Role of Nitric Oxide in the Regulation of Activity of Proteinase Inhibitors α_1-Antitrypsin and α_2-Macroglobulin by Capsaicin-Sensitive Nerves

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Regulation of activity of serine proteinase inhibitor α_1-antitrypsin and nonspecific proteinase inhibitor α_2-macroglobulin in the blood by nitric oxide was studied in intact rats and animals with damage to capsaicin-sensitive nerves. Nonselective nitric oxide synthase inhibitor L-NAME produced a dose-dependent increase in α_1-antitrypsin activity in intact animals. Neuronal NO synthase inhibitor 7-nitroindazole increased α_2-macroglobulin activity. Deafferentation with capsaicin was followed by a decrease in α_1-antitrypsin activity. Both inhibitors of nitric oxide synthase increased activity of α_1-antitrypsin in capsaicin-receiving rats. Nitric oxide precursor L-arginine had a normalizing effect on reduced activity of α_1-antitrypsin after capsaicin deafferentation. Our results suggest that nitric oxide has a modulatory effect on activity of proteinase inhibitors and is involved in the effector influence of capsaicin-sensitive nerves on α_1-antitrypsin activity.

Key Words: *capsaicin; nitric oxide; α_1-antitrypsin; α_2-macroglobulin*

Much recent attention is paid to the effector influence of C-fibers in afferent peptide-containing nerves, which received the name capsaicin-sensitive nerves (CSN). This influence is realized via the release of neuropeptides from peripheral nerve terminals [14]. The release of neuropeptides is induced by activation of vanilloid receptors (TRPV 1) under the effect of endogenous (protons, lipid metabolites, and inflammatory mediators) and exogenous agonists (capsaicin) [1]. Capsaicin in neurotoxic doses causes damage to afferent nerves, which results in suppression of serine proteinase inhibitor α_1-antitrypsin (α_1-AT) and activation of nonspecific proteinase inhibitor α_2-macroglobulin (α_2-MG) in rat serum [4]. These changes contribute to proteinase/inhibitor imbalance and modulate the development of postdenervation atrophy. α_1-AT and α_2-MG are mainly synthesized in the liver, which receives innervation from capsaicin-sensitive spinal and vagal nerves. The tonic effect of CSN on α_1-AT activity is related to a direct or cytokine-mediated influence of neuropeptides in sensory nerves [4]. Nitric oxide (NO) is expressed in afferent neurons [15] and plays a role or mediates the effect of neuropeptides on vascular tone and inflammation [9,15]. Previous studies showed that NO plays a role in the effector influence of CSN [9,15]. NO concentration in the expired air decreases in patients with α_1-AT deficiency [11]. These data suggest that α_1-AT activity is regulated by the nitrergic mechanisms. NO synthesis is catalyzed by the family of NO synthases (NOS), which comprises constitutive neuronal (type I) and endothelial NOS (type III) and inducible NOS (type II). NO is produced from L-arginine in the presence of O_2. NO synthesis in the organism may occur during oxygen-independent reduction of NO metabolites, ni-

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trites (NO_2^-) and nitrates (NO_3^-), by heme-containing proteins with reductase activity. The NO synthase and nitrite reductase pathways for NO synthesis are interrelated in metabolic processes and constitute the general NO cycle [2]. The increased synthesis of NO due to activation of NO synthase by L-arginine has a protective effect during liver ischemia [5,13] and pulmonary inflammation [7]. Activation of the nitrite reductase pathway is induced by sodium nitrite [6].

The effect of the NO synthase pathway for NO synthesis on serine proteinase inhibitor α_1-AT and nonspecific proteinase inhibitor α_2-MG in the blood was studied under normal conditions and during damage to CSN.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-220 g. CSN were damaged by subcutaneous injection of capsaicin (30+50+70 mg/kg, Sigma) under light ether anesthesia. NO synthesis blockade was induced by intraperitoneal injection of nonselective inhibitor L-NAME (ICN) and neuronal NO synthase (nNOS) inhibitor 7-nitroindazole (7-NI, Sigma). NO precursor L-arginine (Sigma) was injected intraperitoneally to increase the concentration of NO. L-NAME and 7-NI were administered individually or in combination with capsaicin (1 h before each dose or 3 and 9 days after the last dose of capsaicin). The test substances were injected in the following single doses: L-NAME, 75 or 100 mg/kg; 7-NI, 25 mg/kg; and L-arginine, 20-100 mg/kg.

The rats were decapitated under ether anesthesia 1 day after treatment with the last dose of NO modifiers (individual administration). Otherwise, the animals were decapitated on day 1, 3, or 13 after combined treatment with the test substances. Experimental groups consisted of 7-8 rats. Our study was performed according to the principles of humanity (European Community Directives) and approved by Biomedical Ethics Committee (Institute of Physiology).

Proteinase inhibitor activity in blood plasma was estimated from the inhibition of degradation of N-α-benzoyl-L-arginine hydrochloride ethyl ester (Sigma) by trypsin (α_1-AT) and complex of trypsin and α_2-MG. Spectrophotometric measurements were performed on a SF-46 (LOMO) or UV-2501 PC device (Shimadzu) at 253 nm. The data were expressed in inhibitory units per ml serum (IU/ml) [4].

The results were analyzed by Student's *t* test.

RESULTS

Administration of L-NAME to intact animals was followed by an increase in α_1-AT activity, which depended on the total dose and duration of treatment (Fig. 1, *a*). α_2-MG activity increased only after 2-fold treatment with L-NAME in a dose of 100 mg/kg. α_2-MG activity did not differ from normal after increasing the period of treatment (Fig. 1, *b*). nNOS inhibitor 7-NI in the specified doses had no effect on α_1-AT activity, but increased α_2-MG activity.

Damage to CSN by capsaicin in neurotoxic doses is associated with neuropeptide depletion. The content of neuropeptides decreases most significantly on days 10-14 after capsaicin administration. α_1-AT activity decreased (Fig. 2, *a*), while α_2-MG activity increased at the early stage of CSN damage (Fig. 2, *b*). This parameter returned to normal on day 13 [13].

The role of NOS blockade in CSN damage was studied 3 days after combined treatment with NOS

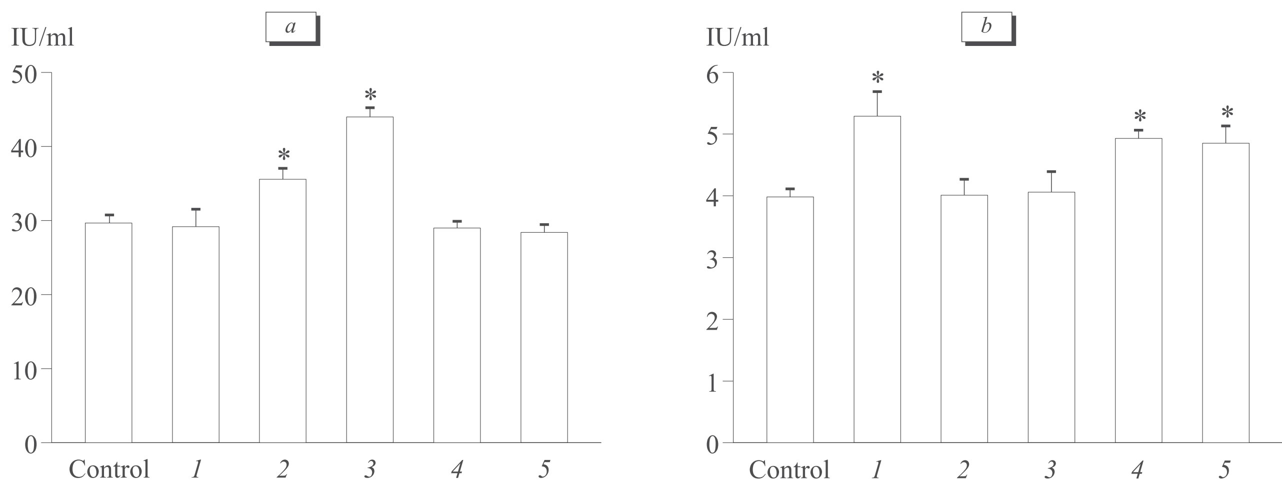

Fig 1. Effect of NO synthesis inhibitors on activities of α_1-AT (*a*) and α_2-MG (*b*) in the blood of intact rats. (*1-3*) L-NAME: (*1*) 100 mg/kg×2; (*2*) 100 mg/kg×4; (*3*) 75 mg/kg×8. (*4*, *5*) 7-NI: (*4*) 25 mg/kg×2; (*5*) 25 mg/kg×4. *$p<0.05$ compared to the control.

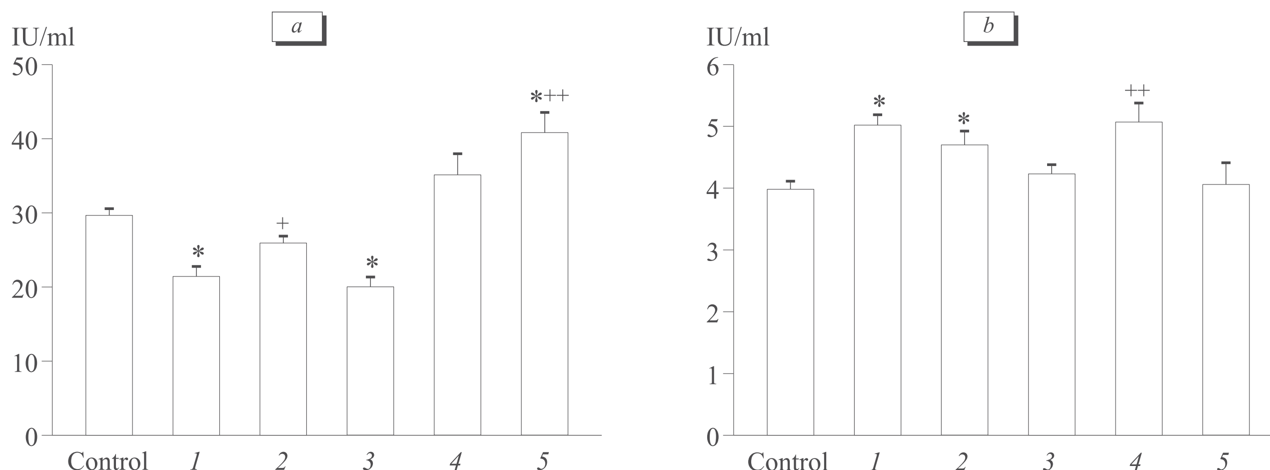

Fig. 2. Effect of NO synthesis inhibitors on activities of α_1-AT (*a*) and α_2-MG (*b*) in the blood of rats with CSN damage. (*1*, *2*) Day 3 after administration of capsaicin: (*1*) capsaicin; (*2*) 7-NI (25 mg/kg, 1 h before each dose of capsaicin)+capsaicin. (*3-5*) Day 13 after administration of capsaicin: (*3*) capsaicin; (*4*) capsaicin+7-NI after 9 days (25 mg/kg×3); (*5*) capsaicin, +L-NAME after 9 days (100 mg/kg×3). $p<0.05$: *compared to the control; +compared to *1*; ++compared to *3*.

inhibitor and capsaicin. Otherwise, the measurements were performed on day 9 after capsaicin administration. This period corresponds to the development of deafferentation syndrome. Combined treatment with 7-NI and capsaicin reduced the effect of capsaicin on α_1-AT activity (day 3; Fig. 2, *a*). Administration of 7-NI in the same dose on day 9 after deafferentation was followed by an increase in α_1-AT activity to the control level (as compared to capsaicin-receiving animals; Fig. 2, *a*). L-NAME injection during this period also increased activity of α_1-AT, which surpassed the control level (Fig. 2, *a*).

α_2-MG activity increased on day 3 after individual or combined administration of 7-NI and capsaicin (Fig. 2, *b*). α_2-MG activity was high in animals receiving 7-NI on day 9 after deafferentation. α_2-MG activity in animals receiving L-NAME during this period did not differ from the control.

We showed that nNOS blockade had no effect on α_1-AT activity in intact animals, but abolished the inhibitory effect of capsaicin on this enzyme. Our results indicate that nNOS of afferent nerves play a role in the damaging effect of capsaicin. Activity of this inhibitor increases after administration of L-NAME to intact animals, as well as in combined treatment with capsaicin and antagonist. These changes reflect the proinflammatory effect of NOS blockade [7,10].

Administration of L-arginine to intact animals had no effect on proteinase inhibitor activity (Fig.

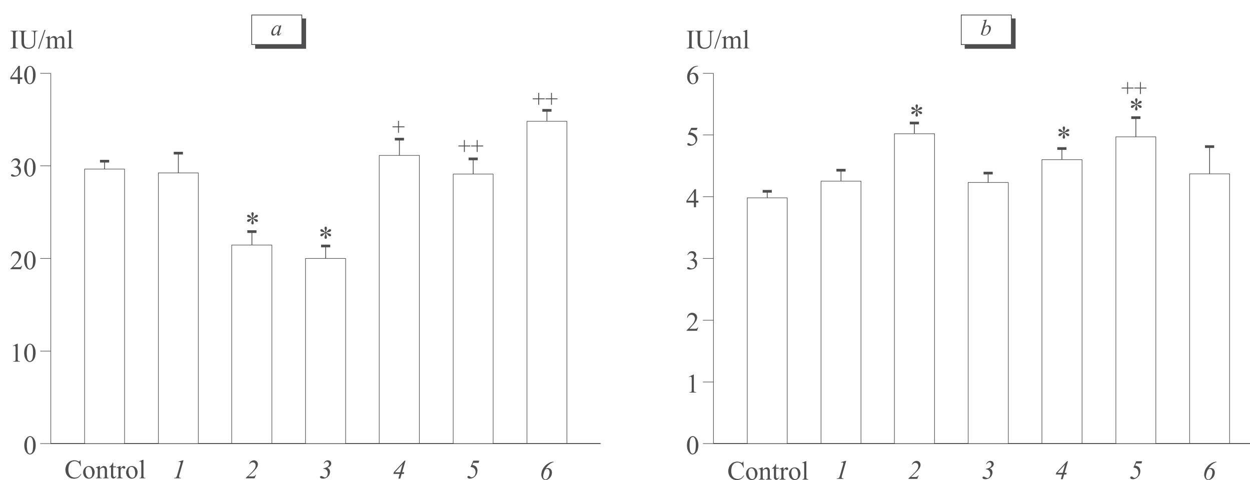

Fig. 3. Effect of L-arginine on activities of α_1-AT (*a*) and α_2-MG (*b*) in the blood of rats with CSN damage. (*1*) L-Arginine, 20 mg/kg×4; (*2*) day 3; (*3*) day 13 after the last dose of capsaicin. (*4*, *5*) Combined administration of L-arginine (100 mg/kg, 1 h before each dose of capsaicin) and capsaicin: (*4*) day 3; (*5*) day 13 after the last dose of capsaicin. (*6*) Administration of L-arginine (100 mg/kg×8) on day 3 after capsaicin treatment. $p<0.05$: *compared to the control; +compared to *2*; ++compared to *3*.

3). Combined treatment with L-arginine and capsaicin had normalizing effect on α_1-AT activity, which was low after administration of capsaicin (days 3 and 13). Long-term administration of L-arginine after treatment with capsaicin was accompanied by further increase in α_1-AT activity above the control level.

α_2-MG activity increased in the early and delayed period after combined treatment with L-arginine and capsaicin. However, α_2-MG activity in animals subjected to long-term administration of L-arginine after capsaicin treatment did not differ from the control (Fig. 3).

Administration of L-arginine to deafferented animals had a normalizing effect on α_1-AT activity. Hence, the NO-mediated effector influence of CSN on α_1-AT activity returns to normal under these conditions.

Morphological study of the liver tissue in rats was performed after administration of capsaicin and variations in NO concentration. It was shown that capsaicin causes degeneration and necrosis of hepatocytes and neutrophil infiltration. NOS antagonists potentiated the effect of capsaicin. Administration of L-arginine decreased the incidence of necroses and severity of neutrophil infiltration in the liver induced by capsaicin [3]. Our results are consistent with published data that L-arginine-induced increase in NO production has the antiinflammatory and cytoprotective effect [7,13] and improves the state of antioxidant protection [5]. These changes probably contribute to prevention of α_1-AT inactivation.

Functional activity of α_1-AT is determined by binding of its reactive site to the target and translocation of the complex into molecular β-sheet. α_1-AT activity depends on a variety of factors, including the formation and disintegration of proteinase complexes, chemical modification in oxidation, and polymerization of mutant α_1-AT [8]. NO has various effects on α_1-AT activity. The release of considerable amounts of NO due to strong stimulation of pain receptors by capsaicin and increased expression of NOS is accompanied by the formation of peroxynitrite, which can inactivate α_1-AT. At the same time, the increase in NO concentration is followed by the formation of cysteine(232)-nitrosylated S-NO-α_1-AT. Modified α_1-AT gains additional inhibitory activity and has the cytoprotective effect *in vivo* [12]. The mechanisms for modulation of α_1-AT activity by L-arginine require further investigations.

The test substances induced opposite changes in activity of α_1-AT and α_2-MG. Hence, these inhibitors are regulated by different mechanisms. As differentiated from α_1-AT, α_2-MG binds the majority of proteinases and small molecules of various classes (cytokines, growth factors, and neuropeptides). Moreover, α_2-MG accelerates clearance of these factors. α_2-MG activity increases in the early period after administration of capsaicin and NOS antagonists with proinflammatory properties, which is probably related to its function as reactant of acute inflammation in rodents. Combined treatment with L-arginine and capsaicin did not abolish the effect of capsaicin on α_2-MG. After long-term administration of L-arginine to deafferented rats, α_2-MG did not differ from the control.

Our results indicate that the L-arginine—NO synthase pathway plays a role in the regulation of α_1-AT activity. It is important to evaluate the role of nitrite reductase and nitrate reductase pathways for NO synthesis in α_1-AT homeostasis.

We conclude that NO has a modulatory effect on proteinase inhibitors and is involved in tonic influence of CSN on α_1-AT activity.

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