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The Effect of N-Stearoylethanolamine on Free Amino Acid Levels in Plasma and Liver of Rats with an Experimental Burn

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Abstract—The effect of an endocannabinoid congener, N-stearoylethanolamine (NSE), on the content of plasma and liver pools of free amino acids (AA) was studied in burned rats. After application of a thermal skin burn (stage III) animals perorally received an aqueous suspension of NSE (10 mg/kg of body weight) during 7 days or were treated with the aqueous NSE suspension (10 mg/ml) applied onto the burn wound, or received a combined treatment. It has been originally demonstrated for the first time, that the treatment of burned rats with NSE prevented the decrease in total AA concentration in blood plasma and the increase in hepatic AA concentration due to modulation in concentrations of glycogenic AA. In burned animals the ratio of plasma and liver homogenate Phe/Tyr and Gly/Val increased while the Fischer ratio (Ile+Leu+Val/Phe+Tyr) decreased, and after the treatment with NSE these parameters remained at the level of intact animals. These data demonstrate that NSE possesses adaptogenic properties, and it is involved in the organism response to the burn. This prevents changes in blood plasma and hepatic pools of free AA of NSE-treated rats with the burn wound compared with untreated animals.

Keywords: N-stearoylethanolamine, free amino acids, burn.

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INTRODUCTION

During the last 20 years increasing evidence has been accumulated in the literature that N-acylethanolamines (NAE) may be referred to compounds exhibiting protector and adaptogenic properties. Under normal conditions these minor lipids exist at extremely low concentrations, which however, may be increased by several orders of magnitude under pathological conditions. These compounds were found to possess a wide range of biological activity in various pathological processes; for example, they exhibit membrane-protective, antioxidant, cardioprotective, immunomodulating, and other properties [1, 2].

Endogenous C18 NAE include 18:3 NAE, 18:2 NAE, 18:1 NAE, and 18:0 NAE. Like the endocannabinoid, anandamide (20:4 NAE), some of these compounds act as weak ligands of the cannabinoid CB1 receptor; they can also activate the TRPV1 receptors and thus play a role of endogenous modulators of TRPV1. It was also demonstrated that such endocannabinoids as anandamide, palmitoylethanolamine, 2-arachidonoylglycerol, etc. are natural activators of α-, β(δ), and γ-subtypes of nuclear PPAR receptors [3]. In contrast to unsaturated NAE, the saturated 18:0 NAE, N-stearoylethanolamine (NSE) does not activate either TRPV1 or CB receptors; however, due to its features NSE may be referred to the compounds with cannabinoid mimetic properties [4]. Although saturated NAE, such as NSE, do not attract much attention due to lack of information about their specific properties, they show tissue distribution similar to that of anandamide. It should be noted that by analogy with predominance of the content of saturated fatty acid over the content of unsaturated (e. g., arachidonic) acids the content of saturated NAE is higher than that of anandamide. Our studies at the Department of Lipid Biochemistry (Palladin Institute of Biochemistry, National Academy of Science of Ukraine) have demonstrated earlier that saturated NAE exhibit neuroprotective and cardioprotective effects similar to anandamide; these effects are associated with membrane protective properties of these compounds. We also found marked anti-inflammatory activity in NSE and demonstrated that it could accelerate burned wound healing [5]. These results became a basis for development of a novel drug with anti-inflammatory and anti-allergic effects [6].

Altered free AA concentrations are one of the earliest biochemical changes, which occur in the body after a burn and can be susceptible for evaluation. Significant changes in plasma free AA have been described in patients with burns accompanied by subsequent inflammation. Some authors observed severe hypoaminoacidemia [7], whereas other authors

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observed an increase in concentrations of some free AA [8].

In this study we have investigated the effect of NSE on the free AA content in blood and liver of rats with an experimental thermal burn.

MATERIALS AND METHODS

White albino rats (250–300 g) were used in experiments. Animals were subdivided into six groups. The first group consisted of intact rats ($n = 12$). Animals of the second group ($n = 11$) received a suspension of NSE per os at the dose of 10 mg/kg. Animals of all other groups were subjected to a controlled burn, induced by application of a metal cylinder (base square of 12.56 cm^2) heated to 100°C on the depilated skin of a back for 15 s under general nembutal anesthesia. Animals of the third group subjected to thermal burns did not receive any treatment (control; $n = 12$). Burned animals constituting the fourth group ($n = 12$) received daily peroral administration of the aqueous suspension of NSE (the does of 10 mg/kg). Burned animals of the fifth group ($n = 11$) received NSE applications on the burned wound during 7 days (aqueous NSE suspension, 10 mg/ml). Burned rats of the six group ($n = 10$) received daily peroral administration of the aqueous suspension of NSE (the does of 10 mg/kg) and also NSE applications on the burned wound (aqueous NSE suspension, 10 mg/ml). The NSE treatment was started immediately after the burn. Animals were decapitated on the 8th day after burn application under nembutal anesthesia.

Blood and liver were taken immediately after decapitation. Blood mixed with 10% sodium citrate (5 : 1) was separated into plasma and erythrocyte fraction by centrifugation at 1500 *g* for 15 min at 4°C. Liver homogenates (10%) were prepared under cooling after removal of blood and using a hand-held homogenizer with a glass pestle and saline.

The amount of blood plasma and liver homogenate free AA was determined using an automated amino acid analyzer T-339 (Microtehno, Czech Republic) after protein precipitation with sulfosalicylic acid [9]. Blood plasma and liver homogenate were mixed with 3% sulfosalicylic acid (1 : 1), mixed, centrifuged at 1500 *g* for 20 min and 1 ml of the supernatant (acid-soluble) fraction was taken for subsequent AA analysis.

Statistical analysis was performed using the Student's *t*-test, differences were considered as statistically significant at $p \le 0.05$.

RESULTS AND DISCUSSION

Two days after the burn the burn wound of control animals represented blisters with fluid, whereas burn wounds of NSE treated rats were covered with crust and their subsequent healing occurred faster compared with control (untreated) burned animals.

Blood plasma and liver free amino acids in intact rats

	Plasma, μmol/ml	Liver, μmol/g of tissue
Ala	0.737 ± 0.030	12.56 ± 0.77
Arg	0.214 ± 0.015	0.16 ± 0.01
Asp	0.081 ± 0.009	8.87 ± 0.73
Cys	0.019 ± 0.003	0.41 ± 0.07
Glu	0.391 ± 0.051	11.97 ± 1.30
Gln	0.569 ± 0.022	14.06 ± 0.92
Gly	0.578 ± 0.026	14.32 ± 1.00
His	0.115 ± 0.013	2.68 ± 0.22
Ile	0.154 ± 0.008	2.80 ± 0.37
Leu	0.261 ± 0.019	6.23 ± 0.83
Lys	0.378 ± 0.047	7.63 ± 0.69
Met	0.094 ± 0.006	2.60 ± 0.23
Orn	0.033 ± 0.005	5.51 ± 0.47
Phe	0.033 ± 0.003	2.82 ± 0.35
Pro	0.137 ± 0.020	2.89 ± 0.37
Ser	0.267 ± 0.039	8.58 ± 0.63
Thr	0.298 ± 0.043	5.37 ± 0.43
Tyr	0.055 ± 0.008	2.27 ± 0.26
Var	0.286 ± 0.020	5.26 ± 0.53

Note: Data represent mean ± SEM for the group of 12 rats.

Table shows free AA content in blood plasma and liver homogenate of intact rats.

Figure 1 shows, that under conditions of our experiment the burn was accompanied by a decrease in the sum of free AA in blood plasma (Fig. 1a, column 2) and a significant increase of the sum of AA in rat liver (Fig. 1b, column 2). This is consistent with data from other laboratories [10]. On the 8th day after the burn the treatment of burned animals with NSE normalized the levels of most AA both in blood plasma and the liver; they basically reached levels of intact animals (Figs. 1a and b, columns 3–5).

Thus, all modes of NSE administration to burned rats prevented the trauma-induced decrease of the sum of free AA in plasma and an increase in this parameter in rat liver.

Figure 2 shows the content of individual free AA in blood plasma (a) and liver (b) of rats subjected to the burn trauma and NSE treatment. Peroral administration of NSE to control rats (Fig. 2a, column 1) resulted in significant increase of Cys, Gln, and Ser in blood plasma, whereas plasma concentrations of other AA investigated in this study remained unchanged. The burn was accompanied by a significant decrease of all free AA in plasma except Asp, Cys, Orn and Thr, while plasma Phe increased compared with intact animals (Fig. 2a, column 2). Peroral treatment of burned rats with NSA caused different but statistically signifi-

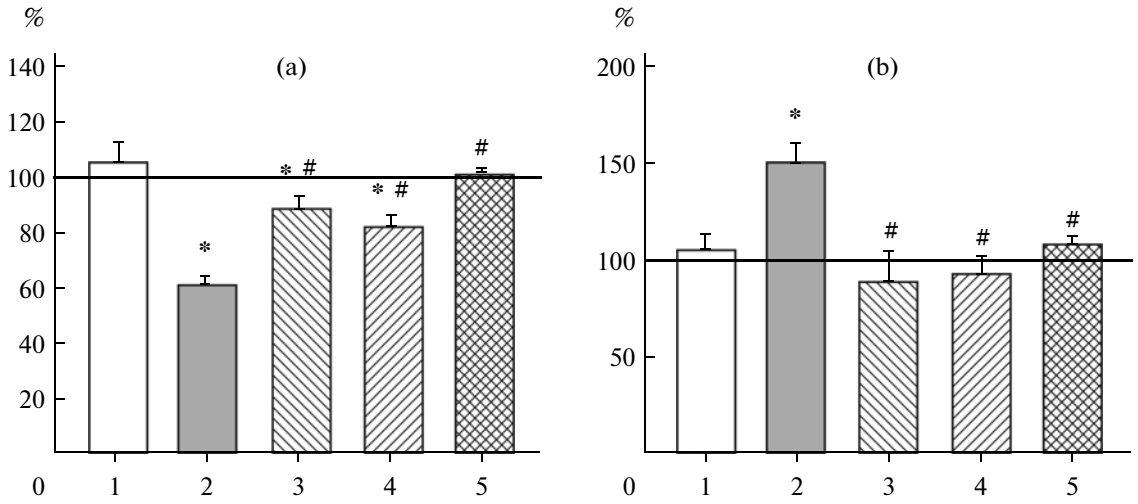

Fig. 1. The sum of free amino acids in blood plasma (a) and liver (b) of rats (% of intact rats).

1—Healthy rats perorally treated with the NSE suspension (10 mg/kg) during 7 days;

2—Burned rats;

3—Burned rats perorally treated with the NSE suspension (10 mg/kg)

4—Burned rats treated with daily application of the aqueous NSE suspension (10 mg/ml) onto the wound;

5—Burned rats subjected to peroral treatment with the NSE suspension (10 mg/kg) combined with application of the aqueous NSE suspension onto the wound.

*—Statistically significant differences ($p < 0.05$) compared with the intact group (punctured line on the diagram); #—statistically significant differences ($p < 0.05$) compared with burned (untreated) animals (column 2).

cant increase of Gln, His, and Val compared with untreated burned rats (Fig. 2a, column 3). The plasma levels of Ala, Arg, Asp, Cys, Gly, Leu, Met, Orn, Phe, and Tyr remained unchanged; the plasma levels of Glu, Ile, Lys, Pro, Ser, and Thr reached the level of control rats in NSE treated burned animals.

Application of the aqueous NSE suspension (10 mg/ml) to the burn wound did not influence the content of blood plasma Ala, Arg, Cys, Glu, Gly, Ile, Leu, Met, Orn, Phe, and Pro, but cause a significant increase of Gln, His and Val compared with untreated burned rats; the content of Asp, Lys, Ser, Thr, and Tyr reached the level of intact animals (Fig. 2a, column 4).

Peroral administration of NSE combined with application of the aqueous NSE suspension to the burn wound completely prevented the decrease in Ala, Arg, Gly, Ile, Leu, Lys, Tyr, and Val and the increase of Phe in blood plasma of burned rats (Fig. 2a, column 5). The content of Asp, Cys, and Glu in blood plasma of the treated rats was higher than in intact animals, whereas the level of Cys, Gln, Met, Pro, and Ser was higher than in untreated burned rats. The content of blood Orn was significantly lower by 70 and 117% than in intact and burned rats, respectively.

The burn trauma of rats was accompanied by a significant increase in hepatic content of Ala, Arg, Asp, Gln, Glu, Gly, Phe, Pro, and Ser, and a significant decrease in Leu, Lys, and Met content, while Cys, His, Ile, Orn, Thr, Tyr, and Val remained unchanged compared with intact animals (Fig. 2b, column 2).

The fact of intensification of metabolic processes in trauma, infection, or burn was originally found in 1932 [11]; it is known that after severe burns the level of metabolic processes in the body may increase by more than 100% [12]. Such so-called hypermetabolic state may be induced by both afferent factors, which are released in the lesion area and cause changes in medullar and hypothalamic centers (lipid peroxides, prostaglandins, oxygen free radicals, cytokines, endotoxins), and also by efferent factors (including hormones, released after excitation of medullar and hypothalamic nuclei, and also cortisol, catecholamines, glucagon, growth hormone, etc.). Organisms respond to a burn trauma by increased glucose utilization; this is accompanied by increased catabolism of fat and proteins [13]. Subsequent events include the increase in glycogenesis in skeletal muscles, protein lysis, and insulin resistance. The liver is characterized by increased glycogenolysis, gluconeogenesis, and protein synthesis.

The statistically significant increase in hepatic levels of Ala, Gln, Gly, Pro, and Ser (Fig. 2b, column 2) with simultaneous decrease of plasma concentrations of these AA (Fig. 2a, column 2) observed in burned versus intact rats (correlations coefficients r of -0.460; -0.610, -0.599, -0.457, and -0.569, respectively) suggest impairments in metabolism of these free AA in burned rats; this is consistent with results obtained by other researchers [14].

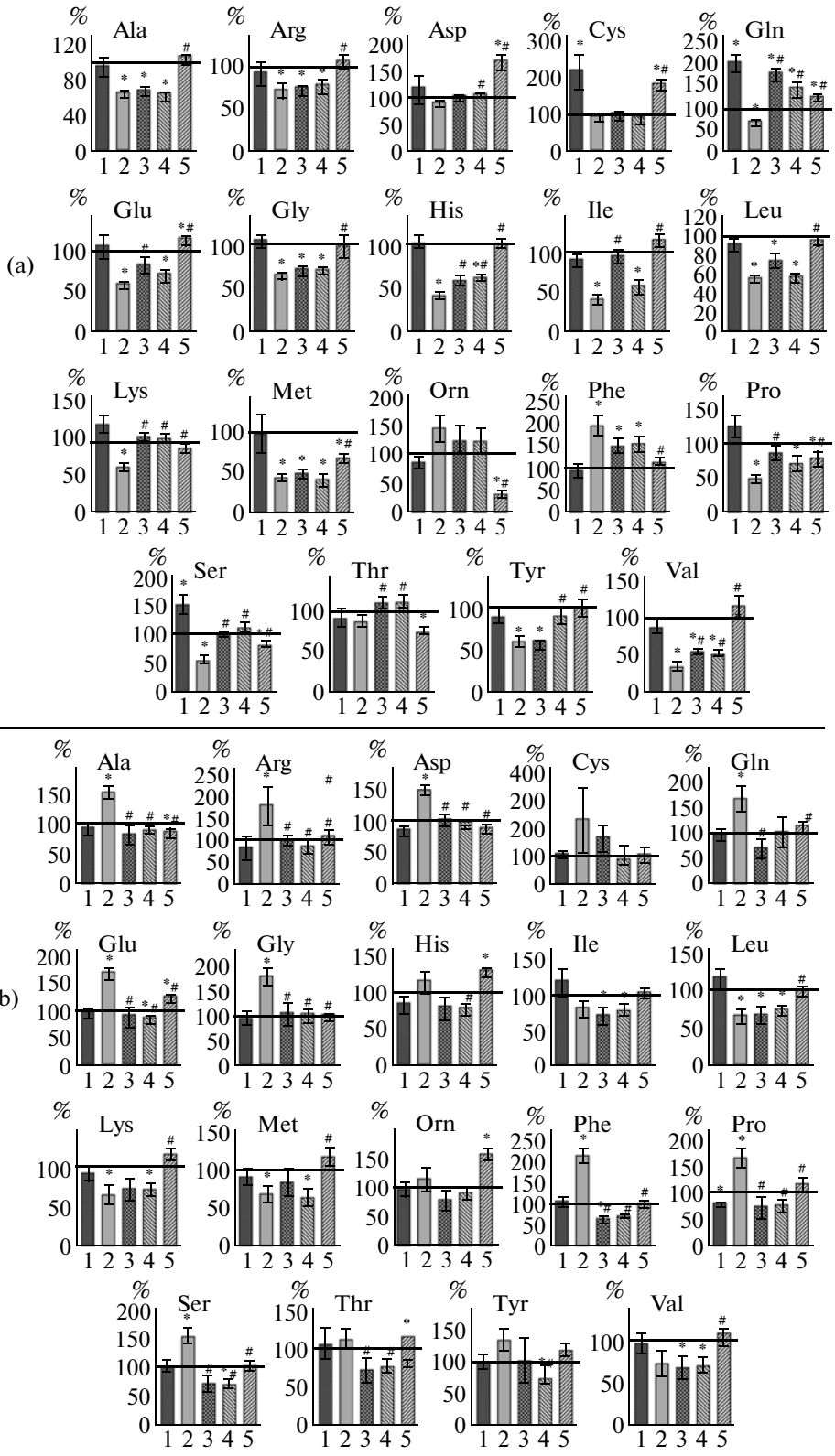

Fig. 2. The effect of the NSE treatment on free AA content in blood plasma (a) and liver (b) of rats (% of intact rats). All designations are the same as at Fig. 1.

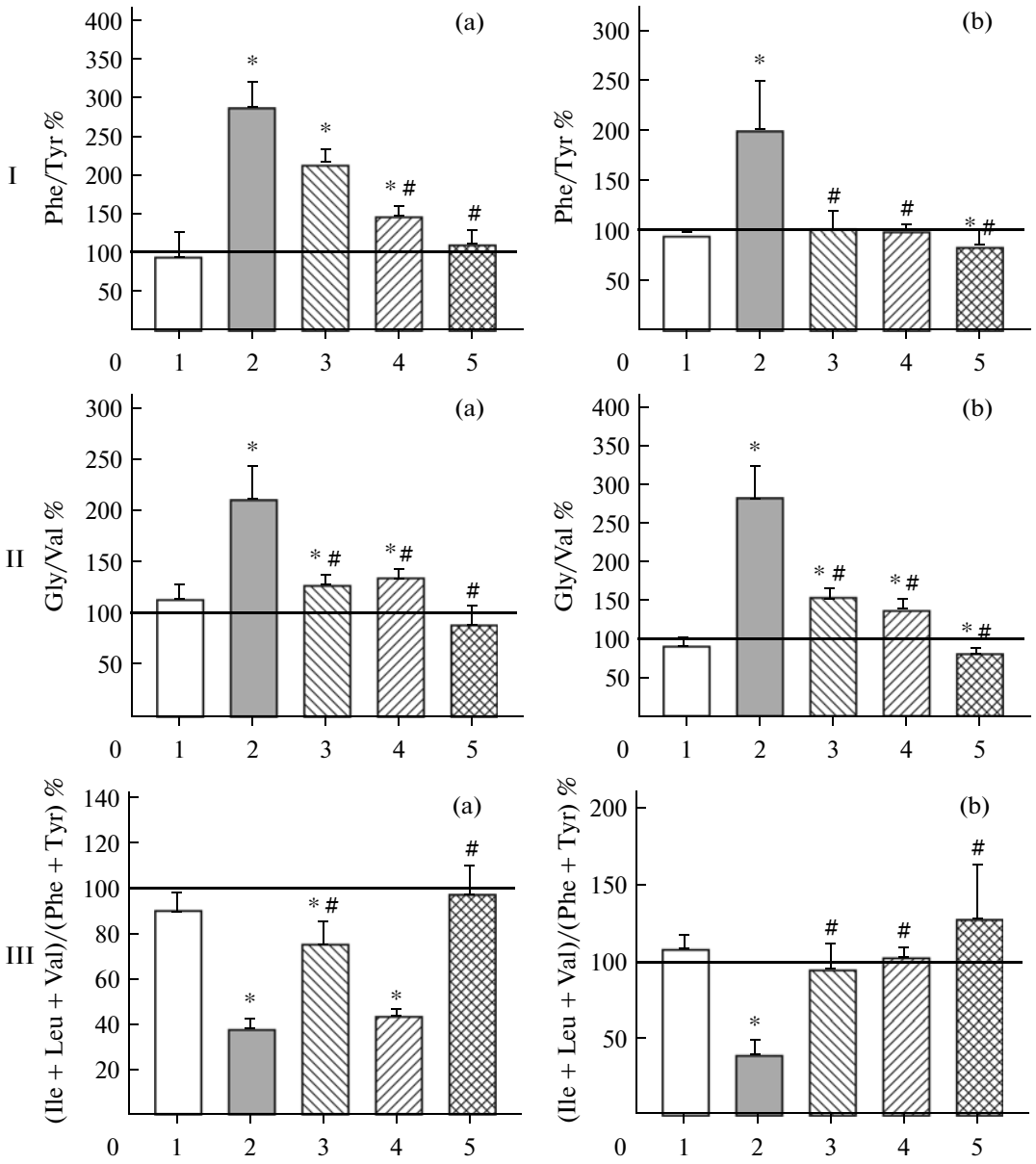

Fig. 3. The ratios Phe/Tyr (I), Gly/Val (II) and the Fischer ratio (III) in blood plasma (a) and liver (b) of rats (% of intact rats). All designations are the same as at Fig. 1.

In most cases peroral administration of NSE to burned rats or its application onto the burn wound or combination of these treatments (Fig. 2b, columns 3–5) normalized hepatic levels of most free AA. Especially pronounced effect was observed after the combined treatment (peroral NSE administration + NSE application on the wound burn) (Fig. 2b, column 5). Examination performed on the 8th day after the burn revealed that the hepatic levels of most free AA studied (Arg, Asp, Cys, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Tyr, and Val) were at the level of intact rats and Orn and Thr levels even exceeded concentrations of these AA in intact animals (by 55.82 and 17.44%, respectively). This may be positive for hepatic cells (ornitine is a known hepatoprotector, threonine together with methionine and tryptophan maintains lipotropic function of the liver) and also for the burn-damaged skin.

Earlier it was shown that the ratio Phe/Tyr may serve as an indicator of the multiple system organ dysfunction in various pathologies [15]. A direct correlation between the Phe/Tyr value in blood plasma of patients and their mortality caused by various complications in the post-burn period has been elucidated [10]. On the 8th day of our experiments the ratio Phe/Tyr in blood plasma (Fig. 3, I, a, column 2) and the liver (Fig. 3, I, b, column 2) was significantly higher in burned rats compared with intact rats. The

combined treatment of burned rats with NSE (peroral administration and application onto the wound) completely prevented the increase of the blood plasma Phe/Tyr ratio in burned rats (Fig. 3, I, a, column 5). In the liver of NSE-treated burned rats the Phe/Tyr ratio remained at the level of intact rats regardless of the mode of NSE administration (Fig. 3, I, b, columns 3–5).

The ratio Gly/Val is used as a protein deficiency marker at various pathological conditions [16]. Figure 3II shows that in NSE-treated burned rats the ratio Gly/Val was significantly lower than in untreated animals (both in blood plasma and the liver); this suggests that NSE can normalize protein imbalance in burned rats.

It is known that in man and animals the increase in energy metabolism supplied by proteins results in a decrease of plasma levels of branched-chain AA (Ile, Leu, Val). Intensity of branched chain AA oxidation determines a degree of total protein catabolism in the body [10]. Thirty five years ago Fischer et al. proposed a "unified" hypothesis on the pathogenesis of hepatoencephalopathies; the hypothesis was based on the fact of the decreased levels of plasma branched chain AA and increased levels of aromatic AA in liver dysfunctions [17]. This hypothesis has been confirmed in numerous experimental and clinical studies, which confirmed appearance of imbalance between content of free aromatic AA and branched chain AA. This imbalance results in the decrease of the Fischer ratio (defined as the ratio of the molar sum of branched chain AA to that of aromatic AA) in liver dysfunctions of various genesis [18, 19].

We observed a significant decrease in the Fischer ratio (Ile+Leu+Val)/(Phe+Tyr) in blood plasma and the liver of burned rats compared with intact animals (Fig. 3, III, a, column 2). This suggests impairments of liver functions in burned rats. The combined treatment of burned rats with NSE normalized the Fischer ratio in blood plasma, which remained at the level of intact animals (Fig. 3, III, a, column 5). In the liver positive effect of NSE on this parameter was observed regardless the mode of NSE administration ((Fig. 3, III, b, columns 3–5).

Thus data of Figs. 1–3 provide convincing evidence that NSE administration prevents changes of free AA levels in plasma and liver of rats with the thermal skin burn. This suggests that unfavorable consequences of increased metabolic processes seen in burned rats may be corrected by the treatment NSE. It is known that the increased metabolic response to burns involves various cytokines (TNFα, IL-1, IL-6, IL-8, IL-10, IFN-γ, etc.) [20], intensification of LPO processes [21], and also impaired regulation of nitric oxide synthesis [22]; this is associated with the damage of many organs in this pathology.

Earlier we already demonstrated that in rats NSE accelerated thermal burn wound healing by inhibiting production of pro-inflammatory cytokines (TNFα, IL-6), normalization of content of the stable nitric oxide metabolite, nitrite-anion and also activity of constitutive and inducible NO synthases; NSE also abolished the imbalance between LPO and antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) in plasma, erythrocytes, liver and spleen [5]. All these data suggest that under conditions of burn injury N-stearoylethanolamine possessing antioxidant and membrane tropic characteristics acts as a compound with adaptogenic and protective properties, which increases burn wound healing and decreases manifestations of imbalance of free amino acids.

CONCLUSIONS

We have demonstrated here for the first time that the endogenous cannabinoid mimetics, N-stearoylethanolamine, accelerates thermal wound healing in rats. N-stearoylethanolamine decreased the hypermetabolic reaction of the body to the burn by preventing burn-induced alterations in the content of blood plasma and hepatic pools of free amino acids. Our results suggest that pharmacological preparations based on N-stearoylethanolamine may be widely used for correction and treatment of various pathologies including burns.

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