ПЕПТИДЫ ВРОЖДЕННОГО ИММУНИТЕТА — ЭНДОГЕННЫЕ ИММУНОМОДУЛЯТОРЫ И УЧАСТНИКИ НЕЙРОИММУННЫХ ВЗАИМОДЕЙСТВИЙ

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THE CORTICOSTATIC EFFECT OF HUMAN LACTOFERRIN DEPENDS ON THE AMINO ACID COMPOSITION OF THE N-TERMINUS OF THE MOLECULE

G.M. Aleshina

Institute of Experimental Medicine, Saint Petersburg, Russia

КОРТИКОСТАТИЧЕСКОЕ ДЕЙСТВИЕ ЛАКТОФЕРРИНА ЧЕЛОВЕКА ЗАВИСИТ ОТ АМИНОКИСЛОТНОЙ ПОСЛЕДОВАТЕЛЬНОСТИ НА N-КОНЦЕ МОЛЕКУЛЫ

Г.М. Алешина

ФГБНУ «Институт экспериментальной медицины», Санкт-Петербург

Исследовано действие различных структурных вариантов лактоферрина человека на стресс-индуцированные изменения уровня кортикостерона в крови у крыс. Использована модель комбинированного эмоционально-физического стресса — плавание в холодной воде (1–4 °C) в течение 2 мин. Уровень кортикостерона в плазме определяли методом иммуноферментного анализа. Ранее нами было установлено, что превентивное внутрибрюшинное введение нативного лактоферрина снижает стресс-индуцированное повышение концентрации кортикостерона в крови крыс через 30 минут после стресса. В настоящей работе показано, что трансгенный лактоферрин человека, у которого отсутствуют четыре остатка аргинина на N-конце, не оказывает такого действия. Полученные результаты позволяют сделать вывод о ключевой роли N-концевых аминокислотных остатков в реализации кортикостатической активности лактоферрина.

Ключевые слова: лактоферрин; кортикостерон; стресс.

The effect of various structural variants of human lactoferrin on stress-induced changes in the corticosterone level in the blood in rats was studied. A model of combined emotional and physical stress — swimming in cold water (1–4 °C) for 2 minutes. The level of corticosterone in plasma was determined by enzyme immunoassay. We have previously found that preventive intraperitoneal administration of native lactoferrin reduces the stress-induced increase in corticosterone concentration in the blood of rats 30 minutes after stress. This work shows that transgenic human lactoferrin, which lacks four arginine residues at the N-terminus, does not have this effect. The obtained results allow us to conclude about the key role of N-terminal amino acid residues in the implementation of the corticostatic activity of lactoferrin.

Keywords: lactoferrin; corticosterone; stress.

Introduction. Lactoferrin (LF) is one of the most multifunctional mammalian proteins. It has been shown that he is involved in iron metabolism, exhibits antimicrobial and anti-inflammatory [1, 2]. Recently, there are works that indicate its participation in neuroendocrinoimmune interactions. We have previously shown that preventive intraperitoneal administration of native LF reduced the stress-induced increase in corticosterone concentration in the blood of rats [3], but the mechanism of such an action remained unclear. The aim of this work is to compare the corticostatic action of native and transgenic human lactoferrin, which have difference in amino acid composition for the N-terminus, in experiment *in vivo*.

Materials and methods. The experiments were performed on male rats Wistar line weighing 150–200 g. The animals were kept in vivarium at room temperature with a 12-hour light/dark cycle, free access to food and water, on a standard diet.

Human milk lactoferrin (purity not less than 98% and iron saturation — 10–15%) was isolated by methods of ion-exchange chromatography and gel filtration [4]. Transgenic lactoferrin was produced by “CAPRABEL” (Belorussian State University and Applied Scientific Animal Breeding Centre of the Belorussian National Academy of Sciences).

The model of acute stress — swimming in cold (1–4 °C) water in 2 min. Lactoferrins were ad-

Fig. 1. The concentration of corticosterone in the blood plasma of rats (30 minutes after stress application). Groups of animals: 1 — intact animals, 2 — animals after stress and the administration of water, 3 — animals after stress and the administration of native LF, 4 — animals after stress and the administration of transgenic LF. * $p < 0.05$ vs group 1; # $p < 0.05$ vs group 2

ministered intraperitoneally 5 min prior the stress application in a dose 200 $\mu\text{g/kg}$ weight. Blood was collected in 30 min stress. Plasma corticosterone levels were evaluated by IFA kit for corticosterone (DRG). Injected proteins contained less than 0.2 UE/ml LPS (measured by LAL-test). Experiments were carried out in the same time period (11.00 am — 2.00 pm) to avoid the influence of circadian changes of corticosterone level.

Statistical evaluation was carried out using the software package Statistica 10.0.

Comparisons were made using the Mann-Whitney test. $p < 0.05$ was considered to be significant. Data are given as mean $\pm$ S.E.M.

Results and discussion. A comparative study of the stress-induced level of corticosterone in rats under conditions of experimental stress and the administration of human LF preparations with different of the N-terminus amino acid composition

of the molecules was carried out. The results are presented in Figure 1.

It has been established that the preventive administration of human LF obtained from the milk of transgenic goats did not reduce the stress-stimulated level of corticosterone in the blood of rats, in contrast to the native LF. The main difference between the structure of transgenic LF and the native one is the absence of a block of four arginine residues at the N-terminus. LF is not the only antimicrobial protein that has a corticostatic effect. For some defensins — neutrophilic antimicrobial peptides, an *in vitro* corticostatic effect on adrenal cell culture was revealed, which, according to the authors, was due to defensins competitively binding to the ACTH receptor due to the presence of an arginine residue block near the N-terminus of the peptide molecule [5]. At the same time, it was shown that preventive administration of defensins, for which an *in vitro* corticostatic effect was detected, reduced the stress-stimulated increase in corticosterone levels in experimental animals under stress conditions [6].

It is possible that the corticostatic action of the native human LF is also due to its competitive binding to the ACTH receptor, due to the presence of a block of several arginines at the N-terminus of the molecule (<http://www.ncbi.nlm.nih.gov/protein/AAG48753.1>).

At the same time, other mechanisms for the implementation of the corticostatic effect cannot be ruled out. It has been shown that bovine LF can also have a corticostatic effect under conditions of experimental stress in rats [7], although it does not have an arginine residue block at the N-terminus, but this effect of bovine LF was manifested when it was administered intraperitoneally in concentration 100 mg/kg animal weight, i.e. 500 times more than in our experiment.

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