LEU-ENKEPHALIN AS A TREATMENT OF INFLAMMATORY PARKINSONISM (A CASE REPORT)

Author: Ines Niehaus

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Abstract

Introduction: The neurotransmitter leu-enkephalin is a pentapeptide with the amino acid sequence of tyrosine, glycine, glycine, phenylalanine, leucine. Leu-enkephalin is neuroprotective in femtomolar concentrations in inflammatory lipopolysaccharide (LPS) animal models of parkinsonism and reduces the sezernation of anti-LPS- IgM-antibodies of B-cells. LPS are part of outer membranes of Gram-negative bacteria.
Method: An oral treatment with leu-enkephalin in µmol concentration was started in a case of lipopolysaccharide-induced parkinsonism.
Results: The first effect was a shortly pain in bones with transient depression of blood pressure. Muscle relaxing effects started after 30 min. Strong improvement of activity of sensory nerves occured within 5 hours. LPS-induced conduction blocks of N. peronaeus were shown in sensory nerve action potentials before. Stiffness and hypokinesia were reduced. Walking abilities improved with reoccuring of the missed left arm swing. One dose of the daily therapy showed long-lasting positive effects up to 72 hours. The patients chronically elevated anti-LPS-IgM-antibodies levels were lowered.
Conclusions: The negatively charged LPS is able to bind to positively charged pentapeptide sequences of important neuronal receptor proteins:
1) $alpha_2$-$delta_2$-subunit of voltage-gated calcium channels
2) NADPH oxidase NOX_5
3) Enzyme myosin light chain kinase 2 of skeletal muscles
Leu-enkephalin might be able to prevent the binding of LPS to these peptides with restoring of the protein function including reduced amount of LPS-caused conduction blocks. Leu-enkephalin in µmol concentration shows excellent anti-parkinsonian and anti-inflammatory effects of much more intensity than L-dopa and pramipexole administered for years in the patient.

Lipopolysaccharides (LPS), Sepsis, and Neurodegeneration
LPS are highly biohazardous bacterial O-antigens. E. coli LPS injected in ng in humans or animals are causing transient mild sepsis-like symptoms with stimulation of the immune system (B-cells, phagocytes). LPS in µmol doses are causing sepsis and lethal septic shock due to failure of multiple organ systems. Sepsis survivors are suffering from long-term neurological seroquaeles like critical illness encephalopathy, polyneuropathy and also parkinsonism.[1] Sepsis-induced polyneuropathy mostly severe occurs in Nervus peronaeus with nerve conduction blocks and myositis with muscle atrophy and weakness in feet and legs like in the described patient.

Leu-Enkephalin
The neurotransmitter leu-enkephalin is a pentapeptide with the amino acid sequence of tyrosine, glycine, glycine, phenylalanine, leucine.

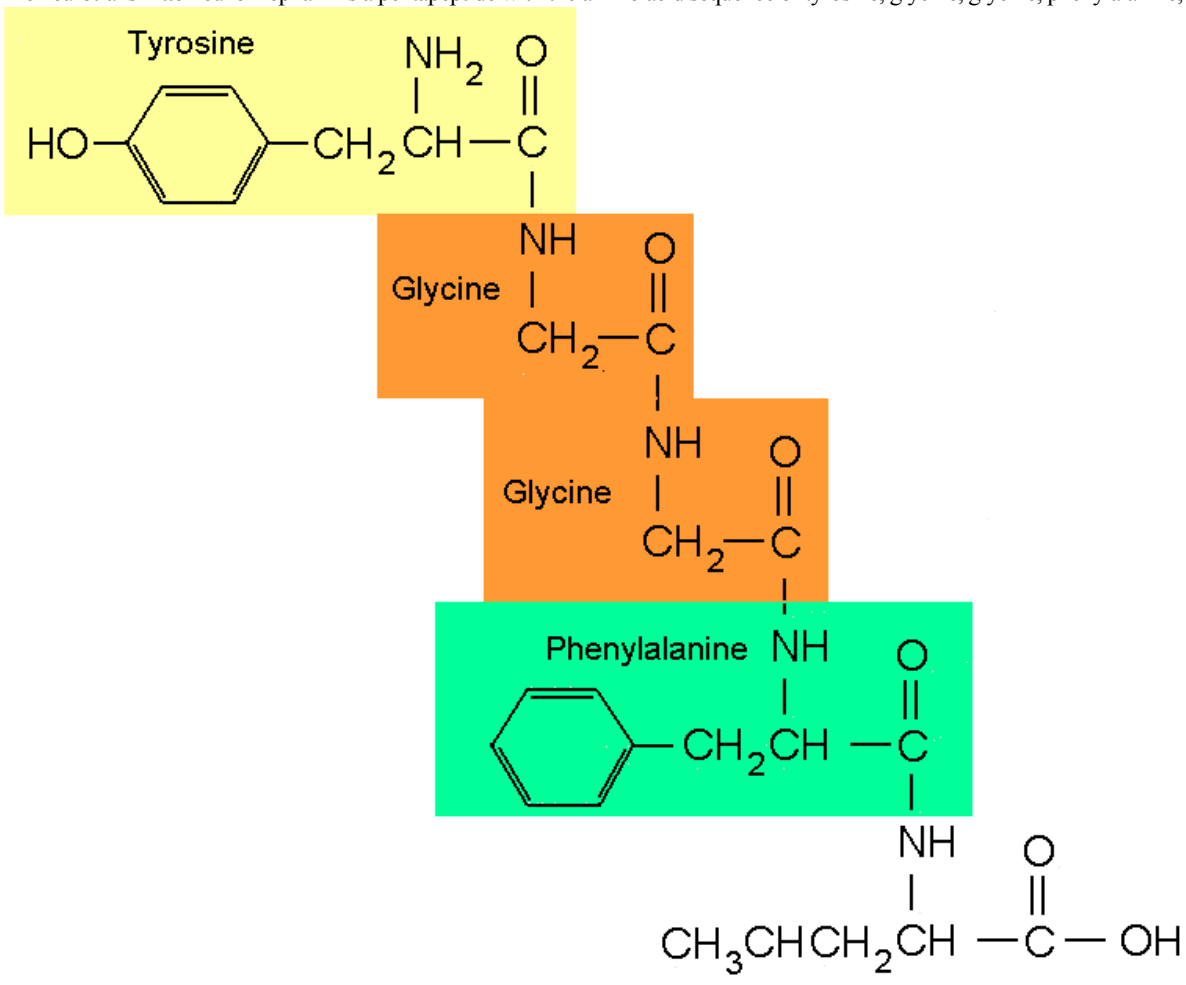

Fig.1 : Chemical structure of Leu-Enkephalin

Fig 1.: Chemical structure of Leu-Enkephalin

Storage of Methionine-Enkephalin in the brain

Met-Enkephalin deficiency is known in brains of patients with PD. In healthy subjects met-enkephalin levels were highest in the caudate nucleus and putamen and lowest in the hippocampus and cerebral cortex. In PD 80-95% reduced Met-enkephalin concentration was known in the SN and ventral tegmental area but not in the caudate nucleus and putamen despite the lack of dopamine in all four regions of the brain. Enkephalin immunoreactivitity in mesencephalon has been observed in nerve terminals surrounding the DA neurons - maybe in astrocytes.[2].

LPS-induced PD in rats the astrocytes are also degenerated, maybe also the stores of enkephalins in astrocytes.

Leu-Enkephalin improves the cerebral blood flow

The opioid neuropeptide leu-enkephalin improves pial microcirculation and cerebral blood flow in moderate to severe brain ischemia [3]. Restored the cerebral blood flow and vasomotor reaction of the pial micro vessels, rapidly and intensively improved peripheral and central lymph circulation against the background of decreased cardio - and homodynamic parameters.

Leu-Enkephalin stimulates the lymph flow

Lymphostimulation is a effective method of treating ischemia. Leu-Enkephalin Is a most effective immunostimulator with direct lymph stimulating effects. The peripheral Leu-Enkephalin-induced lymph stimulation is attended by preventaion or restoration of the damaged local circulation in the ischemized brain cortex. [3]

In rats, the response of pial vessels to I.p. injected Leu-Enkephalin (40µg/kg) is studied after and before bilateral occlusion of common carotid arteries. Leu-Enkephalin preserves the circulation stability inspite of lowered arterial pressure, bradycardia, increased local circulation in the brain cortex by 50-70%, and intensification of the lymph flow in micro- to macro vessels and absence of mortality in the first hours of occlusion. The lymph flow was measured by puncture of the thoracic lymphatic duct. Initially inactive mesenteric microvessels of the small intestine began to contract intensively with acceleration of the lymph flow velocity in mesenteric micro vessels. [4]

Microglial NADPH oxidase mediates Leu-Enkephalin dopaminergic neuroprotection

Leu-Enkephalin is neuroprotective to LPS-induced damages of dopaminergic neurons at femtomolar concentration (10^{-15} to 10^{-13}) through anti-inflammatory properties. The tetrapeptide des-tyrosine leu-enkephalin and the tri-peptide glycine-glycine-phenylalanine is also neuroprotective. But they are only neuroprotective in PHOX+ cell cultures. PHOX is a catalytic subunit gp91 of the NADPH-oxidase complex. NADPH oxidase is an inducible elektron transport system in phagocytic cells causing free radicals H_20_2. [6]

Enkephalins are able to reduce the LPS-induced stimulation of B-cells.

Receptors for opioid peptides like met and leu-enkephalins are present on immune cells like B-cells. IgM-production of LPS-stimulated B-cells were inhibited by low concentrations of Met-enkephalin (10^{-16} to 10^{-10}) from 30-50% in compared with LPS-stimulated controls, where as higher concentration 10^{-8} are not effective. The results show that IgM and IgG3 production was inhibited by ultralow concentration of Met-Enk.[5]

Parkinson's disease (PD) and LPS:

In rats, intranigral injections of LPS results in a rapid inflammatory activation of microglia followed by an acute and permanent damage of dopaminergic neurons. Other neurons are not affected perhaps due to the highest density of microglia in the SN with increased sensitivity to the LPS-induced inflammation.[7]

In mice, a single systemic i. p. injection of LPS results in a chronic neuroinflammation with activated microglia and increased TNF-alpha in the brain and a delayed and progressive loss of the dopaminergic neurons (a first detectable loss of 22% after 7 months, 47% loss after 10 months). The pattern of damages of the dopaminergic neurons in the striatum is symmetrically. Microglia cells were activated 3 h post LPS injection not only in the SN but also in the hippocampus and cortex![8]

Case Report: Results of Examinations in the Case Report:

FTIR-analysis of blood sample in 2003:

The result of a Fourier transform infrared spectroscopy (FTIR-analysis) showed a high content of Salmonella minnesota S-LPS (ca. 1/3 of the blood sample was contaminated with LPS). The LPS was 100% identified as Salmonella minnesota S-LPS.[9]

Positron emission tomography with Fluoro-Dopa on February 20th, 2001:

The F-Dopa-PET shows in 2001 ca. 70% loss of dopaminergic function marked by severe reduction of the decarboxylase activity in both Nuclei caudatii (quotient Nucl. caud right/Occ: 1.52; Nucl. caud. left/Occ: 1.54) without difference of sides and a moderate reduction of the functionality of putamen (quotients: putamen right/Occ:1.78; putamen left/Occ: 1.71).

LAL test of cerebrospinal fluid on April 3rd, 2000:

The result of a chromogenic limulus lysate assay (LAL) of the cerebrospinal fluid was 6600 pg LPS/ml CSF.

Positron emission tomography with FDG on July, 10th 1998

Cerebral glucose metabolism was determined with [Fluorine-18] fluoro-2-deoxy-D-glucose (FDG) using positron emission tomography. In summary the reduction of glucose utilization was ca. 70% in the gyrus frontalis, ca. 80% in the gyrus prae- and postcentralis, and ca. 75% in the gyrus temporalis (normal range 100%).

Summary of the Case Report

This is the first case report describing a treatment with leucine enkephaline in µmol concentration in a 36-yrs old female patient 14 years living with a long-lasting persistent endotoxemia causing chronically systemic and neuronal inflammation with progressive parkinsonism after one single accidental contamination with 10 µg highly purified Salmonella minnesota S-LPS in 1995. The LPS has neither been detoxified nor eliminated from the body proven by a LAL test of the CSF in 2001 (6600 pg LPS/ml CSF) and by a FTIR-analysis of a blood sample in 2003.

Symptoms of Parkinsonism in May 2001 without any treatment

Rigidity in 4 extremities most severe in the neck (typical for encephalic PD), bradykinesia, cogwheel phenomenon; resting tremor; diadochokinesia; missing arm swing and dragging of the leg on the left side; tendency of micrography in handwriting, fine motor skills restricted and general retardation of movements. A treatment with L-Dopa and amantadine is improving symptoms but the patient is unable to tolerate any kinds of dopamine agonists because of severe negative side effects (vertigo, nausea, weakness, sleeping attacks).

Effectiveness of Leu-Enkephalin in the Case Report
Long-lasting improvement of symptoms of Parkinsonism due to orally administered leucine enkephalin in µmol concentration in an aqueous solution.

First effects after first three doses
The first effect was a shortly pain in bones especially of the hips with transient depression of blood pressure. Muscle relaxing effects started after 30 min. Strong improvement of activity of sensory nerves occured within 5 hours. LPS-induced conduction blocks of N. peronaeus were shown in sensory nerve action potentials before.

Stiffness and hypokinesia were reduced. Walking abilities improved with reoccuring of the missed left arm swing. One dose of the daily therapy showed long-lasting positive effects up to weeks. The patients chronically elevated anti-LPS-IgM-antibodies levels were lowered.

Long-term effects
after 11 doses (last one in the middle of January 2009):
- Permanent improvement of the movability of the left side with re-occuring left arm-swing during walking
- left leg is less cramped, longer condition in standing position with less or reduced pain in the leg
- possibility for walking for a longer distance (up to 3 km)
- improved balance, no more falls
- improving pattern of walking

Future treatment options:

The long-term effects of a treatment with leucine enkephalin in aequos µmol concentration will be investigated in the 36-years old female patient with LPS-induced inflammatory parkinsonism.

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Please send any comments or questions to the author and website editor.

e-mail: Ines_Niehaus@gmx.de

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