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The effects of L-arginine and L-NAME supplementation on redox-regulation and thermogenesis in interscapular brown adipose tissue

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Summary

Changes in inducible nitric oxide synthase (iNOS) protein levels and its relationship with the hyperplasia and uncoupling protein 1 (UCP1) levels were examined in interscapular brown adipose tissue (IBAT) of adult rat males receiving L-arginine (L-Arg; 2.25%) or *N*-nitro-L-arginine methyl ester (L-NAME; 0.01%) as a drinking liquid and maintained at low (4±1°C) or room (22±1°C) temperature for 45 days.

Cold generally diminished both iNOS immunopositivity and protein level in IBAT, as well as the rate of apoptosis. Among groups acclimated to cold, higher iNOS immunopositivity and protein levels were detected only in the L-Arg-treated group. Furthermore, chronic L-Arg treatment increased IBAT mass and UCP1 protein content, while L-NAME had an opposite effect, decreasing both IBAT mass and UCP1 protein level, as compared to the control maintained at 4±1°C.

These data suggest that nitric oxide (NO) produced by iNOS could also contribute to overall NO-associated regulation of thermogenesis in IBAT. Namely, that iNOS, i.e. NO, in correlation with enhanced thermogenesis, additionally induced IBAT hyperplasia and UCP1 level compared to that induced by low temperature. Cooperative action of decreased apoptosis accompanied by increased tissue hyperplasia and UCP1 level, observed in IBAT of cold-acclimated rats, would be a way of meeting the metabolic requirements for increased thermogenesis.

Key words: interscapular brown adipose tissue, inducible nitric oxide synthase, nitric oxide, uncoupling protein 1, cold, apoptosis, MnSOD, glutathione, rat.

Introduction

Brown adipose tissue (BAT) represents the main site of non-shivering thermogenesis in mammals (Himms-Hagen, 1990). Thermogenesis in brown adipocytes is related to the presence of a specific mitochondrial protein, UCP1 (uncoupling protein 1; Nicholls and Locke, 1984). When mammals are chronically exposed to cold, the capacity of BAT non-shivering thermogenesis increases in association with tissue hyperplasia, accompanied by increased blood flow, mitochondriogenesis and pronounced UCP1 synthesis (Suter, 1969). Sympathetic innervation and released noradrenaline play an essential role in the triggering of thermogenic processes in BAT.

Nitric oxide (NO) is a gaseous messenger molecule implicated in numerous biological functions in both physiological and pathological conditions. Production of NO is catalyzed by three NO synthase (NOS; EC 1.14.13.39) isoforms (Förstermann et al., 1995). The endothelial and neuronal NOS (eNOS and nNOS, respectively) are regulated by second messengers and the third one, inducible NOS (iNOS) is inducible in a wide range of cells and tissues. It has been shown that brown adipocytes express the eNOS (Giordano et all., 2002) and iNOS (Nisoli et al., 1997) and that NO produced by these isoforms is involved in regulation of BAT function (Kikuchi-Utsumi et al., 2002; Nisoli et al., 1997, 2003). Recent findings justified the role of NO in BAT non-shivering thermogenesis (Saha and Kuroshima, 2000). It can mediate increased blood flow to BAT following noradrenergic stimulation (Nagashima et al., 1994; Kikuchi-Utsumi et al., 2002) and take part in differentiation and proliferation (Nisoli et al., 1998) in brown adipocyte cultures. An obligatory role of NO produced by eNOS in mitochondrial biogenesis has been established very recently (Nisoli et al., 2003, 2004; Clementi and Nisoli, 2005). However, the ability of iNOS, i.e. NO, to enhance interscapular BAT (IBAT) hyperplasia and UCP1 expression in IBAT of rats during cold acclimation has not so far been examined. This prompted us to examine iNOS level in relation to the IBAT hyperplasia and UCP1 protein level in IBAT of cold-acclimated rats receiving L-Arg or l-NAME as a drinking liquid for 45 days.

Materials and methods

Animals

Mill Hill hybrid hooded, 4-month-old rat *Rattus norvegicus* Berkenhout 1769 males were divided into three main groups. One group was receiving L-NAME-HCl (0.01%), an inhibitor of NOS and the second one L-Arg-HCl (2.25%), a NOS substrate, in tapwater for 45 days. At the doses used, L-NAME and L-Arg had no toxic effects (Saha et al., 1996). The third group served as a control. All three groups were additionally divided into two subgroups, one housed at 22±1°C and another kept in a cold room at 4±1°C. The rats were maintained in individual cages with food and water, i.e. drinking liquids *ad libitum*. Each experimental group consisted of six animals.

The animals were killed by decapitation, the IBAT dissected out within 3 min after death, minced and thoroughly rinsed with physiological saline to remove traces of blood. The tissue was homogenized at 0–4°C (using a Ka/Werke Ultra/Turrax homogenizer, Janke and Kunkel, Stenssen, Germany) in 0.25 mol l^{-1} sucrose, 0.1 mmol l^{-1} EDTA and 50 mmol l^{-1} Tris HCl buffer, pH 7.4. The homogenates were sonicated as suggested by Takada et al. (1982).

Activity of manganese superoxide dismutase (MnSOD; EC 1.15.1.1)

Superoxide dismutase (SOD) activity was examined by a modified method of Misra and Fridovich (1972). Total specific SOD and CuZnSOD activities after inhibition with 4 mmol l^{-1} KCN were measured and MnSOD activity calculated. Enzymatic activity was expressed in U mg^{-1} protein. SOD units were defined as the amount of the enzyme inhibiting epinephrine oxidation by 50% under the appropriate reaction conditions.

Determination of glutathione (GSH)

The content of glutathione (GSH) was examined in the tissue after deproteinization with sulfosalicylic acid. Total GSH was measured by enzyme-recycling assay after Griffith (1980) and expressed in nmol GSH g^{-1} tissue.

Immunohistochemistry

Immediately after dissection and washing, the IBAT samples were fixed in 10% formaldehyde at 4°C overnight and processed routinely for embedding in paraffin. 5 μm thick serial IBAT sections were deparaffinized and rehydrated. Immunoreactivity was assessed by the avidin–biotin–peroxidase method (following the manufacturer's instructions; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were incubated with 0.3% H_2O_2 in methanol for 10 min at ambient temperature to block endogenous peroxidase, followed by three 5 min washes in 0.015 mol l^{-1} phosphate-buffered saline (PBS; pH 7.4) and incubated with 1.5% normal goat serum (ABC Staining System, Santa Cruz Biotechnology) in PBS for 60 min at ambient temperature to block non-specific sites. The primary antibody against iNOS (Santa Cruz Biotechnology) was a polyclonal antibody produced in rabbits. The sections were incubated with the primary antibody in PBS (diluted 1:200 v/v) overnight at 4°C, followed by two 5 min PBS washes, incubated with 1:200 IgG biotinylated serum goat anti-rabbit (ABC Staining System, Santa Cruz Biotechnology) in PBS for 60 min at ambient temperature, followed by two 5 min PBS washes. After that, AB reagent (ABC Staining System, Santa Cruz Biotechnology) was added for 30 min at ambient temperature, followed by three 5 min PBS washes and incubation with 0.02% H_2O_2 and 0.075% diaminobenzidine (Sigma) in 0.05 mol l^{-1} Tris buffer, pH 7.6, for 10 min in a dark room. Rinsing in distilled water and counterstaining with Hematoxylin completed the experimental schedule. Negative controls were prepared by omitting the primary antibody.

SDS-PAGE and western blotting

For UCP1 and iNOS analyses by western blotting, proteins were dissolved according to Laemmli (1970) and 10 μg protein samples boiled and electrophoresed in 15% and 7.5% SDS-polyacrylamide gel for UCP1 and iNOS analysis, respectively. After that proteins were transferred onto nitrocellulose membranes. After a brief rinse in TBS (200 mmol l^{-1} Tris, 1.5 mol l^{-1} NaCl, pH 7.4), the membranes were incubated in blocking serum (TBS containing 5% BSA) for 1 h at ambient temperature to block the unbound sites. The membranes were further incubated with rabbit polyclonal antibody against UCP1 (Sigma-Aldrich Inc., St Louis, MO, USA) and with rabbit polyclonal antibody against iNOS (Chemicon International Inc., Temecula, CA, USA). The incubation with primary antibodies was performed in TBS-T (0.2% Triton X-100 in TBS) with 5% BSA at 1:1000 v/v with antibody against UCP1 and primary antibody against iNOS, as recommended by the manufacturer (i.e. 5 μl ml^{-1}), overnight in a cold room. After multiple washes in TBS-T the membranes were incubated with horseradish peroxidase-conjugated IgG secondary antibody (Santa Cruz Biotechnology) at 1:2000 v/v. For UCP1 detection, peroxidase activity was revealed using 4-chloro-1-naphthol and H_2O_2 as a substrate. For iNOS detection, the membrane was covered by luminol-based chemiluminescent substrate for 3 min. Immediately after, a piece of X-ray Hyperfilm MP (Amersham API, Indianapolis, IN, USA) was placed over the blot and exposed for 1 min. The film than was developed, scanned and quantitative analysis of immunoreactive bands was done by an ImageQaunt software (Piscataway, NJ, USA). Volume is the sum of all the pixel intensities within a band; 1 pixel=0.007744 mm^2.

Detection of apoptosis

IBAT sections 5 μm thick were used for immunohistochemical detection of apoptosis by TUNEL labeling of the nuclei showing specific oligonucleotide sequences resulting from DNA strand breaks. Staining was performed with the In Situ Cell Death Detection Kit POD (Boehringer Mannheim, Germany) according to the manufacturer's instructions.

Protein content was estimated by the method of Lowry et al. (1951).

Student's *t*-test was used for data comparison between different groups according to Hoel (1966). The $P<0.05$ level was chosen as the point of minimal acceptable statistical significance.

Results

The results of immunohistochemically located iNOS are shown in Fig. 1. In animals maintained at ambient temperature, brown adipocytes showed cytoplasmic staining with iNOS antibodies. The reaction product was also detected in plasma

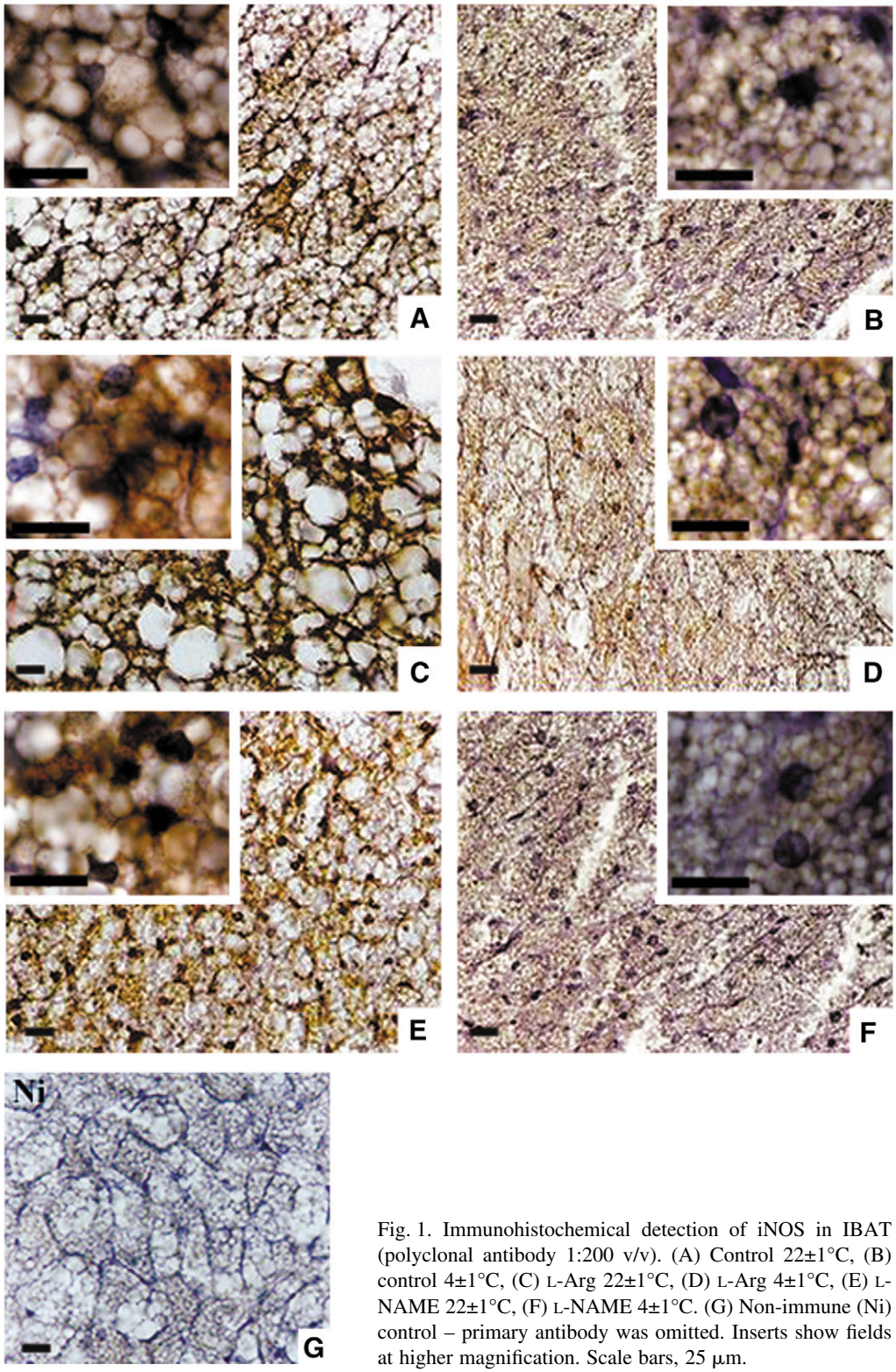

Fig. 1. Immunohistochemical detection of iNOS in IBAT (polyclonal antibody 1:200 v/v). (A) Control 22±1°C, (B) control 4±1°C, (C) L-Arg 22±1°C, (D) L-Arg 4±1°C, (E) L-NAME 22±1°C, (F) L-NAME 4±1°C. (G) Non-immune (Ni) control – primary antibody was omitted. Inserts show fields at higher magnification. Scale bars, 25 μm.

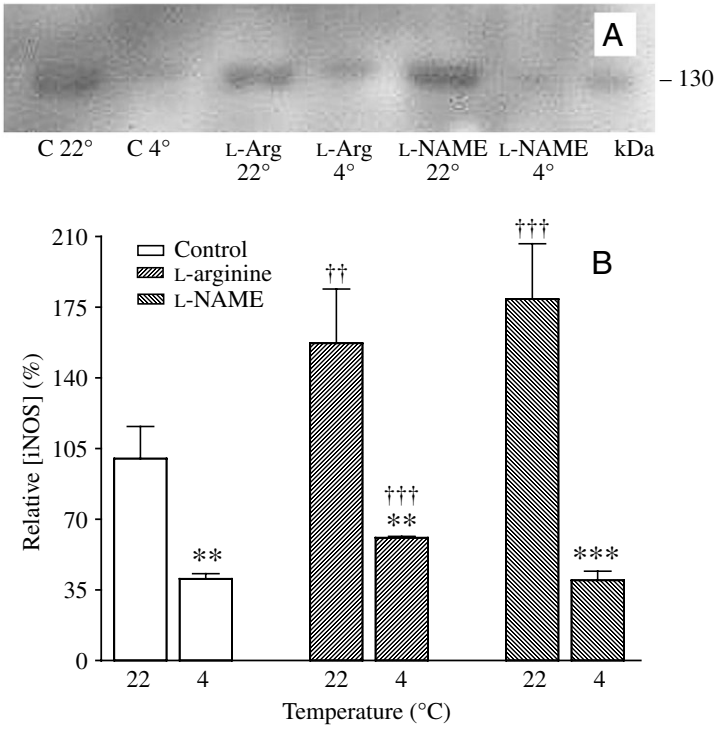

Fig. 2. The effects of L-Arg and L-NAME supplementation and different temperatures (±1°C) on the iNOS protein level in IBAT. (A) Western blotting was performed using specific antibody against iNOS. The results of three observations from a representative experiment and the position of molecular mass markers (kDa) are shown. C, control. (B) Data obtained after quantification of iNOS bands by ImageQuant software. iNOS concentration is expressed in relative to the levels in controls acclimated to room temperature (taken as 100%). The values represent the means ± S.E.M. from three independent experiments. *Comparison of the same treatments at different temperatures, $^{**}P<0.025$; $^{***}P<0.005$; †comparison of different treatments with the control kept at the same temperature, $^{\dagger\dagger}P<0.025$; $^{\dagger\dagger\dagger}P<0.005$. Volume is the sum of all the pixel intensities within a band; 1 pixel=0.007744 mm^2.

membranes (Fig. 1A). In rats kept at low temperature (Fig. 1B) brown adipocytes were weakly stained with iNOS antibodies. Immunopositivity was localized both in the plasma membrane and the cytoplasm of brown adipocytes.

L-Arg treatment additionally emphasized immunopositivity with iNOS antibodies as compared to the appropriate controls. In animals acclimated to ambient temperature, brown adipocytes were stained more intensely and diffusely compared to the control group acclimated to the same temperature; Fig. 1C). Immunopositivity was strong in the cytoplasm and some adipocytes also exhibited nuclear staining. In L-Arg-treated rats maintained at low temperature (Fig. 1D), a stronger immunopositivity was detected than in either the control or L-NAME-treated group. L-Arg-treated group acclimated to low temperature, was the only one among all groups kept in cold with somewhat higher iNOS immunopositivity. However, immunopositivity of this group was lower (Fig. 1D) in comparison with that observed in animals receiving L-Arg but acclimated to ambient temperature (Fig. 1C).

In L-NAME-treated rats kept at ambient temperature, a massive dislocation of iNOS immunopositivity to the brown adipocyte nuclei was observed (Fig. 1E). The reaction product was detected both in plasma membranes and the cytoplasm. The same treatment of the animals acclimated to cold revealed a rather poor staining of the cytoplasm and plasma membranes, while no nuclear staining with iNOS antibodies was detected (Fig. 1F).

The iNOS immunoblot (Fig. 2A) confirmed the immunohistochemical results, showing very faint bands in all groups acclimated to cold but strong bands in the groups kept at ambient temperature. Nevertheless, among cold-

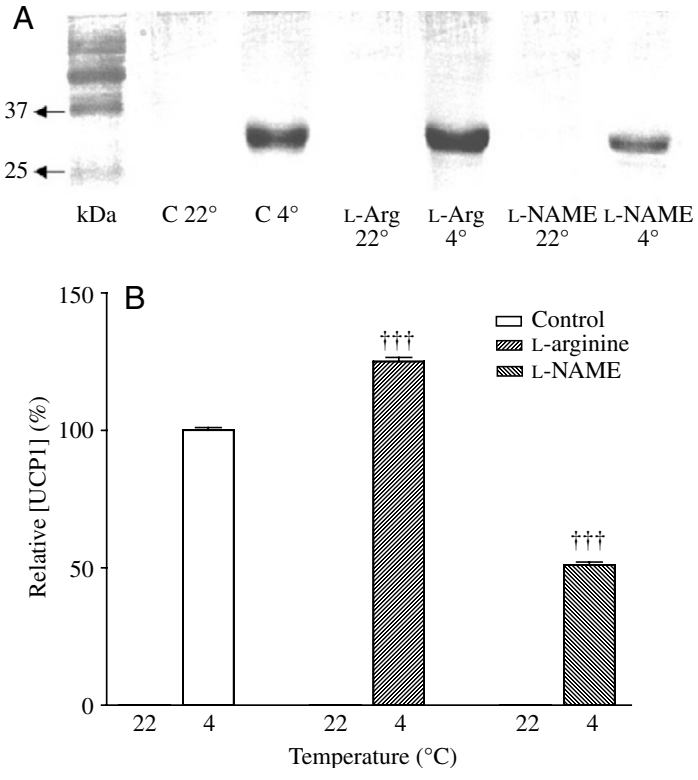

Fig. 3. The effects of L-Arg and L-NAME supplementation and different temperatures on UCP1 protein level in IBAT. (A) Western blotting was performed using specific antibody for UCP1. The results of three observations from a representative experiment and the position of molecular mass markers (kDa) are shown. C, control. (B) Data obtained after quantification of UCP1 bands by ImageQuant software. Relative UCP1 concentration was expressed in relation to the control cold-acclimated group taken as 100%. The values represent the means ± S.E.M. from three independent experiments. †Comparison of different treatments with the control kept at the same temperature, $^{\dagger\dagger\dagger}P<0.005$. Volume is the sum of all pixel intensities within a band; 1 pixel=0.007744 mm^2.

Table 1. *Changes of IBAT mass and protein content*

Group	Control		L-Arg		L-NAME	
	22±1°C	4±1°C	22±1°C	4±1°C	22±1°C	4±1°C
IBAT mass (mg)	224±24	729±27***	192±6	802±55***	225±25	600±28***,†††
Protein content (mg g^{-1} tissue)	1.05±0.08	3.13±0.09***	1.22±0.06	2.64±0.10***,†	1.23±0.12	2.85±0.09***,†

*Comparison of the same treatments at different temperatures, ***P<0.005; †comparison of different treatments with the control kept at the same temperature, †P<0.05, †††P<0.005.

acclimated groups, the strongest band was detected in the L-Arg-treated group. Also, the bands in both L-Arg-treated and L-NAME-treated groups maintained at ambient temperature were stronger than in the corresponding control. Quantification of iNOS bands by ImageQuant software (Fig. 2B) revealed that the iNOS protein level of cold-acclimated rats was significantly higher in L-Arg-treated groups than in the control (P<0.005), while the level of this protein in L-NAME-treated rats was decreased, but the difference was not statistically significant (Fig. 2B). In IBAT of both L-Arg- and L-NAME-treated rats maintained at room temperature, iNOS level was significantly higher than in the corresponding controls (P<0.025 and P<0.005, respectively).

The results of IBAT mass and protein concentration in different groups of animals are summarized in Table 1. It can be seen that IBAT mass, as well as protein content, were increased (P<0.005) in all groups exposed to low temperature in comparison with the corresponding controls kept at room temperature. The IBAT mass showed a rising trend in L-Arg-treated group acclimated to low temperature, whereas that of L-NAME-treated group acclimated to low temperature was significantly decreased (P<0.005) in comparison with the controls acclimated to low temperature.

Western blot analyses revealed that UCP1 occurred only in IBAT of rats acclimated to low temperature (Fig. 3A). Quantification of UCP1 bands by ImageQuant software showed a significantly higher UCP1 level in L-Arg-treated rats than in the control (P<0.005), while the level of this protein was significantly decreased in L-NAME-treated rats compared to the control (P<0.005; Fig. 3B). No UCP1 was detected in IBAT of animals of any group maintained at room temperature.

As evident from Fig. 4, MnSOD activity in IBAT of all cold-acclimated groups was significantly decreased in comparison with the corresponding controls acclimated to room temperature (P<0.005). Also, in rats acclimated to room temperature, MnSOD activity in both L-Arg- and L-NAME-treated groups was significantly decreased (P<0.025, P<0.05, respectively), as compared to the control acclimated to room temperature.

The changes in GSH content in IBAT are depicted in Fig. 5. As seen, GSH content in IBAT was significantly higher in all animals acclimated to low temperature in comparison with the corresponding controls kept at room temperature with different statistical significance. Among groups acclimated to cold, GSH content was significantly decreased in L-Arg-treated rats (P<0.025), but significantly increased in L-NAME-treated cold-exposed animals in comparison with the control acclimated to low temperature (P<0.025).

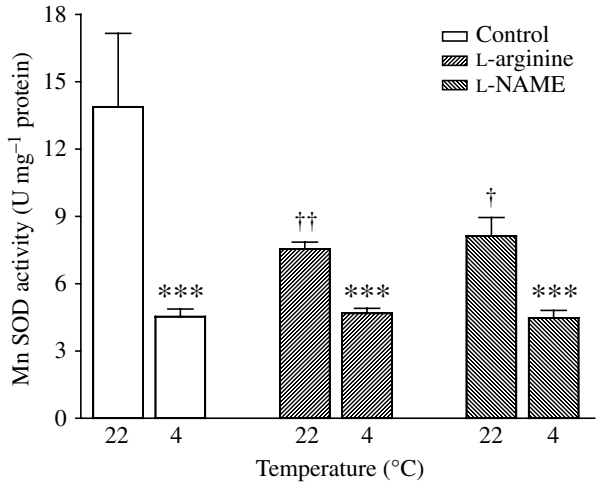

Fig. 4. MnSOD activity in the IBAT. *Comparison of the same treatments at different temperatures, ***P<0.005; †comparison of different treatments with the control maintained at the same temperature, †P<0.05; ††P<0.025.

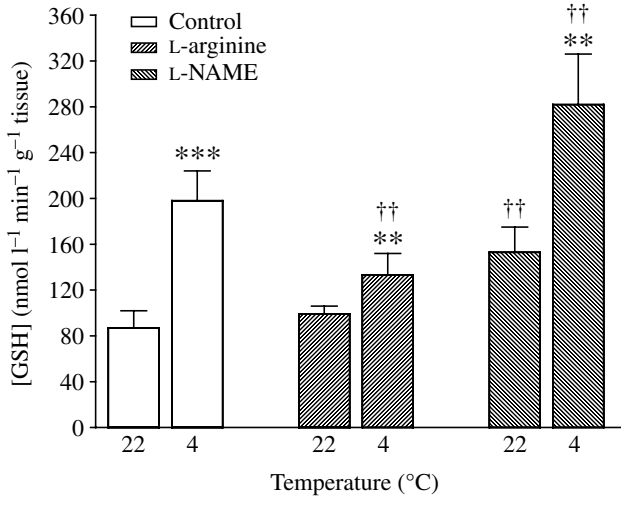

Fig. 5. Glutathione (GSH) content in the IBAT. *Comparison of the same treatments at different temperatures, **P<0.025; ***P<0.005; †comparison of different treatments with the control acclimated to the same temperature, ††P<0.025.

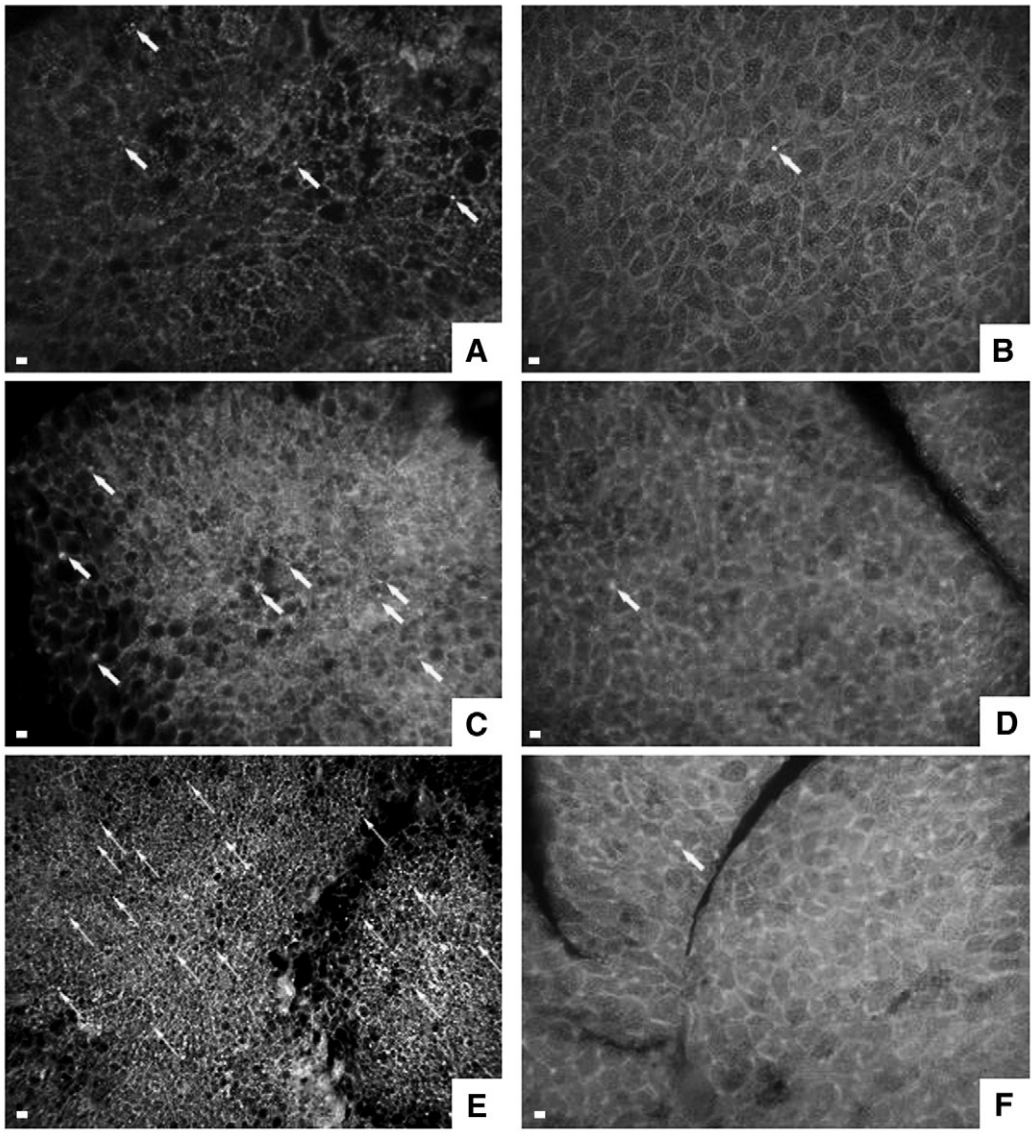

Fig. 6. IBAT TUNEL staining. (A) Control, 22±1°C, (B) control, 4±1°C, (C) L-Arg, 22±1°C, (D) L-Arg, 4±1°C, (E) L-NAME, 22±1°C, (F) L-NAME, 4±1°C. Arrows indicate apoptotic nuclei (see text for details). Scale bars, 20 μm.

The results on TUNEL detection of apoptosis in IBAT are presented in Fig. 6. The number of apoptotic (TUNEL-positive) nuclei was significantly decreased in all groups of rats acclimated to low temperature, as compared to the corresponding groups maintained at room temperature. In the rats acclimated to room temperature, TUNEL positivity in both treated groups (L-Arg, Fig. 6C and L-NAME, Fig. 6E) was significantly increased in comparison with the corresponding controls kept at room temperature (Fig. 6A). The highest number of TUNEL-positive nuclei was detected in L-NAME-treated rats acclimated to room temperature (Fig. 6E).

Discussion

In the present work, cold-acclimated adult rat males were chronically treated with L-Arg and L-NAME in order to study the role of iNOS in IBAT hyperplasia and UCP1 protein level in this tissue.

Our results show that iNOS isoform is expressed in IBAT and that long-term exposure to cold led to a decrease in the level of iNOS protein. Chronic treatment with L-Arg of animals exposed to cold resulted in an increased iNOS level and immunopositivity compared to the control acclimated to cold, while L-NAME had an opposite effect, i.e. it caused decreased iNOS levels and immunopositivity as compared to cold-acclimated control.

We also show that the IBAT mass was significantly increased in animals acclimated to cold. This is in accordance with the data of the others (Bukowiecki et al., 1986; Puerta et al., 1990), as well as with our earlier results (Buzadžić et al., 1999), demonstrating that IBAT of cold-acclimated rats undergoes hyperplastic changes. The IBAT mass was

increasing in L-Arg-treated groups acclimated to low temperature compared to the corresponding controls, and significantly decreased in L-NAME-treated groups acclimated to cold in comparison with the cold-acclimated controls. This agrees well with the results of Saha et al. (1996), who found that L-NAME administered in drinking water for 4–6 weeks caused decreased IBAT mass. In accordance with previous observations, we presumed that NO additionally intensified the cold-induced increase of IBAT mass. This is in accordance with Nisoli et al. (1998) and Kikuchi-Utsumi (2002), who reported that NO (exogenous or generated by constitutive eNOS isoforms) plays a significant role in brown adipocytes.

As an integral part of IBAT hyperplasia and in correlation with an enhanced thermogenesis, the UCP1 level was increased upon exposure to cold. Accordingly, we observed that UCP1 was expressed only in IBAT of rats acclimated to low temperature, in agreement with the results of Ricquier et al. (1983). Moreover, we showed that UCP1 level in L-Arg-treated rats acclimated to cold was significantly higher than in the corresponding control. In contrast, the UCP1 level was significantly decreased in L-NAME-treated animals acclimated to low temperature compared to the control kept at the same temperature. These results suggest that NO additionally induced UCP1, compared to induction of UCP1 by cold. To our knowledge, this is the first evidence for *in vivo* regulation of UCP1 expression by NO. Nisoli et al. (1998) observed that NO increased UCP1 expression of cultured brown adipocytes. It is known that NO is capable of interacting with many cellular targets including oxygen, superoxide anion radical ($O_2^{\cdot-}$), thiols, and particularly with reduced glutathione (GSH; Beckman and Koppenol, 1996). We assumed that the effect of NO on UCP1 expression could be mediated by $O_2^{\cdot-}$ or GSH, but it does not seem to be due to its interaction with $O_2^{\cdot-}$. In fact, it is known that uncoupling induced by thermogenesis acts to decrease $O_2^{\cdot-}$ production (Boveris et al., 1972; Cino and Del Maestro, 1989; Skulachev, 1994). The specificity of SODs, essential $O_2^{\cdot-}$ scavengers (Fridovich, 1978), for the reaction with $O_2^{\cdot-}$ has frequently been used to probe for the involvement of this radical in biological systems. Our previous studies (Petrović et al., 1989) along with the findings of the others (Koch et al., 1994; Perera et al., 1995) showed that MnSOD is easily induced by $O_2^{\cdot-}$ and different agents, and more inducible than CuZnSOD. Accordingly, to check the hypothesis that the effect of NO on UCP1 level was mediated by $O_2^{\cdot-}$, we determined MnSOD activity. MnSOD activity was significantly decreased in rats acclimated to cold, possibly representing an adaptive response due to long-term reduced production of $O_2^{\cdot-}$ in IBAT mitochondria, as a consequence of UCP1 induction and uncoupling respiration from phosphorylation, which is the main prerequisite of IBAT thermogenesis. Taken together, these results suggest that in the regulation of UCP1 expression by NO, reaction with $O_2^{\cdot-}$ is not the main pathway. Decreased level of GSH in L-Arg-treated rats maintained at low temperature compared to the control acclimated to the same temperature, suggests that another interaction of GSH with NO took place.

Reduced glutathione (GSH) represents the most important non-enzymatic intracellular antioxidant, the major function of which is to maintain cellular homeostasis. Several authors have suggested that GSH is the prime thiol-containing target for NO in a cell (Kröncke et al., 1998; Reichenbach et al., 2001), leading to formation of nitrosoglutathione (GSNO), which in turn has been shown to enhance expression of the genes involved in differentiation of brown adipocytes (Nisoli et al., 1998). Moreover, Gaudiot et al. (1998, 2000) showed that GSNO acting as a NO donor increases basal lipolysis in white fat cells, thus increasing the concentration of free fatty acids, which are the main fuel for IBAT thermogenesis (Trayhurn, 1979; Bukowiecki et al., 1981) and are also known to activate UCP1 (Skulachev, 1991; Winkler and Klingerbeg, 1994). Since NO action on gene expression has been well documented (Peunova and Enikolopov, 1995; Hobbs, 1997), it may be assumed that NO induces the UCP1 gene immediately. However, the interrelationship of the UCP1 level and NO, i.e. the ability of NO to stimulate UCP1 expression in IBAT mitochondria, requires further study.

We have also shown that long-term exposure of rats to cold diminished iNOS level, as well as iNOS immunopositivity, accompanied by a rapid decrease in the rate of apoptosis in IBAT. The results of Lindquist and Rehnmark (1998) together with our recent data (Korać et al., 2000) demonstrated decreased apoptosis in cold-adapted rats and a significant increase of this process during re-acclimation. Thus, we could hypothesize that the regulation of cell survival is a process involved in cold-induced IBAT hyperplasia, i.e. a decreased extent of apoptosis, observed in cold-maintained rats, could be an additional adaptive change that contributes to enhanced thermogenic capacity. Changes in both iNOS expression and intensity of apoptosis in IBAT were parallel in rats kept at room temperature. In these animals, a marked increase in both the iNOS level and immunopositivity and the rate of apoptosis were detected in both L-Arg- and L-NAME-treated rats compared to the corresponding control. Thus, we suggest that iNOS may be involved in induction of apoptosis in IBAT.

Generally, NO can induce cell death by apoptosis in a variety of different cell types (López-Farré et al., 1996; Messmer et al., 1996). There are various biochemical and cellular mechanisms underlying NO-induced apoptosis, e.g. by inhibiting cytochrome oxidase (Brown, 2001; Cleeterr et al., 1994), stimulating production of reactive oxygen species (ROS) and reactive nitrogen species (RNS; Brown and Borutaite, 2002), peroxynitrite ($ONOO^-$) formation (Foresti and Motterlini, 1999). Also, changes in NO concentration in cells act by shifting cellular redox potential and turning on or off redox-sensitive genes involved in proapoptotic/antiapoptotic signal pathways, respectively (Kröncke et al., 2001). However, further studies along these lines are necessary to determine possible role of NO in inducing of apoptosis in IBAT.

Our results presented here suggest that NO produced by iNOS could also contribute to overall NO-associated regulation of thermogenesis in IBAT, by enhancing the activity

and capacity of IBAT for non-shivering thermogenesis by increasing both the IBAT mass and UCP1 level. Moreover, the cooperative action of decreased apoptosis accompanied by increased tissue hyperplasia and UCP1 level, observed in IBAT of cold-acclimated rats, would be a way to meet the metabolic requirements for increased thermogenesis.

List of abbreviations

BAT	brown adipose tissue
eNOS	endothelial NOS
GSH	glutathione
IBAT	interscapular brown adipose tissue
iNOS	inducible nitric oxide synthase
L-Arg	L-arginine
L-NAME	*N*-nitro-L-arginine methyl ester
GSNO	nitrosoglutathione
nNOS	neuronal NOS
NO	nitric oxide
PBS	phosphate-buffered saline
ROS	reactive oxygen species
RNS	reactive nitrogen species
SOD	superoxide dismutase
UCP1	uncoupling protein 1

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