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LETTER TO THE EDITOR

Alterations in L-arginine-nitric oxide-producing pathway affect antioxidative defense in the rat skin

KEYWORDS
Skin; Antioxidative defense; Nitric oxide; Glutathione

Skin is one of the most exposed tissue to oxidative preasure both from exogenous factors due to its permanent contact with oxygen and from endogenous factors, as to its high metabolic activity [1]. Thus, skin developed diverse protective mechanisms such as melanogenesis, epiderm stratification and antioxidative defense (AD) aimed to the redox homeostasis maintenance [2]. AD is tissue specific and consists of enzymatic-superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione *S*-transferase (GST), thioredoxine reductase (TR) and low-molecular mass components—glutathione (GSH) and vitamins C and E [3]. Organization of skin AD depends on the cell type (keratinocytes, melanocytes, Langerhans cells, fibroblasts, etc.) [4] and corresponds to redox equilibrium changes occuring in aging, psoriasis, thermal injuries, malignancies, etc. [3].

Otherwise, numerous data demonstrate that nitric oxide ($^{\bullet}NO$) plays an important role in the orchestration of skin responses to external stimuli, and in the regulation of physiological and pathological processes [5]. Production of $^{\bullet}NO$ in skin is catalysed by all three izoforms of $^{\bullet}NO$ synthases (NOS)—inducible, endothelial and neuronal. $^{\bullet}NO$ bioactivity mostly depends on the interaction with many cellular targets, superoxide anion radical ($O_2^{-\bullet}$) and GSH, first [6]. Intracellular $O_2^{-\bullet}$ concentration is determined by the extent of its production, but also by its removal by the action of SODs, especially CuZnSOD [7]. Thus, specificities of organization and activity of the AD directly modulate $^{\bullet}NO$ bioactivity and thereby its effects [7]. We have shown previously in brown adipose tissue (BAT) that this interaction depends on functional status of the tissue [6].

Cold-exposure triggers strong physiological response that alludes upregulation in metabolic rate, especially in metabolically active tissue, such as BAT, liver, skeletal and cardiac muscle, small intestine. We have previously shown that increase in interscapular BAT metabolic activity causes alterations in reactive oxygen species production, which is accompanied with adequate changes in AD [6]. Considering that skin, as major sensory tissue, plays an essential part in thermoregulation, we were aimed here to investigate weather alterations in L-arginine-$^{\bullet}NO$-producing pathway affect skin AD in room temperature- and cold-acclimated animals.

The Institute's ethical committee on animal experiments approved the protocol. Males Mill Hill hybrid hooded, 4-month-old rat were divided into three main groups (consisted of six animals). One group received L-arginine·HCl (2.25%) (a NOSs substrate), another N^{ω}-nitro-L-arginine methyl ester (L-NAME·HCl, 0.01%) (a NOSs inhibitor), in drinking water for 45 days. The third group was control. All groups were divided additionally into two subgroups, housed at 22 ± 1 and 4 ± 1 °C. The rats were maintained in individual cages with food and drinking liquids *ad libitum*.

The animals were sacrificed by decapitation, and skin was dissected within 3 min after death. Only white parts from the dorsal side were taken after cutting the hair with an electric clipper. Hypodermis was removed with a scalpel and dermis with epidermis was homogenized, thereafter sonicated and centrifuged ($105{,}000 \times g$, 90 min) [3]. Supernatants were used for determination of AD enzymes activities (MnSOD, CuZnSOD, CAT, GSH-Px, GR, GST and TR) and GSH amount, as done previously [6,3].

Enzymatic activities of MnSOD, GR and TR showed no statistically significant differences between the examined groups (Table 1). However, L-arginine treatment increased activity of CuZnSOD in both room and low temperature-acclimated rats compared both to corresponding controls and L-NAME-treated rats. CAT activity was increased in L-arginine-treated animals compared to the correspond-

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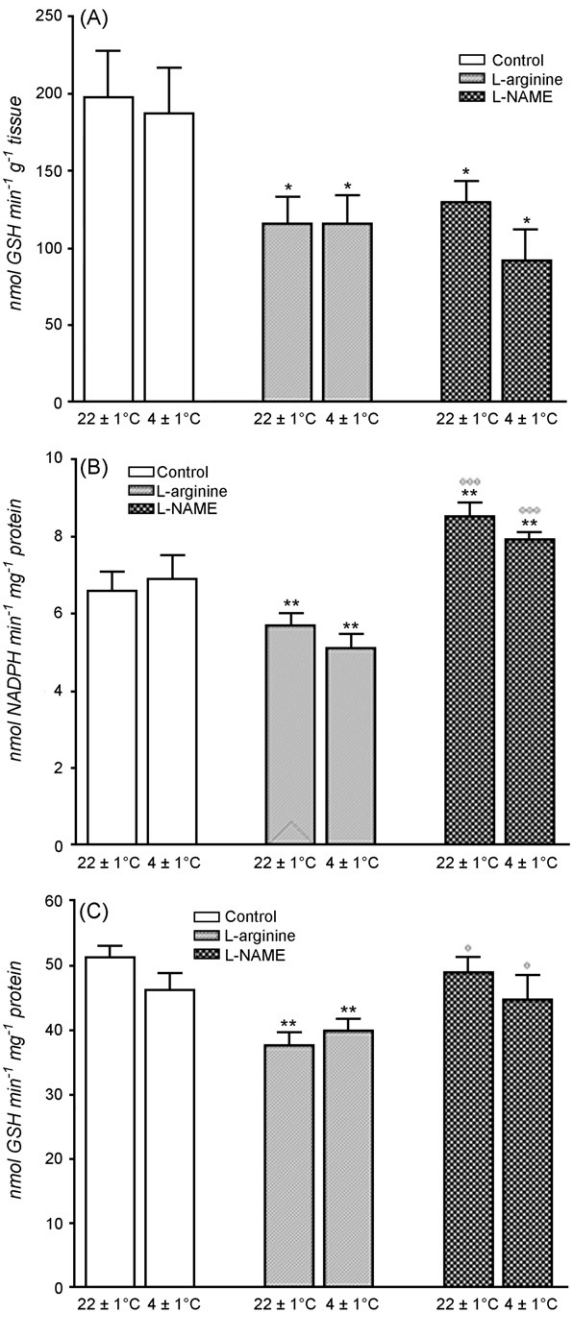

Fig. 1 Changes of glutathione dependent part of antioxidative defense (amount of GSH and GSH-Px and GST activities) in the skin of rats acclimated to different temperatures and treated with L-arginine or L-NAME. GSH amount (A) of both L-arginine and L-NAME treated rats kept at ambient and low temperature was decreased compared to the corresponding controls ($p < 0.05$). GSH-Px activity (B) was decreased in L-arginine treated groups compared to the corresponding controls in both animals maintained at room or low temperature ($p < 0.025$), while L-NAME treatment increased GSH-Px activity compared to both corresponding controls ($p < 0.025$) and L-arginine treated animals ($p < 0.005$). GST activity (C) of both room temperature- and low temperature-maintained rats treated with L-arginine were decreased compared to corresponding controls ($p < 0.025$) and L-NAME-treated animals ($p < 0.05$). The results are the means ± S.E.M. ($n = 6$ in all groups). Comparison of different treatments with the control acclimated to the same temperature: $^{**}p < 0.025$, $^{*}p < 0.05$; comparison of the L-NAME treated group with L-arginine treated group acclimated to the same temperatures: $^{ooo}p < 0.005$, $^{o}p < 0.05$.

ing controls, while the lowerest activities among all groups were seen in L-NAME-treated groups.

L-Arginine and L-NAME treatments decreased GSH content (Fig. 1A) in skin of both the rats kept at ambient and low temperature in comparison with the corresponding controls. L-Arginine decreased activity of GSH-Px compared to the corresponding controls in both animals maintained at room or low temperature, while L-NAME treatment increased GSH-Px activity compared both to the corresponding controls and L-arginine-treated animals (Fig. 1B). L-Arginine decreased skin GST activity of both room and low temperature-maintained rats compared both to the corresponding controls and L-NAME-receiving animals (Fig. 1C).

There are no changes in rats body weight observed after acclimation to cold (data not shown).

Present data clearly demonstrate that long-term acclimation to cold did not affect the AD in the rat skin. However, L-arginine acted inhibiting GSH-dependent part of the AD (decreased activities of GSH-Px and GST accompanied by a reduced GSH content), while increasing SOD/CAT-dependent $O_2^{-\bullet}/H_2O_2$ pathway (increased CuZnSOD and CAT activities). Since L-NAME, a known NOSs inhibitor, prevented L-arginine-induced AD changes, specific response of the AD could be interpreted in terms of the alterations in the L-arginine-$^{\bullet}$NO-pathway in the skin.

Numerous data pointed out to interrelationship of GSH content and GSH-dependent AD part with $^{\bullet}$NO [8]. As we mentioned above, physiological and cytotoxic effects of $^{\bullet}$NO depend on its interaction with molecules within the cells, primarily with GSH and $O_2^{-\bullet}$ [6]. However, results presented here showing L-arginine-induced decrease in the GSH-Px activity additionally demonstrated that the reaction of $^{\bullet}$NO with GSH in the skin is more dominant than $^{\bullet}$NO interaction with $O_2^{-\bullet}$. Moreover, observed decrease in GSH-Px activity and GSH amount were accompanied by a decrease of GST activity. These changes were less expressed in L-NAME-treated animals where recorded GSH-Px activity was higher than the control values. Decreased GSH-Px and GST activities observed in L-arginine-receiving animals could be explained by inaccessibility of the substrate (GSH) for the reaction catalyzed by these two