with activation of oxygen consumption by brown fat tissue [48]. In rats from which interscapular brown fat was removed after acclimation, the calorigenic effect of noradrenaline decreased by 40%. Some data indicate that removal of brown fat from adapted rats decreased the calorigenic effects of adrenaline and noradrenaline more than 1.5-fold [32]. In another article, the same author confirmed that removal of brown fat decreased the calorigenic effect of noradrenaline in adapted individuals by a factor of more than 1.5 [32]. The important role of brown fat in noradrenaline-induced thermogenesis in acclimated animals was confirmed in later studies [17, 21]. However, the real contribution of brown fat to adaptive thermogenesis is quite difficult to evaluate, as it is located not only in the interscapular space, but also in other parts of the body [70]. The possibility that the calorigenic effect of noradrenaline may be due to activation of adrenoreceptors in other tissues cannot be excluded. In fact, microcalorimeter studies reported by Kuroshima et al. [42] showed that noradrenaline stimulated heat production in adipocytes isolated from white fat tissue from cold-adapted rats.

A British group showed [15] that chronic treatment of rats with noradrenaline (an agonist of α- and β-adrenoreceptors) promoted hyperplasia of brown fat, with increases in the number of mitochondria and thermogenin in adipocytes. Chronic administration of phenylephrine (an α-adrenoreceptor agonist) or isoproterenol (a β-adrenoreceptor agonist) did not induce such changes in brown fat tissue. Simultaneous administration of both adrenomimetics produced changes in brown fat tissue analogous to those observed after chronic administration of noradrenaline [15]. These points indicate

that catecholamines imitate the adaptive changes in adipocytes in brown fat. This effect of catecholamines requires activation of α- and β-adrenoreceptors.

In experiments on isolated rat hindlimb muscles, van Hardveld et al. [75] did not detect any increase in the calorigenic effect of catecholamines after cold acclimation. Directly contradictory data were obtained by Shiota and Masumi [67]. Using in vivo experiments on perfused hindlimb skeletal muscle, this group showed that cold adaptation increased noradrenaline-induced O_2 consumption by these muscles. Blockade of α- and β-adrenoreceptors weakened the calorigenic effect of noradrenaline.

Thus, most reports provide evidence that the calorigenic effect of catecholamines is linked with their effects on brown fat, white fat, and skeletal muscle.

Contribution of the sympathoadrenal system to cold acclimation. Chemical sympathectomy (with reserpine) and blockade of peripheral autonomic ganglia eliminated increases in glucose and FFA levels in response to acute cold exposure [25], while demedullation of the adrenals had no effect on this parameter. After these procedures, rats responded to cold (+4°C) by developing hypothermia, while intact individuals retained normal body temperature. The most precipitous hypothermia developed in rats after use of reserpine and ganglion blockade [25]. Blockade of β-adrenoreceptors with propranolol was found to decrease oxygen consumption in cold-adapted rats, but to have no effect on O_2 consumption in intact individuals [2]. These observations indicate that the sympathoadrenal system (SAS) plays an important role in acute adaptation of the body to cold.

Cold-adapted rats showed a three-fold increase in urinary adrenaline excretion, while noradrenaline excretion increased eight-fold [68]. After one month in the Antarctic, polar explorers also showed increased urinary excretion of noradrenaline (+107%) and adrenaline (+70%) [4]. Increased catecholamine (noradrenaline, adrenaline, dopamine) excretion in cold-adapted rats was noted by the authors of later reports [23]. The increase in urinary noradrenaline excretion was particularly marked [23]. An increase in plasma noradrenaline was seen after five days of acclimation to cold, with a simultaneous increase in the level of noradrenaline metabolites [24]. Huttunen et al. [33] published the results of their observations of volunteers swimming in cold water (+4°C) daily for one month and in nonadapted people. In response to immersion in cold water for 36 sec, these and other groups have noted identical increases in plasma noradrenaline concentrations. People swimming in cold water for three months showed a smaller increase in noradrenaline in response to transient exposure to cold. Similar data were obtained from observations of participants in the Indian Antarctic Expedition [31]. During the first seven days, urinary adrenaline excretion increased four-fold, while noradrenaline excretion increased five-fold. After two months in the Antarctic, these measures returned to baseline (pre-expedition) levels. Thus, the response of the SAS to cold depends on the duration of cold acclimation.

Direct evidence for activation of the sympathetic nervous system during cold adaptation in mice was obtained by Kawate et al. [37]. They recorded bioelectrical activity from one of the sympathetic nerves innervating the interscapular brown fat in anesthetized animals. A decrease in body temperature led to an increase in sympathetic activity in adapted and nonadapted mice, though this effect was more marked in acclimated individuals [37].

In 1964, a Canadian group reported convincing evidence that noradrenaline is involved in cold acclimation [45]. Rats were divided into three groups: 1) intact animals; 2) individuals subjected to chronic cold (+6°C for 45 days); and 3) rats with chronic daily administration of noradrenaline (for 45 days). Cold adaptation was found to produce a basal increase in oxygen consumption, while chronic catecholamine administration had no such effect. Subcutaneous noradrenaline increased O_2 consumption in nonadapted individuals by 40%, compared with a 75% increase in adapted rats and 100% in rats given noradrenaline. In intact animals, rectal temperature in response to noradrenaline increased by 1°F (the range 0...+100°F corresponds to –18...+38°C), while increases in the second and third groups were by 2.5°F [45]. All intact animals died on being kept at –20°C for 12 h, compared with 70% of those given noradrenaline and only 20% of adapted rats. Thus, these data indicate that noradrenaline imitates the calorigenic effect of cold acclimation. However, the results provide evidence that catecholamines are not the only humoral factors supporting cold adaptation. Similar results were obtained by LeBlanc et al. [46] in conditions of chronic administration of the β-adrenoreceptor agonist isoproterenol. A later study from the same group showed that s.c. noradrenaline (0.3 mg/kg) increased rectal temperature in adapted rats by 1.5°C 1 h after injection [47], while temperature remained unaltered in control individuals.

Studies reported in 1984 showed [38] that tyramine – an agent inducing noradrenaline release from nerve terminals – increases oxygen consumption and rectal temperature in rats. The calorigenic effect of tyramine was significantly greater in adapted individuals. This indicates that endogenous noradrenaline plays an important role in noncontractile thermogenesis. The increase in the calorigenic effect of noradrenaline (an agonist of α- and β-adrenoreceptors) in adapted individuals has also been confirmed in other investigations [19], though the calorigenic effect of the selective β_3-adrenoreceptor agonist CGP-12177 was particularly marked after acclimation [19]. It should be borne in mind that chronic administration of any receptor agonist induces receptor desensitization, i.e., decreased sensitivity to agonist [1]. Here there is a directly opposite situation, i.e., enhancement of the calorigenic effect of noradrenaline. In this regard we note the studies of Thomas et al. [72]. These investigators incubated 3Y3-F442A cells with the β-agonist

isoproterenol for 30 h. After this procedure, the expression of β_3-adrenoreceptors (measured in terms of β_3-adrenoreceptor mRNA) increased by 165%, while β_1-adrenoreceptor expression decreased by 70%. This indicates that in some cases, prolonged stimulation of a receptor may lead to its upregulation, i.e., an increase in the density of the receptors on the cell membrane.

Noradrenaline released from sympathetic nerve terminals rather than noradrenaline circulating in the bloodstream appears to play an important role in adaptive thermogenesis. Thus, in 1984, Rothwell and Stock [62] published results from experiments on rats with surgical denervation of the interscapular brown fat. Sympathectomy before seven-day cold exposure completely prevented acclimatory hypertrophy of fat tissue and the increase in the content of uncoupling proteins (UCP) in adipocytes. UCP-1 (thermogenin) levels were measured in this study in terms of the number of binding sites for labeled GDP in adipocytes [62]. Uncoupling proteins are known to: 1) uncouple oxidative phosphorylation and 2) increase heat production by mitochondria [5], so an increase in the quantity of UCP-1 is evidence for cold acclimation. Desautels et al. [18] showed in 1986 that adaptive hyperplasia of brown fat and an increase in thermogenin content were not seen in animals with surgical sympathectomy of the interscapular fat. In experiments on adipocyte cultures, Puigserver et al. [57] showed that incubation of cells with noradrenaline leads to an increase in the UCP-1 level at 24 h of incubation. This effect reached a maximum at five days. These data would seem to indicate that noradrenaline plays a key role in adaptation to cold, while adrenaline does not. However, some data contradict this view [66]. A US group conducted experiments on mice with knockout of the gene responsible for synthesis of the enzyme phenylethanolamine-N-methyltransferase [66], which is involved in adrenaline synthesis. Knockout mice responded to 24-h cold with no increase in UCP-1 mRNA in brown fat tissue. This is evidence that adrenaline also plays an important role in cold-induced UCP-1 synthesis in brown fat tissue. The molecular mechanism supporting this UCP-1 expression remains incompletely understood and is now being studied by investigators addressing the problem of cold adaptation.

Thus, analysis of published data provides evidence that noradrenaline released from sympathetic nerve terminals and adrenaline circulating in the blood play an important role in cold adaptation.

Roles of uncoupling proteins and β-adrenoreceptors in the calorigenic effect of catecholamines. A Finnish group sought to identify which types of β-adrenoreceptors are involved in cold adaptation [40]. During adaptation, rats received daily i.p. injections of the β_1- and β_2-adrenoreceptor antagonist propranolol or the β_1- and β_2-adrenoreceptor antagonist pindolol. Blockade of β_1- and β_2-adrenoreceptors did not affect adaptive hyperplasia of the interscapular brown fat or the adaptive increase in total protein content in brown fat tissue. Unfortunately, the authors did not evaluate the animals' resistance to cold. Nonetheless, these points provide indirect evidence that β_1- and β_2-adrenoreceptors are not involved in the mechanism of cold adaptation in rats. Experiments using isolated brown fat adipocytes showed [7] that the greatest increase in respiration was obtained in these cells by incubating adipocytes with selective β_3-adrenoreceptor agonists and that less marked effects were seen with β_1- and β_2-agonists. A Swedish group addressed the receptor nature of the calorigenic effect of catecholamines and evaluated this effect in terms of the respiration of isolated rat brown fat adipocytes [79]. This study used the selective β_1-adrenoreceptor agonist dobutamine, the β_2 agonist salbutamol, and the selective β_3-adrenoreceptor agonists BRL-37344 and CGP-12177, as well as the β_1-, β_2-, and β_3-adrenoreceptor agonist isoproterenol. Only agents interacting with β_3-adrenoreceptors increased adipocyte respiration. The authors concluded that the calorigenic effect of catecholamines was associated with stimulation of β_3-adrenoreceptors on brown fat tissue adipocytes [79]. It remained unclear whether the calorigenic effect of catecholamines in other tissues was linked with activation of β_3-adrenoreceptors. A Japanese group showed that chronic administration of isoproterenol to rats induced hyperplasia of the interscapular brown fat, with an increase in their uncoupling protein UCP-1 content [53]. Chronic administration of the selective β_3-adrenoreceptor agonists CL316,243, BRL 26830A, and ICI D7114 had the same effect [53]. These data were supported by Cinti et al. [14]. Nagase's group showed that chronic administration of the β_3 agonist CL316.243 to mice induced a six-fold increase in the quantity of UCP in brown fat, with simultaneous increases in the expression of uncoupling proteins in white fat and skeletal muscle [54]. However, data obtained by other authors [26] indicate that single doses of the β_3 agonist trecadrine to rats increased the level of uncoupling proteins in brown fat tissue but not in skeletal muscle. It may be that increased UCP-3 expression in muscles requires prolonged occupation of β_3-adrenoreceptors by agonist. An American group showed that single doses of the β_3 agonist CL316,243 to mice increased the quantities of UCP-23 mRNA and UCP-3 mRNA in skeletal muscles [11]. This effect was not seen in in mice with knockout of the β_3-adrenoreceptor gene. The effect of occupation of β_3-adrenoreceptors depends not only on the duration of receptor stimulation, but also on the properties of the β_3-adrenoreceptor agonist. Increases in UCP-2 and UCP-3 expression in mouse skeletal muscle after courses of CL316.243 were supported by a later study [55]. An American group showed that chronic (14 days) administration of noradrenaline and the β_3-adrenoreceptor agonist CL3126,243 to rats increased the quantity of UCP-1 protein in brown fat tissue by 60% and 230%, respectively [52]. Experiments with cultured brown fat adipocytes showed that UCP-1 expression increased on stimulation of any of the three β-adrenoreceptors, the greatest effect being

seen on activation of all three β-adrenoreceptors with isoproterenol [61]. UCP-1 protein is not the only end effector of the calorigenic action of catecholamines. Thus, Gong et al. [28] showed that two doses of the β_3 agonist CL214613 increased UCP-3 expression in white fat tissue.

In 1995, observations of healthy volunteers showed that ephedrine increased thermogenesis [49]. Prior use of nadolol (an antagonist of β_1- and β_2-adrenoreceptors) decreased the thermogenic action of ephedrine by 57%. The effects of ephedrine are known to be associated mainly with mobilization of endogenous catecholamines [6], so there are grounds for suggesting that the thermogenic effect of endogenous catecholamines in humans results from occupation of β_3-adrenoreceptors and one of the other two types of adrenoreceptor – β_1 or β_2. A Dutch group showed that administration of isoproterenol to volunteers increased energy consumption, while blockade of β_1-adrenoreceptors by atenolol halved the level of isoproterenol-induced energy consumption [9]. The selective β_2 agonist salbutamol also increased oxygen consumption. Thus, the data reported by these authors showed that thermogenesis in humans involves all three types of β-adrenoreceptor. In 2011, Wijers et al. reported a sensational finding [76]: β-adrenoreceptors are not involved in thermogenesis in humans. During a cold test (+16°C), volunteers were given the β-antagonist propranolol. This agent was found to have no effect on energy consumption. In this regard, it should be noted that propranolol blocks only β_1- and β_2-adrenoreceptors and has virtually no interaction with β_3-adrenoreceptors [77]. Thus, the studies of Wijers et al. provide indirect evidence that the decisive role in thermogenesis in humans is played by β_3-adrenoreceptors.

Results from experiments on mice lacking all three types of adrenoreceptor – β_1, β_2, and β_3 – were published in 2002 [35]. These individuals were found not to be resistant to acute cold. Mattson et al. [50] reported results from experiments on mice with knockout of the β_3-adrenoreceptor gene in 2011. These animals were found to survive for seven weeks at +4°C, i.e., deletion of the β_3-adrenoreceptor gene had no effect on acclimation in these animals. Injections of noradrenaline to nonadapted normal mice and knockout animals increased oxygen consumption in both groups, though this was less marked in knockout individuals, while the UCP-1 protein level, conversely, was higher in mice with deletion of the β_3-adrenoreceptor gene. Expression of β_1-adrenoreceptors was increased in brown fat in knockout individuals. Experiments on isolated brown fat adipocytes showed that isoproterenol increased cAMP synthesis in these cells. The most marked effect of isoproterenol was obtained in normal wild-type cells [50]. Isoproterenol-induced cAMP synthesis in knockout adipocytes was not apparent in conditions of selective blockade of the β_1-adrenoreceptors by ICI-89406. The authors concluded that in mice with knockout of the β_3-adrenoreceptor gene, the key role in thermogenesis was played by β_1-adrenoreceptors. Unfortunately, the authors did not evaluate the survival of knockout mice for seven weeks at +4°C in conditions of chronic blockade of β_1-adrenoreceptors. Data reported by Ueta et al. [73] showed that intravenous infusion of noradrenaline or the β_1-adrenoreceptor agonist dobutamine increased the temperature of the interscapular brown fat in mice. Observations of mice with knockout of the β_1-adrenoreceptor gene showed that infusion of dobutamine had no effect on brown fat temperature in these animals and the calorigenic effect of noradrenaline decreased by a factor of four [73].

Thus, analysis of published data provides evidence that the calorigenic effect of catecholamines in rats is linked with activation of β_3-adrenoreceptors, while in mice it is associated with stimulation of β_1-adrenoreceptors and in humans with activation of β_3-adrenoreceptors and, to a lesser extent, occupation of β_1- and β_2-adrenoreceptors by endogenous catecholamines. Uncoupling proteins play an important role in the calorigenic effect of catecholamines.

Adaptation-related sensitization and desensitization of β-adrenoreceptors. Cold adaptation leads to changes in the properties of β-adrenoreceptors. Using the β antagonist ($\beta_2 > \beta_1 > \beta_3$) [^{3}H]dihydroalprenolol, a Japanese group evaluated the state of β-adrenoreceptors after cold acclimation [43]. These studies showed that prolonged cold exposure (four weeks) of the body decreased the affinity of β-adrenoreceptors for ligands in brown fat without altering the density of β-adrenoreceptors [41]. This is evidence for desensitization of β-adrenoreceptors, which is a typical result of receptor stimulation [1]. However, this result contradicted published data on an increase in the calorigenic effect of noradrenaline in adapted individuals. Directly contradictory results were obtained by a Canadian group [8]. Using [^{3}H] dihydroalprenolol, they found that cold adaptation leads to an increase in the density of β-adrenoreceptors on adipocyte membranes in brown fat tissue. Their studies established that cold acclimation leads to an increase in the sensitivity of adenylate cyclase in fat cells to noradrenaline and isoproterenol [8], which is evidence for receptor sensitization. These data indicate that the cold-induced calorigenic effect of catecholamines may result from sensitization of β-adrenoreceptors on cells in brown fat tissue. Like other investigators, Svartengren et al. [71] used [^{3}H]dihydroalprenolol. Their experiments showed that adaptation had no effect on either the density (B_{max}) of β-adrenoreceptors on the membranes of adipocytes in brown fat tissue or their affinity (K_d or K_i) for β-adrenoreceptor ligands. At the same time, half-maximal stimulation of adenylate cyclase in adipocytes from adapted individuals required a noradrenaline concentration 2.5 times that needed for the same effect in cells from intact rats. Half-maximal activation of respiration in adipocytes from adapted rats required a noradrenaline concentration five times greater than for cells from control animals [71]. The authors came to the conclusion that β-adrenoreceptors and the intracellular signal systems became uncoupled in

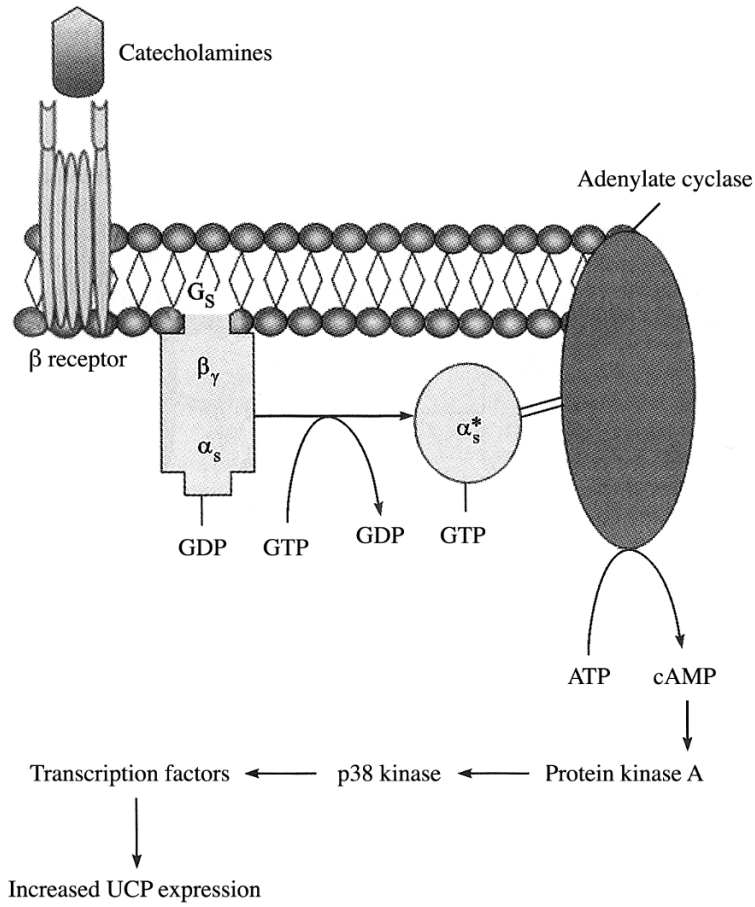

Fig. 1. Scheme showing intracellular signal pathways in prolonged exposure to stress. G_s protein, α_s subunit, and β_γ subunit of G_s protein.

brown fat adipocytes from cold-acclimated animals, leading to desensitization of β-adrenoreceptors in fat cells. As noted above, this contradicts published data showing increases in the calorigenic effect of catecholamines in adapted individuals. Using the same methodological approaches as these authors, Rothwell et al. [63] showed that cold acclimation leads to a decrease in the density of β-adrenoreceptors in brown fat cells, while the density of β-adrenoreceptor in cardiomyocytes remained unaltered. After adaptation, the affinity (K_d) of β-adrenoreceptors in adipocytes to selective β_1- and β_2-adrenoreceptor agonists did not change [63]. Unfortunately, the authors of this report did not assess the functional state of β-adrenoreceptors, so it remained unclear whether uncoupling of these receptors from the intracellular signal systems occurred on acclimation.

Nedergaard et al. [56] assessed the calorigenic effect of noradrenaline in terms of the respiration of isolated brown fat adipocytes. Cells from cold-adapted hamsters and rats were ten times less sensitive to noradrenaline than cells from control individuals. This points to desensitization of adipocyte adrenoreceptors and contradicts the data presented above on adaptive increases in the calorigenic effect of noradrenaline. These data were supported by a later study reported by Unelius et al. [74], which was also performed on isolated brown fat adipocytes from cold-adapted hamsters and intact animals. The calorigenic effect of catecholamines was assessed in terms of cell respiration. Adaptation was found to decrease the calorigenic effects of noradrenaline (an agonist of α- and β-adrenoreceptors) and isoproterenol (an agonist of β_1-, β_2-, and β_3-adrenoreceptors), the calorigenic effect of the β_3 agonists BRL-37344 and CGP-12177 being particularly marked. The authors tried to identify the level at which desensitization of the adrenergic response of adipocytes occurred. Cells were incubated in the presence of forskolin (an adenylate cyclase activator). Adaptation was found to lead to a significant decrease in the calorigenic effect of forskolin [74]. Experiments using the enzyme-resistance analog of cAMP, 8-Br-cAMP, showed that the calorigenic effect of this intracellular messenger decreased. As the cAMP receptor is protein kinase A [6], it can be suggested that the affinity of protein kinase A for cAMP within cells decreases or that the quantity of this enzyme decreases. The adaptation-related decrease in the calorigenic effect of β_3-adrenoreceptors was supported in experi-

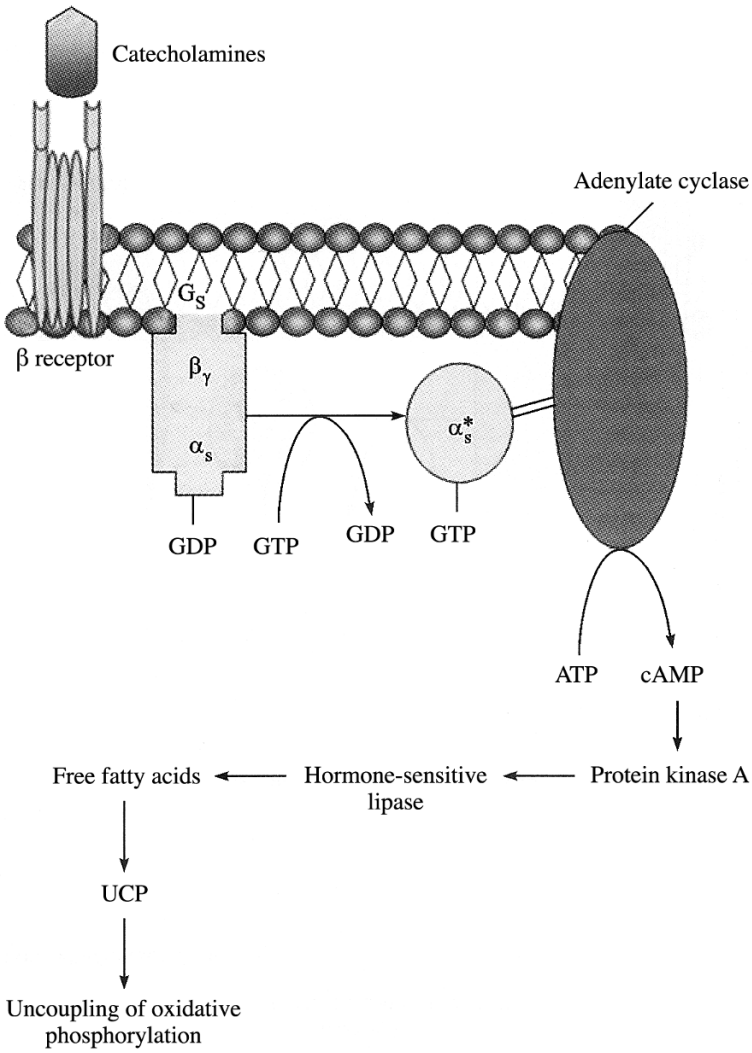

Fig. 2. Scheme showing intracellular signal pathways on acute exposure to cold. G_s protein, α_s subunit, and β_γ subunit of G_s protein.

ments on isolated brown fat adipocytes in later studies reported by a Swedish group [79].

Cold adaptation was followed by desensitization of brown fat adipocyte β-adrenoreceptors and uncoupling of β-adrenoreceptors from the intracellular signal systems in these cells. This contradicts published data on adaptation-related increases in the calorigenic effect of catecholamines and thus far remains unexplained.

The signal mechanism of the calorigenic effect of stimulation of β-adrenoreceptors. β-Adrenoreceptors are known to be linked with adenylate cyclase via G_s proteins [6], so there is clear interest in the studies of Granneman and Bannon [29], who found that the level of G_s proteins in brown fat tissue of cold-adapted rats was twice that in intact individuals. This is indirect evidence of an increase in the sensitivity of fat cells to hormones operating via activation of G_s proteins.

The calorigenic effect of stimulation of β-adrenoreceptors is linked with increases in cAMP synthesis. In fact, studies have demonstrated that treatment of rats with forskolin (an adenylate synthase activator) increase oxygen consumption [64].

Working from the data presented above, a chain of signal events can be constructed, as seen in experimental animals after prolonged cold stimulation of the SAS (Fig. 1): catecholamines → β-adrenoreceptors → G_s proteins → adenylate cyclase → cAMP → increased UCP expression. Before the report of Cao et al. [13], the intermediate steps between the increase in the cAMP level and the increase in the UCP level remained unknown. Data from these investigators showed that cells contain at least three signal mechanisms regulating cAMP-dependent UCP expression. The first signal pathway is: catecholamines → β-adrenoreceptors → adenylate cyclase → cAMP → protein kinase A →

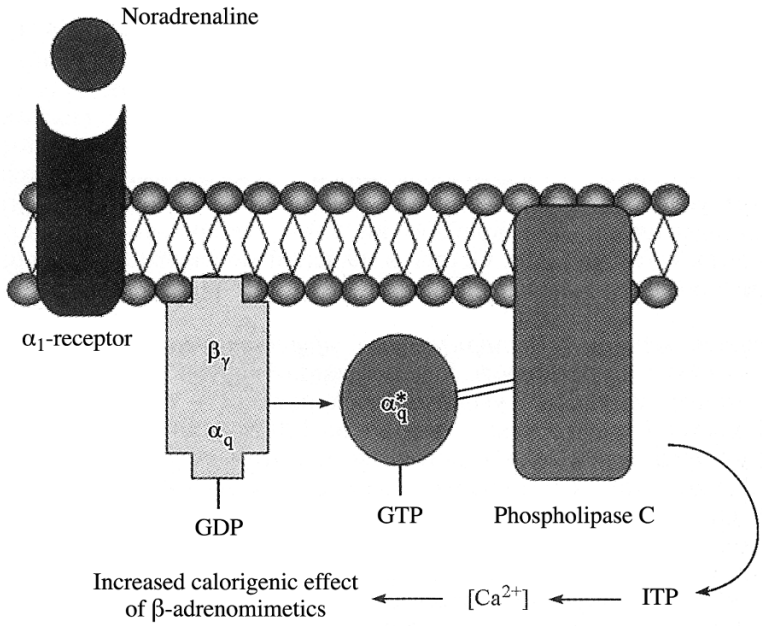

Fig. 3. Scheme showing intracellular signal pathways in the calorigenic effect. G_s protein, α_s subunit, and β_γ subunit of G_s protein.

CREB → increased UCP expression, where CREB (cAMP response element-binding protein) is a transcription factor interacting with the enhancer of the UCP gene. An enhancer is a short DNA segment able to bind transcription factors to increase the transcription of a gene or group of genes [3]. Phosphorylation of CREB by protein kinase A leads to an interaction between CREB and the enhancer of the UCP gene and an increase in the transcription of the UCP gene. The second signal pathway includes the following signal chain: catecholamines → β-adrenoreceptors → adenylate cyclase → cAMP → protein kinase A → p38 MAPK → ATF-2 → increased UCP expression, where ATF-2 is activating transcription factor 2 and MAPK is mitogen-activated protein kinase. p38 kinase phosphorylates ATF-2, such that it interacts with the enhancer of the UCP gene and increases UCP mRNA synthesis. The third signal pathway includes: catecholamines → β-adrenoreceptors → adenylate cyclase → protein kinase A → p38 MAPK → PGC-1-α → increased UCP expression, where PGC-1-α is the transcription factor peroxisome proliferator activator receptor-γ (PPAR-γ) coactivator 1-α (PGC-1-α). Phosphorylation of PGC-1-α leads to interaction of this transcription factor with the enhancer of the UCP gene and acceleration of the transcription of the UCP gene [13]. It should be noted that this proposed scheme for catecholamine-induced enhancement of UCP expression remains to be confirmed in independent experiments. In addition, this scheme does not explain the delayed calorigenic effect of catecholamines in adapted animals, when increases in oxygen consumption are seen within a few minutes of catecholamine treatment, excluding any increase in the quantity of UCP protein. Some investigators separate the views of Cao et al. regarding a mechanism for the chronic calorigenic effect [65], believing that the acute calorigenic effect of catecholamines is mediated by the following scheme (Fig. 2): catecholamines → β-adrenoreceptors → adenylate cyclase → cAMP → protein kinase A → hormone-sensitive lipase → FFA → UCP → uncoupling of oxidative phosphorylation. Although this mechanism of catecholamine-induced heat production has not been confirmed, it does have grounds as it is in good agreement with published data on the properties of UCP [5].

The role of α-adrenoreceptors in the calorigenic effect of catecholamines. The discussion above related to the role of β-adrenoreceptors in cold acclimation. Data on the role of α-adrenoreceptors in cold adaptation are quite sparse. The first study of this question was published in 1977 by Fregly et al. [22]. Cold-adapted rats were given the β-agonist isoproterenol. This was found to increase rectal temperature, while the α agonist phenylephrine (Mesaton) at a dose of 0.1 mg/kg had no effect on body temperature in adapted individuals. This is evidence that α-adrenoreceptors are not involved in cold-related thermogenesis. In 1994 Borst et al. reported experiments on nonadapted rats [10]. These showed that phenylephrine (3 or 10 mg/kg) produced a 3.5-fold increase in oxygen consumption. The β_3-adrenoreceptor agonist CGP 12177A had the same effect. The authors did not identify any synergism when these two agents were combined, though there was a tendency to an increase in the calorigenic effect in response to combined use of the two adrenomimetics. The α-adrenoreceptor agonist phenoxybenzamine eliminated the calorigenic effect of phenylephrine, while the β antagonist propranolol had no such effect. Thus, stimula-

tion of α-adrenoreceptors can increase oxygen consumption. Why are the results reported by Fregly et al. and Borst et al. so far apart? The reason would appear to lie in the doses used. Fregly et al. used phenylephrine at a dose of 0.1 mg/kg, while Borst et al. used a dose 30 times greater – 3 mg/kg.

There are even fewer reports on the role of α_2-adrenoreceptors in thermogenesis. A Spanish group established that treatment of intact rats with the α_2-adrenoreceptor antagonist yohimbine decreased body temperature by 1.5°C and blocked the calorigenic effect of the β_3 agonist trecadrine [27]. Analogous effects were noted in relation to oxygen consumption. In experiments on isolated white fat adipocytes, yohimbine had no effect on cell respiration but inhibited trecadrine-induced increases in cell respiration. Thus, stimulation of α_2-adrenoreceptors by endogenous catecholamines promotes increases in heat production. In addition, α_2-adrenoreceptors evidently take part in a synergistic interaction with β_3-adrenoreceptors in controlling cell respiration and thermogenesis. The molecular nature of this interaction remains unclear, as does the role of α_2-adrenoreceptors in cold acclimation.

The signal mechanism of the calorigenic effect of stimulation of α-adrenoreceptors. In 1984, Nedergaard's group observed that cold-adapted animals showed increases in the density of α_1-adrenoreceptors on the membranes of brown fat cells – by 100% in rats and by 40% in hamsters [58]. There was no change in the affinity of α_1-adrenoreceptors for ligand. These points provide evidence that α_1-adrenoreceptors take part in adaptation to cold. This same group subsequently established that adaptation of rats to cold leads to a twofold increase in the level of α_{1A}-adrenoreceptor mRNA in brown rat adipocytes, while the quantities of α_{1B}-adrenoreceptor mRNA and α_{1D}-adrenoreceptor mRNA decreased [39]. The increase in α_{1A}-adrenoreceptor expression evidently increased the common pool of α_1-adrenoreceptors in fat cells. Experiments on isolated hamster brown fat adipocytes reported by Zhao et al. [78] addressed the effects of adrenoreceptor stimulation on cell respiration and cAMP synthesis. The abilities of selective β_1-, β_2-, and β_3-adrenoreceptors and noradrenaline to stimulate cell respiration and cAMP synthesis were compared. The most marked increase in cAMP synthesis occurred when β_3-adrenoreceptors were occupied, while the greatest increase in oxygen consumption was obtained with the α- and β-adrenoreceptor agonist noradrenaline. Forskolin increased cAMP synthesis to a greater extent than noradrenaline, while noradrenaline produced the greatest stimulation of cell respiration. The selective α-adrenoreceptor agonist cirazoline had no effect on the cAMP level or oxygen consumption by fat cells. At the same time, cirazoline increased forskolin-stimulated adipocyte respiration [78]. α-Adrenoreceptors, operating via G_q proteins, are coupled with phospholipase C, which synthesizes diacylglycerol, which activates protein kinase C [6]. In addition, phospholipase C synthesizes inositol-1,4,5-triphosphate, which induces Ca^{2+} mobilization from the endoplasmic reticulum, leading to an increase in the intracellular calcium concentration $[Ca^{2+}]_i$ [6]. The protein kinase C inhibitor chelerythrine had no effect on the potentiating action of cirazoline. The calcium ionophore A23187 imitated the potentiating action of cirazoline on oxygen consumption by cells [78]. Thus, events in the cell develop as follows (Fig. 3): noradrenaline → α_1-adrenoreceptors → G_q protein →phospholipase C → inositol-1,4,5-triphosphate → $[Ca^{2+}]_i$ → increased calorigenic effect of β-adrenoreceptors. The mechanism of the Ca^{2+}-dependent potentiation of the calorigenic effect of β-adrenomimetics remains unknown. These data provide evidence of synergism between α_1- and β-adrenoreceptors in controlling thermogenesis in brown fat adipocytes. At the same time, these data diverge from the results reported by Borst et al. [10], who did not detect this synergism. It is possible that the cause of this is that the experiments of Zhao et al. were in vitro experiments on adipocytes, while Borst et al. performed in vivo experiments, such that the calorigenic effect involved not only adipocytes, but also other cells.

Conclusions

Thus, it has now been convincingly demonstrated that noradrenaline released from sympathetic nerve terminals and adrenaline secreted by the adrenal cortex play an important role in cold adaptation. Most data indicate that the calorigenic effect of noradrenaline increases during acclimation. Catecholamines stimulate thermogenesis in brown fat tissue and, in all probability, in white fat tissue and skeletal muscle. The adrenergic increase in cold tolerance is linked with increases in UCP expression in brown fat tissue, white fat tissue, and skeletal muscles. Data have been obtained showing that the following signal pathways have roles in mediating the calorigenic effect of catecholamines during prolonged exposure to cold: catecholamines → β-adrenoreceptors → adenylate cyclase, cAMP → protein kinase A → p38 kinase → transcription factors → increased UCP expression. Acute exposure to cold involves the signal pathway: catecholamines → β-adrenoreceptors → adenylate cyclase → cAMP → protein kinase A → hormone-sensitive lipase → free fatty acids → UCP → uncoupling of oxidative phosphorylation. The calorigenic effect of stimulation of α_1-adrenoreceptors is mediated by the following mechanism: noradrenaline → α_1-adrenergic receptors → phospholipase C → inositol-1,4,5-triphosphate → $[Ca^{2+}]_i$ → increased calorigenic effect of catecholamines. After cold adaptation, β-adrenoreceptors in brown fat adipocytes undergo desensitization and β-adrenoreceptors in the intracellular signal systems in these cells become uncoupled. This contradicts published data on adaptation-related increases in the calorigenic effect of catecholamines and remains in need of explanation.

This study was supported by the Russian Scientific Foundation (Grant No. 14-15-00008). The authors would like to thank S. Yu. Tsibul'nikov and N. A. Danil'chenko for technical assistance.

REFERENCES

1. G. R. Burn and D. M. Roberts, "Therapeutic receptors and pharmacodynamics," in: *Basic and Clinical Pharmacology* [Russian translation], B. G. Katzung (ed.), Binom Press, Nevskii Dialekt, Moscow, St. Petersburg (1998), pp. 22–52.
2. V. E. Divert, E. Ya. Tkachenko, and M. A. Yakimenko, "The thermoregulatory effect of blockade of β-adrenoreceptors in cold-adapted rats at rest and at work," *Fiziol. Zh. SSSR*, **71**, No. 6, 777–781 (1985).
3. W. S. Klug and M. R. Cummings, *Concepts of Genetics* [Russian translation], A. A. Lushnikova and S. M. Musatkin (eds.), Tekhnosfera, Moscow (2007).
4. V. V. Kurbanov, V. P. Khmel'kov, T. N. Krupina, et al., "Clinical biochemical aspects of adaptation of humans to the central Antarctic as applied to the tasks of space biology and medicine," *Kosmich. Biol. Aviakosmich. Med.*, **11**, No. 4, 34–41 (1977).
5. L. N. Maslov, Yu. B. Lishmanov, I. G. Khaliulin, et al., "Uncoupling proteins and their role in regulating the resistance of the brain and heart to ischemia and reperfusion," *Ros. Fiziol. Zh.*, **97**, No. 8, 761–780 (2011).
6. B. B. Hoffman, "Substances activating adrenoreceptors and other sympathomimetic substances," in: *Basic and Clinical Pharmacology* [Russian translation], B. G. Katzung (ed.), Binom Press, Nevskii Dialekt, Moscow, St. Petersburg (1998), pp. 154–174.
7. C. Atgie, F. D'Allaire, and L. J. Bukowiecki, "Role of β_1- and β_3-adrenoceptors in the regulation of lipolysis and thermogenesis in rat brown adipocytes," *Am. J. Physiol.*, **273**, No. 4, C1136–C1142 (1997).
8. N. Begin-Heick, I. Noland, M. Dalpé, and N. M. Heick, "Altered effect of insulin and catecholamines in brown adipose tissue of cold-acclimated rats," *Can. J. Physiol. Pharmacol.*, **57**, No. 3, 320–324 (1979).
9. E. E. Black, M. A. van Baak, K. P. Kempen, and W. H. Saris, "Role of α- and β-adrenoceptors in sympathetically mediated thermogenesis," *Am. J. Physiol.*, **264**, No. 1, E11–E17 (1993).
10. S. E. Borst, R. J. Oliver, R. L. Sego, and P. J. Scarpace, "α-Adrenergic receptor-mediated thermogenesis in brown adipose tissue of rat," *Gen. Pharmacol.*, **25**, No. 8, 1703–1710 (1994).
11. O. Boss, E. Bachman, A. Vidal-Puig, et al., "Role of the β_3-adrenergic receptor and/or a putative β_4-adrenergic receptor on the expression of uncoupling proteins and peroxisome proliferator-activated receptor-γ coactivator-1," *Biochem. Biophys. Res. Commun.*, **261**, No. 3, 870–876 (1999).
12. G. M. Budd, J. R. Brotherhood, D. W. Thomas, et al., "Cardiovascular and metabolic responses to noradrenaline in men acclimatized to cold baths," *Eur. J. Appl. Physiol. Occup. Physiol.*, **67**, No. 5, 450–456 (1993).
13. W. Cao, K. W. Daniel, J. Robidoux, et al., "p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene," *Mol. Cell. Biol.*, **24**, No. 7, 3057–3067 (2004).
14. S. Cinti, R. Cancello, M. C. Zingaretti, et al., "CL316,243 and cold stress induce heterogeneous expression of UCP1 mRNA and protein in rodent brown adipocytes," *J. Histochem. Cytochem.*, **50**, No. 1, 21–31 (2002).
15. S. A. Cunningham and D. G. Nicholls, "Induction of functional uncoupling protein in guinea pigs infused with noradrenaline. Studies with isolated brown adipocytes," *Biochem. J.*, **245**, No. 2, 485–491 (1987).
16. F. Depocas, W. A. Behrens, and D. O. Foster, "Noradrenaline-induced calorigenesis in warm- and in cold-acclimated rats: the interrelation of dose of noradrenaline, its concentration in arterial plasma, and calorigenic response," *Can. J. Physiol. Pharmacol.*, **56**, No. 2, 168–0174 (1978).
17. F. Depocas, G. Zaror-Behrens, and S. Lacelle, "Noradrenaline-induced calorigenesis in warm- or cold-acclimated rats. In vivo estimation of adrenoceptor concentration of noradrenaline effecting half-maximal response," *Can. J. Physiol. Pharmacol.*, **58**, No. 9, 1072–1077 (1980).
18. M. Desautels, R. A. Dulos, and B. Mozaffari, "Selective loss of uncoupling protein from mitochondria of surgically denervated brown adipose tissue of cold-acclimated mice," *Biochem. Cell. Biol.*, **64**, No. 11, 1125–1134 (1986).
19. A. Dicker, B. Cannon, and J. Nedergaard, "Cold acclimation-recruited nonshivering thermogenesis: the Syrian hamster is not an exception," *Am. J. Physiol.*, **269**, No. 4, R767–R774 (1995).
20. E. Evonuk and J. P. Hannon, "Cardiovascular function and norepinephrine thermogenesis in cold-acclimated rats," *Am. J. Physiol.*, **204**, No. 5, 888–894 (1963).
21. D. O. Foster, F. Depocas, and M. L. Frydman, "Noradrenaline-induced calorigenesis in warm-and cold-acclimated rats: relations between concentration of noradrenaline in arterial plasma, blood flow to differently located masses of brown adipose tissue, and calorigenic response," *Can. J. Physiol. Pharmacol.*, **58**, No. 8, 915–924 (1980).
22. M. J. Fregly, F. P. Field, E. L. Nelson, et al., "Effect of chronic exposure to cold on some responses to catecholamines," *J. Appl. Physiol.*, **42**, No. 3, 349–354 (1977).
23. M. J. Fregly, F. Rossi, P. Van Vergen, et al., "Effect of chronic treatment with losartan potassium (DuP 753) on the elevation of blood pressure during chronic exposure of rats to cold," *Pharmacology*, **46**, No. 4, 198–205 (1993).
24. K. Fukuhara, R. Kvetnansky, V. K. Weise, et al., "Effects of continuous and intermittent cold (SART) stress on sympathoadrenal system activity in rats," *J. Neuroendocrinol.*, **8**, No. 1, 65–72 (1996).
25. A. Gilgen, R. P. Maickel, O. Nikodijevic, and B. B. Brodie, "Essential role of catecholamines in the mobilization of free fatty acids and glucose after exposure to cold," *Life Sci.*, **1**, No. 12, 709–715 (1962).
26. J. Gómez-Ambrosi, G. Frühbeck, and J. A. Martínez, "Leptin, but not a β_3-adrenergic agonist, upregulates muscle uncoupling protein-3 messenger RNA expression: short-term thermogenic interactions," *Cell. Mol. Life Sci.*, **55**, No. 6–7, 992–997 (1999).
27. J. Gómez-Ambrosi, G. Frühbeck, M. Aguado, et al., "Divergent effects of an alpha2-adrenergic antagonist on lipolysis and thermogenesis: interactions with a beta3-adrenergic agonist in rats," *Int. J. Mol. Med.*, **8**, No. 1, 103–109 (2001).
28. D. W. Gong, Y. He, M. Karas, and M. Reitmen, "Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β_3-adrenergic agonists, and leptin," *J. Biol. Chem.*, **272**, No. 39, 24,129–24,132 (1997).
29. G. Granneman and M. J. Bannon, "Neural control of the α-subunit of G_s messenger ribonucleic acid in rat brown adipose tissue," *Endocrinology*, **125**, No. 5, 2328–2334 (1989).
30. J. P. Hannon and A. M. Larson, "Fatty acid metabolism during norepinephrine-induced thermogenesis in the cold-acclimatized rat," *Am. J. Physiol.*, **203**, No. 6, 1055–1061 (1962).
31. K. Harinath, A. S. Malhotra, K. Pal, et al., "Autonomic nervous system and adrenal response to cold in man at Antarctica," *Wilderness Environ. Med.*, **16**, No. 2, 81–91 (2005).
32. J. Himms-Hagen, "Brown adipose tissue thermogenesis: interdisciplinary studies," *FASEB J.*, **4**, No. 11, 2890–2898 (1990).
33. P. Huttunen, H. Rintamäki, and J. Hirvonen, "Effect of regular winter swimming on the activity of the sympathoadrenal system before and after a single cold water immersion," *Int. J. Circumpolar Health*, **60**, No. 3, 400–406 (2001).
34. L. Jansky, S. Vybíral, M. Trubacová, and J. Okrouhlik, "Modulation of adrenergic receptors and adrenergic functions in cold adapted humans," *Eur. J. Appl. Physiol.*, **104**, No. 2, 131–135 (2008).
35. M. Jimenez, B. Léger, K. Canola, et al., "$\beta_1/\beta_2/\beta_3$-Adrenoceptor knockout mice are obese and cold-sensitive but have normal lipolytic responses to fasting," *FEBS Lett.*, **530**, No. 1–3, 37–40 (2002).
36. R. J. Joy, "Responses of cold acclimatized men to infused norepinephrine," *J. Appl. Physiol.*, **18**, No. 6, 1209–1212 (1963).

37. R. Kawate, M. I. Talan, and B. T. Engel, "Sympathetic nervous activity to brown adipose tissue increases in cold-tolerant mice," *Physiol. Behav.*, **55**, No. 5, 921–925 (1994).
38. M. Kiang-Ulrich and S. M. Horvath, "Metabolic responses to tyramine and cold in young male Sprague–Dawley and Fischer 344 rats," *Am. J. Physiol.*, **246**, No. 2, Part 1, E141–E144 (1984).
39. K. Kikuchi-Utsumi, M. Kikuchi-Utsumi, B. Cannon, and J. Nedergaard, "Differential regulation of the expression of α_1-adrenergic receptor subtype genes in brown adipose tissue," *Biochem. J.*, **322**, No. 2, 417–424 (1997).
40. M. L. Kortelainen, P. Huttunen, and T. Lapinlampi, "Influence of two β-adrenoceptor antagonists, propranolol and pindolol, on cold adaptation in the rat," *Br. J. Pharmacol.*, **99**, No. 4, 673–678 (1990).
41. M. Kurahashi and A. Kuroshima, "Changes in adipocyte β-adrenergic receptor of cold-acclimated rats," *Jpn. J. Physiol.*, **29**, No. 1, 15–23 (1979).
42. A. Kuroshima, M. Kurahashi, and T. Yahata, "Calorigenic effects of noradrenaline and glucagon on white adipocytes in cold- and heat-acclimated rats," *Pflügers Arch.*, **381**, No. 2, 113–117 (1979).
43. A. Kuroshima and T. Yahata, "Thermogenic responses of brown adipocytes to noradrenaline and glucagon in heat-acclimated and cold-acclimated rats," *Jpn. J. Physiol.*, **29**, No. 6, 683–690 (1979).
44. A. Kuroshima, T. Yahata, and T. Ohno, "Effect of noradrenaline on plasma hormones and metabolites in cold-acclimated rats," *Jpn. J. Physiol.*, **38**, No. 2, 199–207 (1988).
45. J. LeBlanc and M. Pouliot, "Importance of noradrenaline in cold adaptation," *Am. J. Physiol.*, **207**, No. 4, 853–856 (1964).
46. J. LeBlanc, J. Vallières, and C. Vachon, "Beta-receptor sensitization by repeated injections of isoproterenol and by cold adaptation," *Am. J. Physiol.*, **222**, No. 4, 1043–1046 (1972).
47. J. LeBlanc, A. Labrie, D. Lupien, and D. Richard, "Catecholamines and triiodothyronine variations and the calorigenic response to norepinephrine in cold-adapted and exercise-trained rats," *Can. J. Physiol. Pharmacol.*, **60**, No. 6, 783–787 (1982).
48. J. Leduc and P. Rivest, "Effects of ablation of interscapular brown fat on cold acclimation in rats," *Rev. Can. Biol.*, **28**, No. 1, 49–66 (1969).
49. Y. L. Liu, S. Toubro, A. Astrup, and M. J. Stock, "Contribution of beta 3-adrenoceptor activation to ephedrine-induced thermogenesis in humans," *Int. J. Obes. Metab. Disord.*, **19**, No. 9, 678–685 (1995).
50. C. L. Mattsson, R. L. Csikasz, E. Chernogubova, et al., "β_1-Adrenergic receptors increase UCP1 in human MADS brown adipocytes and rescue cold-acclimated β_3-adrenergic receptor-knockout mice via nonshivering thermogenesis," *Am. J. Physiol. Endocrinol. Metab.*, **301**, No. 6, E1108–E1118 (2011).
51. K. Moriya, H. Maekubo, and T. Hiroshige, "Effects of norepinephrine on uptake and oxidation of plasma free fatty acids in cold-acclimated rats," *Jpn. J. Physiol.*, **27**, No. 5, 601–617 (1977).
52. J. D. Mulligan, A. A. Gonzalez, A. M. Stewart, et al., "Upregulation of AMPK during cold exposure occurs via distinct mechanisms in brown and white adipose tissue of the mouse," *J. Physiol.*, **580**, No. 2, 677–684 (2007).
53. I. Nagase, N. Sasaki, K. Tsukazaki, et al., "Hyperplasia of brown adipose tissue after chronic stimulation of β_3-adrenergic receptor in rats," *Jpn. J. Vet. Res.*, **42**, No. 3–4, 137–145 (1994).
54. I. Nagase, Y. Yoshida, K. Kumamoto, et al., "Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic β_3-adrenergic agonist," *J. Clin. Invest.*, **97**, No. 12, 2898–2904 (1996).
55. Y. Nakamura, I. Nagase, A. Asano, et al., "β_3-Adrenergic agonist up-regulates uncoupling proteins 2 and 3 in skeletal muscle of the mouse," *J. Vet. Med. Sci.*, **63**, No. 3, 309–314 (2001).
56. J. Nedergaard, "Catecholamine sensitivity in brown fat cells from cold-acclimated hamsters and rats," *Am. J. Physiol.*, **242**, No. 3, C250–C257 (1982).
57. P. Puigserver, D. Herron, M. Gianotti, et al., "Induction and degradation of the uncoupling protein thermogenin in brown adipocytes in vitro and in vivo. Evidence for a rapidly degradable pool," *Biochem. J.*, **284**, No. 2, 393–398, (1992).
58. A. Raasmaja, N. Mohell, and J. Nedergaard, "Increased β_1-adrenergic receptor density in brown adipose tissue of cold-acclimated rats and hamsters," *Eur. J. Pharmacol.*, **1–6**, No. 3, 489–498 (1984).
59. A. D. Rimmer, E. Schonbaum, and E. A. Sellers, "Effects of norepinephrine on blood glucose and free fatty acids in cold-adapted rats," *Am. J. Physiol.*, **203**, No. 1, 95–97 (1962).
60. L. Rochon and L. J. Bukowiecki, "Alterations in adipocyte response to lipolytic hormones during cold acclimation," *Am. J. Physiol.*, **258**, No. 5, C835–C840 (1990).
61. E. M. Rohlfs, K. W. Daniel, R. T. Premont, et al., "Regulation of the uncoupling protein gene (UCP) by β_1, β_2, and β_3-adrenergic receptor subtypes in immortalized brown adipose cell lines," *J. Biol. Chem.*, **270**, No. 18, 10,723–10,732 (1995).
62. N. J. Rothwell and M. J. Stock, "Effects of denervating brown adipose tissue on the responses to cold, hyperphagia and noradrenaline treatment in the rat," *J. Physiol.*, **355**, 457–463 (1984).
63. N. J. Rothwell, M. J. Stock, and D. K. Sudera, "Thermogenic responses to adrenoceptor agonists and brown fat adrenoceptors in overfed rats," *Eur. J. Pharmacol.*, **125**, No. 3, 313–323 (1986).
64. P. J. Scarpace and M. Matheny, "Thermogenesis in brown adipose tissue with age: post-receptor activation by forskolin," *Pflügers Arch.*, **431**, No. 3, 388–394 (1996).
65. H. Sell, Y. Deshaies, and D. Richard, "The brown adipocyte: update on its metabolic role," *Int. J. Biochem. Cell. Biol.*, **36**, No. 11, 2098–2104 (2004).
66. R. J. Sharara-Chami, M. Joachim, M. Mulcahey, et al., "Effect of epinephrine deficiency on cold tolerance and on brown adipose tissue," *Mol. Cell. Endocrinol.*, **328**, No. 2–4, 34–39 (2010).
67. M. Shiota and S. Masumi, "Effect of norepinephrine on consumption of oxygen in perfused skeletal muscle from cold-exposed rats," *Am. J. physiol.*, **254**, No. 4, E482–E489 (1988).
68. L. C. Smith and L. P. Dugal, "Excretion of catecholamines by white rats in the cold," *Can. J. Physiol. Pharmacol.*, **42**, No. 4, 567–577 (1964).
69. R. E. Smith and D. J. Hoijer, "Metabolism and cellular function in cold acclimation," *Physiol. Rev.*, **42**, No. 1, 60–142 (1962).
70. R. E. Smith and J. C. Roberts, "Thermogenesis of brown adipose tissue in cold-acclimated rats," *Am. J. Physiol.*, **206**, No. 1, 143–148 (1964).
71. J. Svartengren, P. Svoboda, and B. Cannon, "Desensitisation of β-adrenergic responsiveness in vivo. Decreased coupling between receptors and adenylate cyclase in isolated brown-fat cells," *Eur. J. Biochem.*, **128**, No. 2–3, 481–488 (1982).
72. R. F. Thomas, B. D. Holt, D. A. Schwinn, and S. B. Liggett, "Long-term agonist exposure induces upregulation of β_3-adrenergic receptor expression via multiple cAMP response elements," *Proc. Natl. Acad. Sci. USA*, **89**, No. 10, 4490–4494 (1992).
73. C. B. Ueta, G. W. Fernandes, L. P. Capelo, et al., "β_1-Adrenergic receptor is key to cold- and diet-induced thermogenesis in mice," *J. Endocrinol.*, **214**, No. 3, 359–365 (2012).
74. L. Unelius, G. Bronnikov, N. Mohell, and J. Nedergaard, "Physiological desensitization of β_3-adrenergic responses in brown fat cells: involvement of a postreceptor process," *Am. J. Physiol.*, **265**, No. 5, C1340–C1348 (1993).
75. C. van Hardeveld, M. Zuidwijk, and A. Kassenaar, "Involvement of adrenergic receptors in skeletal muscle metabolism of the cold-adapted rat," *Horm. Metab. Res.*, **12**, No. 6, 264–269 (1980).
76. S. L. Wijers, P. Schrauwen, M. A. van Baak, et al., "β-Adrenergic receptor blockade does not inhibit cold-induced thermogenesis in humans: possible involvement of brown adipose tissue," *J. Clin. Endocrinol. Metab.*, **96**, No. 4, E598–E605 (2011).

77. J. Zaagsma and S. R. Nahorski, "Is the adipocyte beta-adrenoceptor a prototype for the recently cloned atypical 'beta 3-adrenoceptor'?" *Trends Pharmacol. Sci.*, **11**, No. 1, 3–7 (1990).
78. J. Zhao, B. Cannon, and J. Nedergaard, "α_1-Adrenergic stimulation potentiates the thermogenic action of β_3-adrenoreceptor-generated cAMP in brown fat cells," *J. Biol. Chem.*, **272**, No. 52, 32847–32856 (1997).
79. J. Zhao, B. Cannon, and J. Nedergaard, "Thermogenesis is β_3- but not β_1-adrenergically mediated in rat brown fat cells, even after cold acclimation," *Am. J. Physiol.*, **275**, No. 6, R2002–R2011 (1998).
80. K. D. Zylan and H. J. Carlisle, "Effect of ambient temperature on the paradoxical metabolic responses to norepinephrine," *Pharmacol. Biochem. Behav.*, **43**, No. 2, 577–582 (1992).