

Probl Cryobiol Cryomed 2025; 35(4):244
<https://doi.org/10.15407/cryo35.04.244>

Effect of synthetic leu-enkephalin on the lipid profile in homeothermic animals under conditions of chronic cold stress

V.H. Mirnyy, N.M. Moisieieva,
Yu.S. Akhatova, O.L. Gorina

Institute for Problems of Cryobiology and Cryomedicine
of the National Academy of Sciences of Ukraine,
Kharkiv, Ukraine

Lipid metabolism is one of the most sensitive systems affected by cold stress. A decrease in temperature activates thermogenesis and lipolysis, leading to increased levels of triglycerides and free fatty acids in the blood. With prolonged cold exposure, these changes become chronic and result in an imbalance between atherogenic and protective lipoproteins, increasing the risk of vascular disorders. Endogenous opioids, as key components of the antistress system, may be involved in regulating these derangements. In this context, dalargin, a synthetic analogue of leu-enkephalin, is considered as a potential metabolic corrector. Therefore, the aim of this study was to assess the effect of dalargin on the lipid profile of homeothermic animals under conditions of chronic cold stress.

Chronic cold stress (CCS) was modeled by cyclically interchanging cold exposure (at 0–4 °C) and room temperature for 5 days. Animals were divided into four groups: intact, control (CCS), and two experimental groups, which received dalargin (100 µg/kg) or dalargin with prior administration of naloxone (1 mg/kg) 30 minutes before cold exposure. Blood lipid profile was assessed by measuring levels of triglycerides (TG), total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), and atherogenic index (AI). Statistical significance was evaluated using the Mann–Whitney test; at $p < 0.05$, differences were considered significant.

Animals, exposed to CCS, demonstrated a significant increase in TG, LDL, cholesterol, and AI levels (all $p < 0.05$), indicating lipolysis activation and a shift toward an atherogenic lipid profile. Concurrently, the HDL level significantly decreased ($p < 0.001$) that reduced the ability to reverse cholesterol transport and increased the risk of vascular complications.

Under the dalargin effect, a significant reduction in TG, LDL, and AI levels as well as an increase in HDL were revealed relative to the CCS group ($p < 0.05$), indicating effective correction of lipid imbalance. This effect may be associated with the anti-stress properties of dalargin and its potential ability to modulate neuroendocrine regulation of lipid metabolism. The using of naloxone completely counteracted these processes attesting the participation of opioid receptors in the mode of action of dalargin.

Chronic cold stress induces atherogenic dyslipidemia, which can be effectively corrected by dalargin administration. The findings highlight the active role of the opioid system in lipid metabolism regulation under cold stress and attest to the receptor-specific action of dalargin that may have practical value in the development of antistress metabolic correctors.

Probl Cryobiol Cryomed 2025; 35(4):244
<https://doi.org/10.15407/cryo35.04.244a>

Membrane destabilization by Gramicidin S: evaluating its compatibility with cryoprotective protocols

I.A. Perepelitsa¹, V.P. Berest²
V. N. Karazin Kharkiv National University,
Kharkiv, Ukraine

Cryopreservation is vital to contemporary personalized medicine, cell therapy-based products, assisted reproductive technology, and organ transplantation. Nowadays, long-term storage of biological samples at liquid nitrogen temperatures requires cryoprotectants to reduce ice-related damage, antioxidants to hamper membrane lipid peroxidation, and antibiotics to prevent contamination. More commonly antibacterial substances are used as additives in extenders to ensure the sanitary quality of the livestock semen in reproductive biotechniques (Santos, 2020). However, the deleterious effects of conventional antibiotics on sperm quality as well as their effectiveness under growing bacterial resistance, led to the search of alternative antimicrobials. Potent candidates among them are naturally occurring antimicrobial peptides (AMP) (Hemmati, 2025). As long as the main target for AMP is the cell membrane, we decided to test the time dependence of membrane-active AMP Gramicidin S lytic action against RBC membranes.

Antimicrobial peptides are convenient because the bacterial membrane contains teichoic acid and lipopolysaccharide, which impart a negative charge to the surface, promoting electrostatic interaction with cationic AMP (Chen T, *et al.*, 2019). Positively charged AMP interact with negatively charged phospholipids of the outer layer of the bacterial membrane. Gramicidin S (GrS) is a cyclic peptide antibiotic active against Gram-positive, Gram-negative bacteria and some pathogenic fungi.

We have studied the effect of gramicidin S on the structural integrity of the RBC membrane. For this purpose, erythrocytes were incubated with Gramicidin S at concentrations (2.5, 5, 10, 20, 25 µM). Incubation was performed for 10 min, 1 h and 24 h. Triton X-100 (TX100) was added to assess the stability of the membranes of incubated cells. Gramicidin analogues were used for parallel analysis: LMB002 and LMB033 (Horbatok, 2023).

The results of the study showed that the lag period of hemolysis decreased almost linearly with increasing GrS concentration (2.5–25 µM), reflecting the diffusion-limited mechanism of peptide insertion into the lipid bilayer and preparation of the membrane for the action of detergent. This also applied to the maximum hemolysis rate for GrS, which significantly exceeds that for the open isoforms LMB002 and LMB033, demonstrating a non-monotonic dependence with a peak at medium concentrations (due to deeper insertion into the bilayer and phase separation of lipids).