

In vivo Radiodetoxification of *Salmonella minnesota* Lipopolysaccharides with radio-labeled Leucine Enkephalin cures sensory polyneuropathy: A Case report.

Type of Article: Case Report

Ines Niehaus

Kopperpahler Allee 24 H, D-24119 Kronshagen, Germany

ABSTRACT

Background: Lipopolysaccharides (LPS) which are part of the outer cell wall of Gram negative bacteria can cause generalized inflammation, sepsis and septic shock with multiorgan failure. Other short and long term neurological sequelae of LPS include polyneuropathy, encephalopathy and parkinsonism. The neurotransmitter and delta-opioid receptor agonist leucine enkephalin (Leu-enk) which has neuro- and cytoprotective action has been shown to stimulate dopaminergic neurons and reduce anti-LPS-antibody production of LPS-stimulated B-cells among other actions. This makes Leu-enk a potential agent for the treatment of LPS induced polyneuropathy and parkinsonism. This is the first case report of in vivo radiodetoxification of ***Salmonella minnesota*** lipopolysaccharides (LPS) in a patient with lipopolysaccharide-induced polyneuropathy, encephalopathy and parkinsonism 14 yrs after LPS-induced sepsis by a lab accident in 1995.

Methods: The case records and experience of the patient and a Review of relevant literature was utilised.

Results: The long-term neurological sequelae of sepsis was treated with 1 micromol doses once to two times a week of radio-labeled leucine enkephalin in a 37 years old female patient, with a 14 year history of lipopolysaccharide-induced polyneuropathy, encephalopathy and parkinsonism after LPS-induced sepsis by a lab accident in 1995.

Electroneurography studies were performed before and after the treatment as well as positron emission tomographies of the cerebral cortex and of the striatum. Lipopolysaccharides were also measured via Limulus Lysate Assay of the cerebrospinal fluid and Fourier transform infrared spectroscopy analysis of the blood. The patient experienced remarkable improvement in both sensory and parkinsonian symptoms with the restoration of sustained sensory action potentials in the sensory nerves as shown by electroneurography studies indicative of cure of sensory polyneuropathy.

Conclusion: Radio labelled leucine enkephalin (leu-enk), is effective in treating the acute and chronic polynueropathy and parkinsonian features of gram negative sepsis as shown in this first case report of its use. Lue-enk effects results from various mechanisms which lead to reduced nueroinflamtmntion and improved cerebral blood and lymphatic flow in addition to other moloecular actions.

Keywords: Radio-labeled Leucine Enkephalin; Lipopolysaccharides; Polyneuropathy; Parkinsonism.

INTRODUCTION:

Introduction to Lipopolysaccharides (LPS).

LPS which is a part of the outer cell wall of Gram negative bacteria (Fig. 1), are highly bio-hazardous with a minimal injected pyrogenic dose as low as 4 ng LPS/kg in humans¹⁻³. The injection or release of Higher doses of LPS by Gram negative bacteria results in a systemic inflammatory response syndrome with sepsis-like symptoms, septic shock and failure of multiple organ systems which could result in death, in the worst case¹⁻⁴. A permanent release of LPS in the blood stream can lead to several chronic neurologic diseases, like Alzheimer's Disease, Guillain-Barré-Syndrome (GBS), amyotrophic lateral sclerosis, multiple sclerosis and Parkinson's disease (PD)⁵⁻¹⁴.

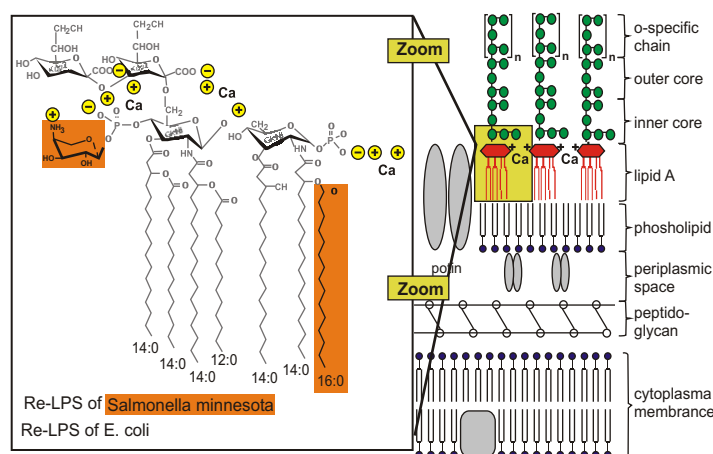


Figure 1. Structure of the Gram negative membrane with calcium-LPS-complexes

Introduction to Parkinson's disease and LPS.

Idiopathic PD is a degeneration of dopaminergic neurons in the striatum and substantia nigra (SN) of unknown cause, leading to the main symptoms of tremor, rigidity (muscle stiffness), and bradykinesia (slowness of movements), in contrast to secondary Parkinsonism (e. g. postencephalitic) with known causes and sometimes additional symptoms. In humans, many cases of PD show general brain inflammation with dramatic increased proliferation of microglia, which express pro-inflammatory cytokines such as TNF-alpha, IL-6, and IFN-gamma¹⁵⁻¹⁶.

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 probably due to the higher density of microglia in the SN and its increased sensitivity to the LPS-induced inflammation¹⁵⁻²¹.

In mice, a single systemic injection of LPS resulted in a chronic neuroinflammation with activated microglia and increased TNF-alpha in the brain in addition to a delayed and progressive loss of the dopaminergic neurons (a first detectable loss of 22% after 7 months and 47% loss after 10 months). The pattern of damage observed in the dopaminergic neurons of the striatum was symmetrical and microglia cells were activated 3 hours post LPS injection, not only in the SN but also in the hippocampus and cortex¹⁸.

Introduction to Leucine Enkephalin.

The neurotransmitter and delta-opioid receptor agonist leucine enkephalin (Leu-enk) is a pentapeptide with the following amino acid sequence, tyrosine, glycine, glycine, phenylalanine, leucine (Fig. 2). The neuro- and cytoprotective Leu-enk increases cerebral blood and lymphatic flow. Leu-enk also confers protection against lipopolysaccharide (LPS)-stimulation of dopaminergic neurons and reduces anti-LPS-antibody production of LPS-stimulated B-cells (Tab. 1). The activity of primary afferent nerve fibres of the spinothalamic tract is also elevated by Leu-enk. The clinical applications of the effects of leu-enk is demonstrated by the Russian synthetic Leu-enk product Dalargin, which has been shown to be helpful in treating septic patients with multiorgan failure²²⁻²³.

leucine enkephalin is thus an agent with potential for use in treating the neurologic and other multiorgan complications of sepsis resulting from the LPS of gram negative bacteria. This is the first case report describing treatment with leucine enkephalin, for a chronic endotoxemia of 15 years duration due to accidental Salmonella LPS injection; which resulted in chronic systemic and neuronal inflammation with septic polyneuropathy, encephalopathy and parkinsonism. The aim of this report is to generate awareness of this potential and examine the mechanisms of leucine enkephalin action.

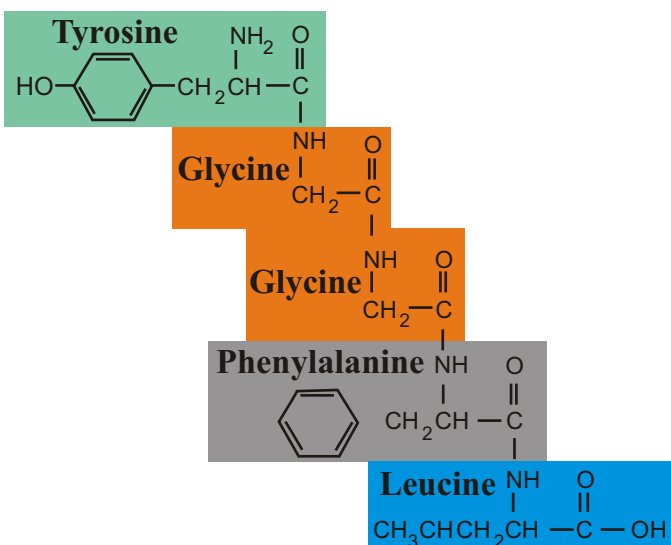


Figure 2. Structure of Leucine Enkephalin

Table 1 : Effects of Leucine Enkephalin (LE) in Humans

Nervous System	Immune System
Neurotransmitter	synthetic LE Dalargin prevents infection in cardio-surgical pat.(23)
Opioid peptide	Dalargin reduces respiratory distress syndrome severity
Binds to G-protein coupled delta-opioid receptors	Dalargin useful in treating postoperative multiorgan failure (22)
Inhibitory and stimulatory effects on neurotransmitter release	improves wound healing in patients with severe burn injuries
primary afferent nerves increase activity in spinothalamic tract	antioxidative properties with e.g. protection of lung and liver
stimulatory effects on primary sensory neurons (33)	Oxidative damage of organ transplants reduced
Stimulatory effect blocked by ganglioside GM1 antibodies	Reduces LPS-induced TNF-alpha production of macrophages
neuroprotective in LPS-stimulated dopaminergic cell cultures (17)	reduces anti-LPS-IgM-antibodies in LPS-stimulated B-cells (20)
neuroprotective in femtomol conc. by inhibiting PHOX (17)	Airway cells increase cAMP with bronchodilatation
Reduction of reactive oxygen species production in microglia	Immune cells (T-/B-cells, macrophages) with opioid-receptors
restores dopamine transporter loss in methamphetamine PD	uptaken by immune cells to 95%
microglia and astrocytes express delta-opioid receptors	produced by macrophages
Circulation	Processing of Leucine Enkephalin
Stimulates peripheral and central lymph circulation (31)	Preproenkephalin mRNA
improvement of cerebral blood flow in brain ischemia (32)	protein proenkephalin
reduces high intraocular pressure	Processing in trans-golgi-network to tyr-gly-gly-phe-leu

Case Report:

A 37 year female laboratory worker developed a 14 year history of persistent endotoxemia, manifesting with septic polyneuropathy, encephalopathy and parkinsonism after a single accidental injection with 10 µg highly purified Salmonella minnesota S-LPS in 1995^{5,6}. The above clinical features resulted from chronic systemic and neuronal inflammation induced by salmonella LPS. Result of a Positron emission tomography scan with [Fluorine-18] fluoro-2-deoxy-D-glucose (FDG), used to determine cerebral glucose metabolism of the patient done in July 1998 showed, a reduction of glucose utilization of ca. 70% in the gyrus frontalis, ca. 80% in the gyrus prae- and postcentralis, and ca. 75% in the gyrus temporalis (normal range 100%). The result of a chromogenic limulus lysate assay (LAL) of the cerebrospinal fluid in April 2000, was 6600 pg LPS/ml CSF, indicating significant levels of LPS endotoxin in the CNS. A Positron emission tomography with Fluoro-Dopa (F-Dopa PET) in February 2001, showed a 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 a significant difference in both sides and a moderate reduction of the functionality of the putamen (quotients: putamen right/Occ: 1.78; putamen left/Occ: 1.71). This is the first report of this unusual symmetrical pattern of damage to the striatum with the damage of the Nucl. Caud greater than the damage in the putamen. In idiopathic PD, asymmetrical damage, with more involvement of the putamen compared to the Nucl. Caud is the usual pattern²⁵. In May 2001, (6 yrs after the lab accident with LPS), without the commencement of any PD drugs, the patients Neurological State was characterized by rigidity in 4 extremities, most severe in the neck (typical for encephalic PD); bradykinesia; cogwheel phenomenon; resting tremor; diadochokinesia; absence of left arm swing and dragging of the leg on the left side; tendency of micrography in handwriting as well as restriction of fine motor skills and general retardation of movements.

Treatment was then commenced with L-dopa/Carbidopa (100 mg L-dopa/25 mg Carbidopa 3-4 times a day) and 100-200 amantadine daily, resulting in some improvement of symptoms. The patient was unable to tolerate dopamine agonists, except very low doses of pramipexole (0.004 mg three times a day) which was commenced in 2007, because of severe negative side effects of the dopamine agonistst like vertigo, nausea, weakness and sleep disturbance. In spite of the commencement of PD drugs in 2001, the features of chronic LPS-induced neuroinflammation was worsened by acute episodes precipitated by varied stresors resulting in severe deterioration of symptoms of parkinsonism such as feverish akinetic crises with inability to walk. In spite of the slow improvement in the patients clinical state after these acute relapse, full recovery from symptoms of relapse to the preceding state was not achieved

Electroneurography which was done in 2006 (11 years after LPS sepsis), showed LPS-induced polyneuropathy with conduction blocks of the sensory tibial, peroneal and sural nerves with missing F-waves of the peroneal nerves (Fig. 3), which is the most sensitive nerve to endotoxins in the blood of

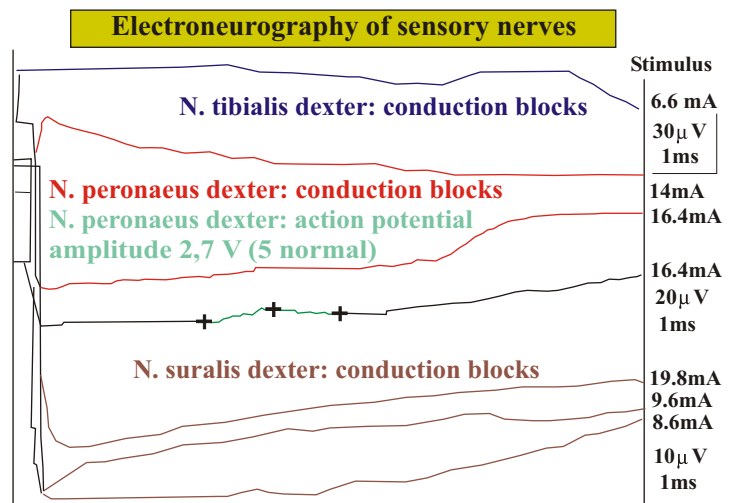


Figure 3. Electroneurography of sensory Nerves

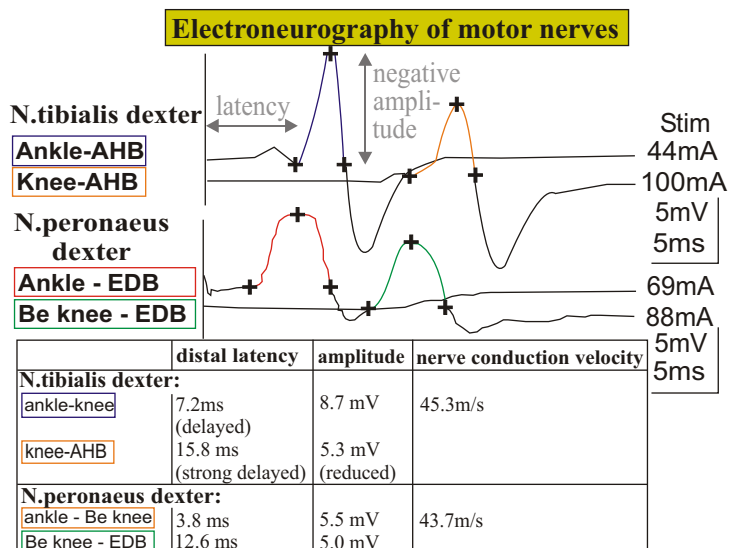


Figure 4. Electroneurography of motor Nerves

septic patients. The action potentials of several motor nerves also showed delayed distal latencies (Fig. 4)

With the persistence of above symptoms and clinical features, treatment with 1 µmol radio-labeled leucine enkephalin (GenScript, NJ, USA) - 1 µmol [H-3]-Leu-enk which was administered in oral aqueous doses one to two times a week in the patient started in December 2008.

Electroneurography studies were performed before and after the treatment as well as positron emission tomographies of the cerebral cortex with FDG and of the striatum with Fluoro-Dopa^{24,25}. Lipopolysaccharides were also measured via Limulus Lysate Assay (Ref. 2) in the cerebrospinal fluid and with FTIR-analysis of the blood²⁶, The evidence that

the LPS has not been detoxified by the body, is shown by the limulus lysate assay test of the cerebrospinal fluid CSF in 2001 (6600 pg LPS/ml CSF) and by a Fourier transform infrared spectroscopy analysis of a blood sample in 2003, which showed a high content of Salmonella minnesota S-

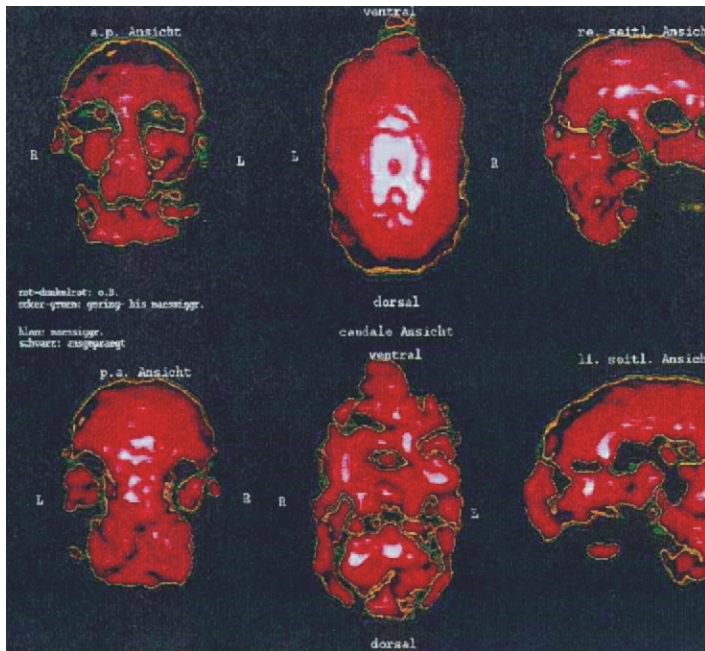
LPS. (ca. 1/3 of the blood sample was contaminated with LPS). The LPS was 100% identified as Salmonella minnesota S-LPS. The above findings were indicative of persistent endotoxemia. Positron emission tomography (PET) with Fluoro-Dopa showed ca. 70% loss of dopaminergic function in the striatum in 2001. Cerebral glucose metabolism which was determined with [Fluorine-18] fluoro-2-deoxy-D-glucose using PET in 1998(Fig.5) showed reduced glucose

Table 2: Effects of Leucine Enkephalin Treatment on different Symptoms

Polyneuropathy	Encephalopathy	Parkinsonism	Others
Pain	++ Concentration	++ Rigidity	++ Myocloni
Paraesthesia	+ Tiredness	0 + Stiffness	++ Seizures
Numbness	++ Short time memory	++ Tremor	0 + Dysphagia
Reduced sensibility	++ Long time memory	0 + Bradykinesia	++ Speaking Difficulties
Weakness	++ Forgetfulness	+ + Cogwheel phenomena	+ + General condition
Muscle atrophy	0 + Velocity of information processing	+ + Headache	++ Arm swing in walking
Walking abilities	++ Headache	+ + Micrography	+ +
Muscle power	++		

in grey colour: effects 5 hours after 1 moldadu
 in black colour: longterm effects of Leu-enk

0 no effect
 + symptoms improvement
 ++ strong symptoms improvement
 +++ very strong symptoms improvement



Annotation: red colour: normal; ochre-green: little damages; blue: moderate damage; black: strong damages.

Figure 5. Positron emission tomogram with FDG in the case report

metabolism in different gyri of the patient, as earlier stated.. Following the commencement of orally administered tritiated radio-labeled leucine enkephalin ([H-3]-Leu-Enk) in 1 micromol concentration in an aqueous solution, the patient experienced improvement in both sensory and parkinsonian symptoms.

A lot of neurological symptoms like reduced sensation, weakness, impaired walking ability, impaired concentration, bradykinesia and rigidity, markedly improved within 5 hours of administration of one dose of leucine enkephalin.

Short term Effects after first three doses of [H-3]-Leu-Enk.

The first observed effects were a short duration of pain in bones especially of the hips with transient depression of blood pressure. Muscle relaxing effects were noticed after after 30 minutes of administration while significant improvement of activity of sensory nerves occurred within 5 hours when compared with the LPS-induced conduction blocks of peroneal nerves which was shown in sensory nerve action potentials earlier (Tab. 2). Stiffness and hypokinesia were reduced. Walking abilities improved with restoration of the missed left arm swing and sustained improvement in the symptoms of Parkinsonism. One dose of the daily therapy showed long-lasting positive effects up to weeks while the patients chronically elevated anti-LPS-IgM-antibodies levels were lowered.

Long-term Effects of Treatment with [H-3]-Leu-Enk: After 11 doses of [H-3]-Leu-Enk (last dose in the middle of January 2009) (Tab. 2). Permanent improvement of mobility of left side of the body, with restored left arm-swing during walking as well as resolution of left leg flexion was noticed. The patient was able to stand for longer periods and walk with reduced leg pain. The patients walking distance increased to 2 3km with improved walking pattern and improved balance. Falls were now a rare occurrence. After 20 doses of [H-3]-Leu-Enk, sensory action potentials in the sensory nerves became sustained as shown by electroneurography studies of September 2009.

DISCUSSION:

Salmonella minnesota S-LPS results in chronic general and neuroinflammation with progressive neurodegeneration following its uptake by cells such as macrophages and microglia, as S-LPS binds to the immune cell without any detoxification or modification^{18,19,27,28}. In contrast, the LPS of E. coli or living Gram negative bacteria undergoes detoxification by Kupffer's cells in the liver over time²⁹.

The intracortical injection of a single dose of 2 micogram LPS in stressed rats, has been shown to strengthen the LPS-induced inflammatory changes such as activated microglia and the loss of astroglia and neurons in the prefrontal cortex. This response shows the synergistic effect between LPS-induced inflammation and stress in neurodegenerative diseases including PD²⁹. The induction of neuroinflammation and PD by acute gram negative sepsis has been demonstrated in a case report where acute sepsis-induced PD was treated with only antibiotics⁵.

The unusual pattern of damage to the striatum with greater involvement of the Nucl. Caud compared to putamen in this case report in contrast to idiopathic PD where the damage of the putamen is greater than that in the Nucl. Caud, might be explained by the neuroanatomy, as the Nucl. Caud. are located nearer to the ventricles which are filled with LPS-containing CSF than the putamen.

The neuro protective and restorative effect of the enkephalins, which is the primary basis for the use of [H-3]-Leu-Enk in the treatment of this case has been established by various studies. Resultantly deficiencies or alterations in the function of enkephalins result in neurological disease like LPS induced PD.

Storage of Methionine-enkephalin in the brain: Met-enkephalin (Met-enk) deficiency has been demonstrated in the brains of patients with PD³⁰. In healthy subjects met-enkephalin levels are highest in the caudate nucleus and putamen and lowest in the hippocampus and cerebral cortex³⁰. In PD a 80-95% reduction in the Met-enk concentration has been observed 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 immunoreactivity in the mesencephalon has also been observed in the nerve terminals surrounding the DA neurons, probably in the astrocytes. The depletion of enkephalin stores in the astrocytes of rats with LPS-induced PD may be responsible for the degeneration of astrocytes.

Leu-Enk improves the cerebral blood flow.

The opioid neuropeptide Leu-enk improves pial microcirculation and cerebral blood flow in moderate to severe brain ischemia³¹. The restoration of cerebral blood flow and vasomotor reaction of the pial micro vessels, results in rapidly and intensively improved peripheral and central lymph circulation even against the background of decreased cardio- and haemodynamic parameters.

Leu-Enk stimulates the lymph flow.

Lymphostimulation is an effective method of treating ischemia. Leu-enk is a most effective immunostimulator with direct lymph stimulating effects. The peripheral Leu-enk-induced lymph stimulation is achieved by the prevention or restoration of the damaged local circulation in the ischemized brain cortex³². In rats, the response of pial vessels to i. p. injected Leu-enk (40 microgram/kg) was studied before and after bilateral occlusion of the common carotid arteries. Leu-enk preserved the circulation stability inspite of lowered arterial pressure and bradycardia. Leu-enk also increased local circulation in the brain cortex by 50-70%, and intensified the lymph flow in micro- to macro vessels. No mortality was reported in the first hour of occlusion. The lymph flow which was measured by puncture of the thoracic lymphatic duct³², showed that initially inactive mesenteric microvessels of the small intestine began to contract intensively with acceleration of the lymph flow velocity in the mesenteric micro vessels.

Microglial NADPH oxidase mediates Leu-enk dopaminergic neuroprotection.

Leu-enk is neuroprotective to LPS-induced damages of dopaminergic neurons at femtomolar concentration (10^{-15} to 10^{-13}) through its anti-inflammatory properties¹⁷. The tetrapeptide des-tyrosine Leu-enk and the tri-peptide glycyl-glycine-phenylalanine have also been found to be neuroprotective. However they are only neuroprotective in PHOX+ cell cultures. PHOX is a catalytic subunit gp91 of the

NADPH-oxidase complex. NADPH oxidase is an inducible electron transport system in phagocytic cells which lead to the production of the free radical hydrogen peroxide¹⁷.

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

Receptors for opioid peptides like met- and Leu-enk 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-enk²⁰.

Leu-Enk neurotransmitter release stimulation in primary sensory neurons.

Physiological nmol to pmol concentration of Leu-enk prolongs the action potential duration of primary sensory neurons by binding to stimulatory G-protein coupled delta-opioid receptors of presynaptic terminals with elevation of cAMP levels and stimulation of calcium influx. The glutamate release is increased in the synaptic cleft (Fig. 5-7). LPS blocks the Leu-enk stimulatory effect by binding to alpha₂-delta₂-subunits of voltage-gated calcium channels and to delta-opioid receptors. Increased levels of Leu-enk by oral uptake are able to restore the function of LPS-blocked G-protein coupling to delta-opioid receptors by causing a new palmitoylation signal to cysteine 333^{33,34}.

The negatively charged LPS binds to positively charged pentapeptide sequences of the following important neuronal receptor proteins: delta-opioid-receptors (Fig. 6-7), alpha₂-delta₂-subunit of voltage-gated calcium channels, NADPH oxidase NOX₅ and

Enzyme myosin light chain kinase 2 of skeletal muscles. Leu-enk might be able to prevent the binding of LPS to these peptides with restoration of the protein function including reduced amount of LPS-caused conduction blocks. Leu-enk in micromol concentration showed excellent anti-

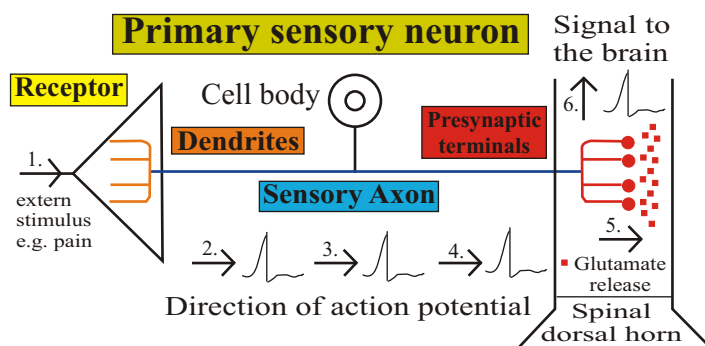


Figure 6. Structure of primary sensory Neuron

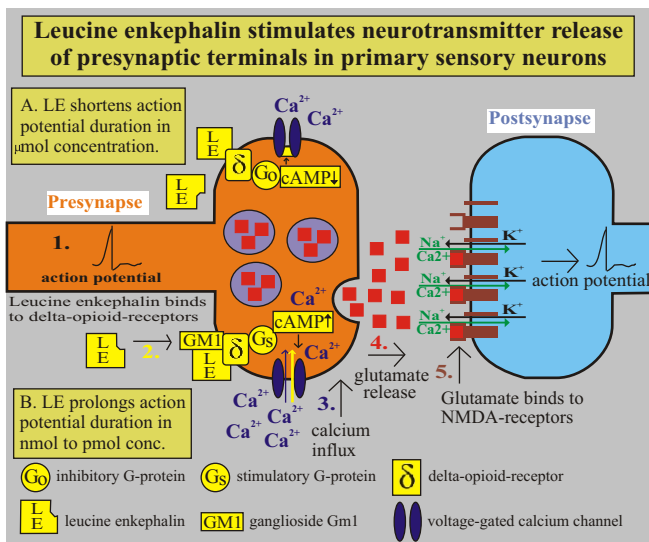
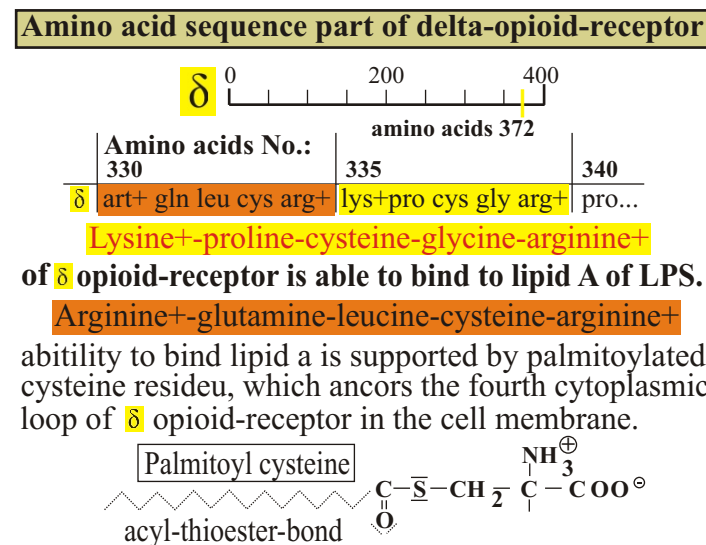


Figure 7. Leucine enkephalin stimulates neurotransmitter release of presynaptic terminals in primary sensory neurons



parkinsonian and anti-inflammatory effects of much more intensity than L-dopa and pramipexole administered for years in the patient.

Figure 8. Part of the amino acid sequence of delta-opioid-receptors:

LPS blocks the Leu-enk stimulatory effect in primary sensory neurons by:

1. Binding directly to delta-opioid-receptors with steric hindering of G-protein coupling
2. Binding to ganglioside GM1 coupled to amino acid arginine No. 192 of the delta-opioid-receptor

Effects of tritiated [H-3]-Leu-Enk.

In vitro radiodetoxification of LPS by beta-radiation with cobalt-60 is known³⁵. Irradiation of LPS reduces the toxicity of LPS due to chemical alterations like decrease of glucosamine, 2-keto-desoxy-octuronic acid (KDO) and fatty acids (Ref. 35,36 Bartok).Positron-protonated peptides mostly fragment to produce N-terminal and C-terminal

fragment ions^{37,38}. The gas-phase structures of the protonated Leu-enk are investigated via infrared multiple-photon-dissociation spectroscopy. The singly protonated Leu-enk precursor is protonated to ion forms with different types of structures:

- linear isomers with a C-terminal oxazolone ring and cyclic peptides structures as well as linear imine-type structures and cyclic structures³⁷.

The oxazolone and imine-type structures are already being used in Schleswig-Holstein, Germany to produce pharmaceutical products like psychopharmaceutical and antibiotic drugs. Though there is a risk of radiation and reduced detoxification by the human liver of these positron-protonated drugs, which could cause oedematic swellings and inflammation with sepsis and even liver failure in the patients taking these drugs. The adverse effects of the positron induced radiation, can be prevented through the use of tritiated Leu-enk as described in this case report.

Effects of [H-3]-Leu-enk on LPS-induced septic polyneuropathy and encephalopathy.

When Salmonella minnesota S-LPS is taken up by cells such as macrophages, microglia and astrocytes, without chemical modification, they become intercalated in the cell membranes and Golgi-apparatus causing chronic inflammation with progressive neurodegeneration in the patient. Lipid A of LPS binds to pentapeptide sequences of B-H-P-H-B or B-H-B-H-B (B: Arg+, Lys+, h: hydrophobic amino acid, p: polar amino acid)³⁹, (Fig. 7). The intracellular C-terminal loop of delta-opioid-receptors is blocked by lipid A bound to lys+pro-cys-gly-arg+ (Fig. 6) resulting in inability to couple to G-proteins with the cessation of Leu-enk signals. Stimulatory Gs-protein coupled delta-opioid-receptors bind to ganglioside GM1 with the amino acid arginine 192. Cells without GM1 or antibody blocked GM1 like in Campylobacter jejuni infections (Guillain-Barré-Syndrome) react to Leu-enk by Go-protein coupled inhibitory signals.

Polyneuropathy.

LPS-blocked delta opioid receptors downregulate preproenkephalin mRNA expression with reduced Leu-enk levels. Polyneuropathy patients have very low blood Leu-enk levels. Increased levels of Leu-enk by oral uptake are able to activate blocked delta-opioid receptors by causing a new palmitoylation signal to cysteine 333 (Fig. 7) with the ability of the C-terminal loop to bind to Gs-proteins again with increased levels of cAMP and increased calcium influx via voltage-gated calcium channels. The Leu-enk stimulatory signal causes expression of GM1. Leu-enk seems to start a self-propagating mechanism with reactivation of LPS-blocked delta-opioid receptors, upregulated preproenkephalin mRNA and GM1 expression leading to normalized Leu-enk levels in the nervous and immune system of the patient. This self propagating action of leu-enk is typified by the long term effects of leu-enk in the patient even after the discontinuation of treatment for months.

Encephalopathy.

Neuroinflammation with memory impairment induced by chronic intraventricular LPS infusion was marked, by

complete block of voltage-gated calcium channel dependent long-term potentiation in rats' hippocampus⁴⁰. This is probably caused by the binding of LPS to pentapeptide arg⁺-pro-arg⁺-tyr-arg⁺ of alpha₂-delta₂-subunits of calcium channels. Leu-enk thus increases the influx of calcium into cells and improves memory function. The expression of the inflammatory status of the dorsal horn ganglions change from alpha₂-delta₂-subunits to alpha₂-delta₁-subunits without LPS-binding pentapeptide. Ultralow fmol concentration of Leu-enk have neuroprotective effects on delta-opioid-receptor though the reduction of reactive oxygen species production in LPS-stimulated mixed microglia dopaminergic cell cultures. Through these mechanisms Leu-enk thus stimulates cerebral blood flow with improved dopamine and glucose metabolism which give explanation to the clinical response of the patient.

CONCLUSIONS

Radio labelled leucine enkephalin (leu-enk), is effective in treating acute and chronic polyneuropathy and parkinsonian features of gram negative sepsis as shown in this first case report of its use. Leu-enk effects results from various mechanisms which lead to reduced neuroinflammation and improved cerebral blood and lymphatic flow in addition to other molecular actions. The effects of leu-enk are also long term and sustained after it is discontinued. The use of this novel treatment approach is advocated in treating the neurological and other complications of sepsis and endotoxemia.

REFERENCES

1. Annane D, Bellissant E, Cavaillon JM. Septic shock. *Lancet*. 2005; 365: 63-78.
2. Brandtzaeg P, Kierulf P, Gaustad P et al. Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. *J Infect Dis*. 1989; 159(2): 195-204.
3. Sikkeland LI, Skogstad M, OvstebÅ, R, Brusletto B et al. Circulating lipopolysaccharides in the blood from "bioprotein" production workers. *Occup Environ Med*. 2008; 65(3): 211-4.
4. DeMaria A Jr, Johns MA, Berberich H et al. Immunization with rough mutants of Salmonella minnesota: initial studies in human subjects. *J Infect Dis*. 1988; 158(2): 301-11.
5. Alasia DD, Asekomeh GA, Unachuku CN. Parkinsonism induced by sepsis: a case report. *Niger J Med*. 2006; 15(3):333-6.
6. Niehaus I. Lipopolysaccharides induce inflammation-mediated neurodegeneration in the substantia nigra and cerebral cortex (a case report). 6th International Conference AD/PD 2003. Monduzzi Editorial, Bologna, Italy. pp 34-38.
7. Niehaus I, Lange JH. Endotoxin: is it an environmental factor in the cause of Parkinson's disease? *Occup Environ Med*. 2003 May;60(5):378.
8. Brown RF, Jackson GD, Martin T et al. Bacterial lipopolysaccharide induces a conduction block in the sciatic nerves of rats. *Lab Anim Sci*. 1999; 49(1):62-9.
9. Lustig S, Danenberg HD, Kafri Y et al. Viral neuroinvasion and encephalitis induced by lipopolysaccharide and its mediators. *J Exp Med*. 1992; 176(3): 707-12.
10. Yuki N, Taki T, Inagaki F et al. A bacterium lipopolysaccharide that elicits Guillain-Barré-syndrome has a GM1 ganglioside-like structure. *J Exp Med*. 1993; 178(5): 1771-5.
11. Mohammadi B, Schedel I, Graf K et al. Role of endotoxin in the pathogenesis of critical illness polyneuropathy. *J Neurol*. 2008; 255(2):265-72.
12. Watanabe K, Kim S, Nishiguchi M et al. Brucella melitensis infection associated with Guillain-Barré-syndrome through molecular mimicry of host structures. *FEMS Immunology and Medical Microbiology* 2005; 45: 121-127.
13. Witt NJ, Zochodne DW, Bolton CF et al. Peripheral nerve function in sepsis and multiple organ failure. *Chest*. 1991; 99(1): 176-84.
14. Folgar S, Gatto EM, Raina G, Micheli F. Parkinsonism as a manifestation of multiple sclerosis. *Mov Disord*. 2003; 18(1): 108-10.
15. Castano A, Herrera AJ, Cano J, Machado A. The degenerative effect of a single intranigral injection of LPS on the dopaminergic system is prevented by dexamethasone, and not mimicked by rh-TNF-alpha, IL-1beta and IFN-gamma. *J Neurochem*. 2002; 81(1):150-7.
16. Villarán RF, Espinosa-Oliva AM, Sarmiento M et al. Ulcerative colitis exacerbates LPS-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson's Disease. *J Neurochem* June 24, 2010; Published online in advance of print.
17. Qin L, Liu Y, Qian X, Hong JS, Block ML. Microglial NADPH oxidase is a novel target for femtomolar neuroprotection against oxidative stress. *Ann N Y Acad Sci*. 2005; 1053: 107-20.
18. Qin L, Wu X, Block ML et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia*. 2007; 55(5):453-62.
19. Qian L, Flood PM, Hong JS. Neuroinflammation is a key player in Parkinson's disease and a prime target for therapy. *J Neural Transm* Jun 23 2010; published online.
20. Das KP, Hong JS, Sanders VM. Ultralow concentrations of proenkephalin and [met⁵]enkephalin differentially affect IgM and IgG production by B cells. *J Neuroimmunol* 1997; 73(1-2): 37-46.
21. Hunter RL, Choi DY, Ross SA, Bing G. Protective properties afforded by pioglitazone against intrastriatal LPS in Sprague-Dawley rats. *Neurosci Lett*. 2008; 432(3): 198-201
22. Smirnova VI, Pulina NN, Kazannov VV et al. Possible use of dalargin in the intensive-care of emergencies. *Vestn Ross Akad Med Nauk* 1994; 6: 48-51.
23. Pashutin SB, Zinovyeva TD, Tsygankova LG, Smirnova VI. Prevention of infectious complications with dalargin in cardio-surgical patient. *Khirurgiya (Mosk)* 1993; 11: 55-59.
24. Holthoff VA, Vieregge P, Kessler J et al. Discordant twins with Parkinson's disease: positron emission tomography and early signs of impaired cognitive circuits. *Ann Neurol*. 1994; 36(2): 176-82.
25. Snow BJ, Vingerhoets FJ, Langston JW et al. Pattern of dopaminergic loss in the striatum of humans with MPTP induced parkinsonism. *J Neurol. Neurosurg. Psychiatr*. 2000; 68(3):313-6.

26. Kim S, Reuhs BL, Mauer LJ. Use of Fourier transform infrared spectra of crude bacterial lipopolysaccharides and chemometrics for differentiation of *Salmonella enterica* serotypes. *J Appl. Microbiol.* 2005; 99(2):411-7.
27. Ge Y, Ezzell RM, Tompkins RG, Warren HS. Cellular distribution of endotoxin after injection of chemically purified lipopolysaccharide differs from that after injection of live bacteria. *J Infect Dis.* 1994; 169(1):95-104.
28. Vasselon T, Hailman E, Thieringer R, Detmers PA. Internalization of monomeric lipopolysaccharide occurs after transfer out of cell surface CD14. *J Exp Med.* 1999; 190(4): 509-21.
29. de Pablos RM, Villaran RF, Arguelles S, Herrera AJ et al. Stress increases vulnerability to inflammation in the rat prefrontal cortex. *J Neurosci.* 2006; 26(21): 5709-19.
30. H. Taquet, Javoy-Agid F, Lesselin F, Agid Y. Methionine-Enkephalin deficiency in brains of patients with Parkinson's Disease. *Lancet* 1981; 317(8234):1367-1368.
31. Khugaeva VK, Aleksandrov PN, Aleksandrin VV. Effect of opioid lymph stimulation on the microcirculation in pial vessels of the ischemized rat brain. *Bulletin Exp. Biol. Med.* 1995; 129(1): 100-105.
32. Khugaeva VK, Bespalova ZD. Effects of Leu-enkephalin analogue on cerebral circulation in cerebral ischemia of different severity. *Bulletin Exp. Biol. Med.* 1998; 11: 1100-1102.
33. Crain SM, Shen KF. Opioids can evoke direct receptor-mediated excitatory effects on sensory neurons. *Trends Pharmacol Sci* 1990; 11(2): 77-81.
34. Petäjä-Repo UE, Hogue M, Leskelä TT et al. Distinct subcellular localization for constitutive and agonist-modulated palmitoylation of the human delta-opioid receptor. *J Biol Chem.* 2006; 281(23): 15780-9
35. Dunzendorfer S, Lee. TLR4 is the signaling but not the lipopolysaccharide. *J Immunol* 2004; 173(2): 1166-70.
36. Munford RS, DeVeaux LC, Cronan JE Jr, Rick PD. Biosynthetic radiolabeling of bacterial lipopolysaccharide to high specific activity. *J Immunol. Methods* 1992; 148: 115-120
37. Polfer NC, Oomens J, Suhai S, Paizs B. Infrared spectroscopy and theoretical studies on gas-phase protonated Leu-enkephalin and its fragments: direct experimental evidence for the mobile proton. *J Am Chem Soc* 2007; 129: 5887-5897
38. Florian Schinle. Mass selective mid-IR MPD spectroscopy in a penning trap: Investigation of leucine enkephalin. Poster presentation at the European FT-MS Conference in Lausanne May 2010
39. Frecer V, Ho B, Ding JL. Interpretation of biological activity data of bacterial endotoxins by simple molecular models of mechanism of action. *Eur J Biochem.* 2000; 267(3):837-52.
40. Min SS, Quan HY, Ma J et al. Chronic brain inflammation impairs two forms of long-term potentiation in the rat hippocampal CA1 area. *Neurosci Lett.* 2009; 456(1):20-4.