Leucine Enkephalin as Treatment of septic Polyneuropathy and Encephalopathy

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Abstract

This is the first case report of effective treatment of long-term neurological sequelaes of sepsis with leucine enkephalin (LE). The 37 years old female patient is suffering from lipopolysaccharide-induced polyneuropathy, encephalopathy and parkinsonism 14 yrs after sepsis. Acute and long-term neurological sequelaes of sepsis occur in up to 70% of the patients.
LE is used in orally administered doses of 1 micromol every third day. A lot of neurological symptoms like reduced sensibility, weakness, walking abilities, concentration, bradykinesia and rigidity are markedly improved 5 hours after taken one dose of LE and also after months.
The neurotransmitter LE with the amino acid sequence tyrosine, glycine, glycine, phenylalanine, leucine is neuro- and cytoprotective. LE increases the cerebral blood and lymphatic flow. The Russian synthetic LE product Dalargin is helpful in treating postoperative patients with multiorgan failure.
LE stimulates the neurotransmitter release of primary sensory neurons. Physiological nmol to pmol concentration of LE 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. LPS is blocking the LE stimulatory effect by binding to alpha$_2$-delta$_2$-subunits of voltage-gated calcium channels and to delta-opioid receptors. Increased levels of LE 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.

Introduction

Lipopolysaccharides (LPS):
LPS are part of the outer cell wall of Gram negative bacteria (Fig. 1).[1] LPS are highly biohazardous (4 ng LPS/kg i.v. in humans as minimal pyrogenic dose)! Higher doses of LPS cause a systemic inflammatory response with sepsis-like symptoms, septic shock, multiple organ system failure, and lethality in worst cases. Long-term neurological sequelaes occur in up to 70% of septic patients.[7]

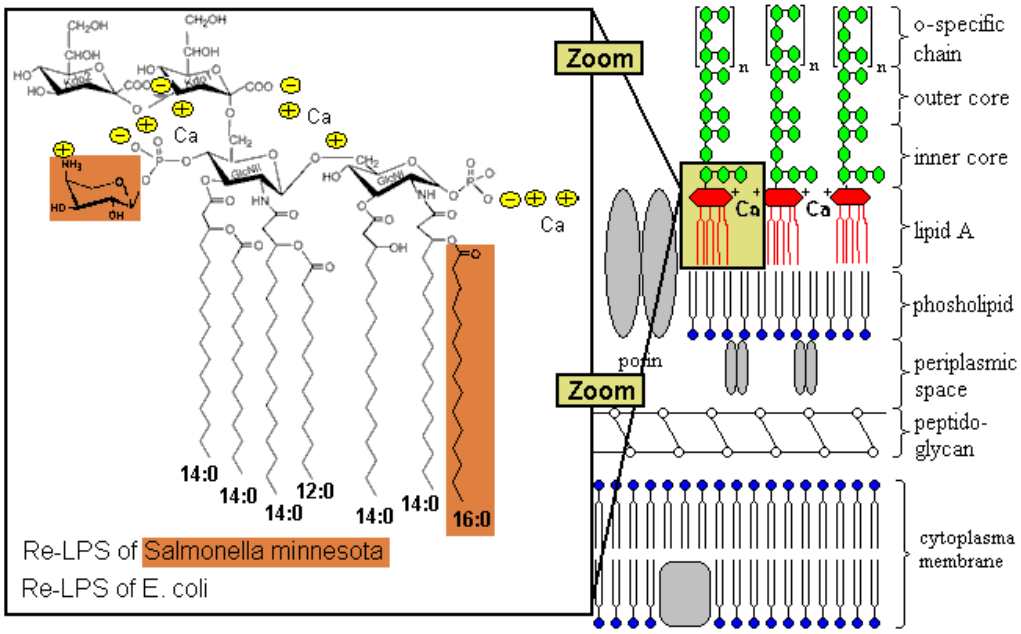


Fig. 1: Structure of the Gram negative membrane with calcium-LPS-complexes

Leucine Enkephalin:
Leucine enkephalin is a pentapeptide (Fig. 2) with the amino acid sequence of tyr-gly-gly-phe-leu.

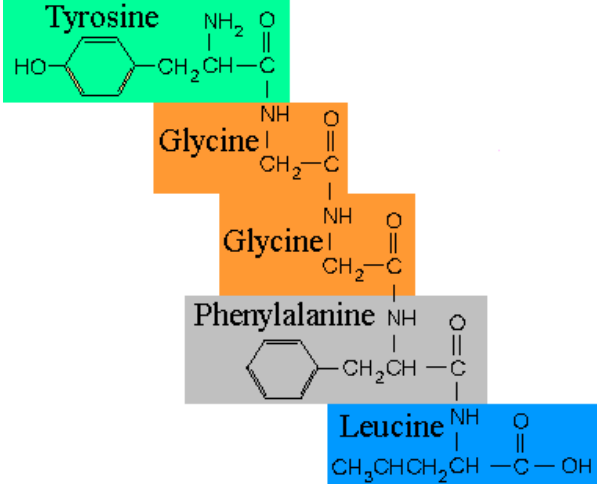


Fig. 2: Structure of Leucine Enkephalin

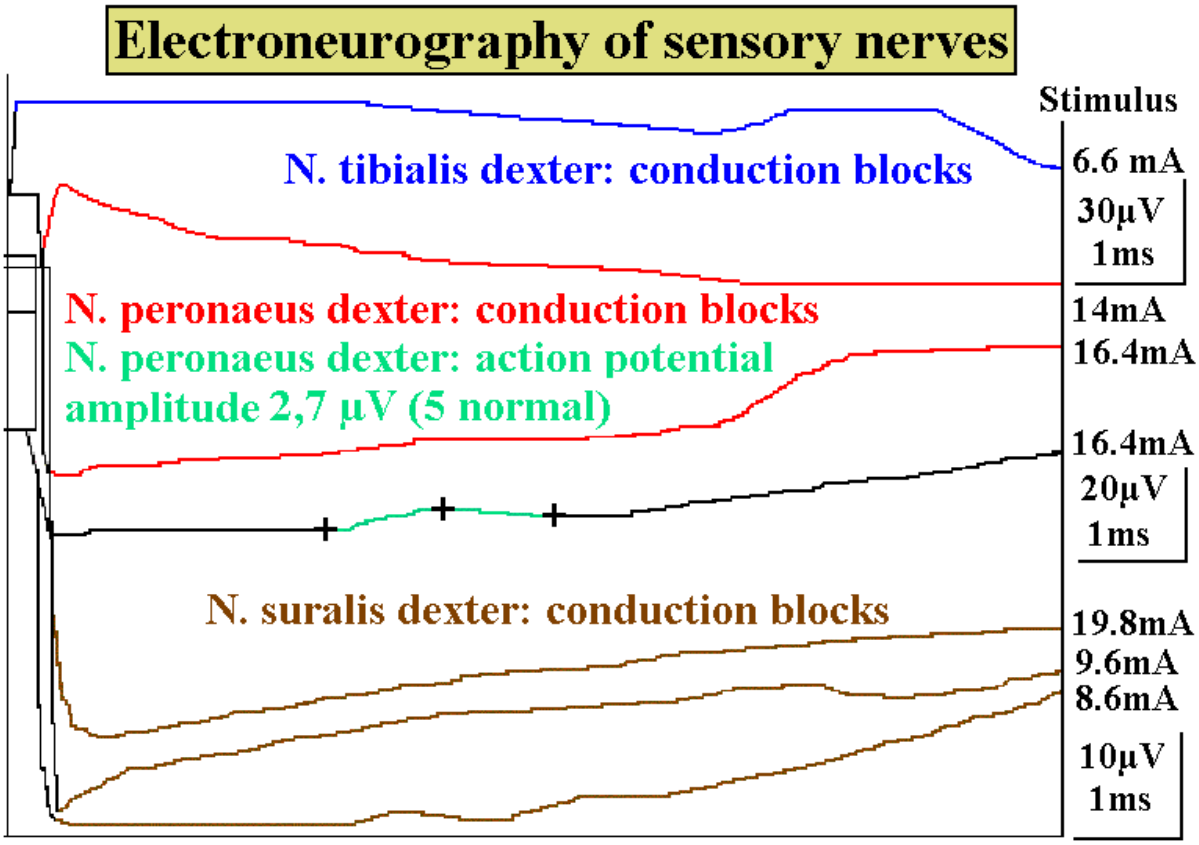
Tab.1 : Effects of Leucine Enkephalin (LE) in Humans

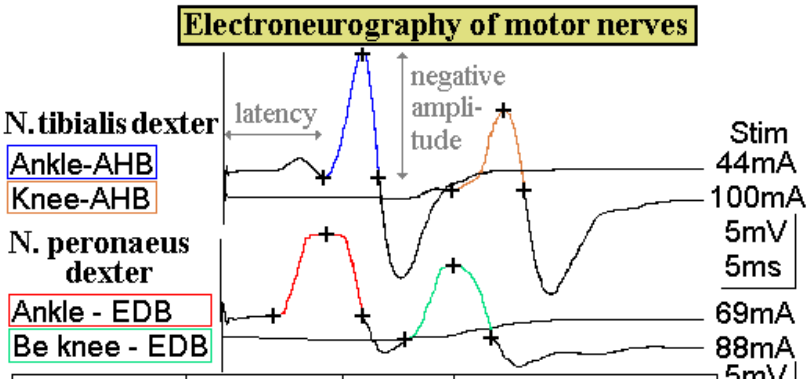
Nervous System	**Immune System**
neurotransmitter	synthetic LE Dalargin prevents infection in cardiosurgical pat.
opioid peptide	Dalargin reduces respiratory distress syndrome severity (12)
binds to G-protein coupled delta-opioid receptors	Dalargin useful in treating postoperative multiorgan failure (11)
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 (6)	oxidative damage of organ transplants reduced
stimulatory effect blocked by ganglioside GM1 antibodies	reduces LPS-induced TNF-αlpha production of macrophages
neuroprotective in LPS-stimulated dopaminergic cell cultures	reduces anti-LPS-IgM-antibodies in LPS-stimulated B-cells
neuroprotective in femtomol conc. by inhibiting PHOX (9)	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 methamphetamin 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	preproenkephalin mRNA
improvement of cerebral blood flow in brain ischemia (13)	protein proenkephalin
reduces high intraocular pressure	processing in trans-golgi-network to tyr-gly-gly-phe-leu
antiarrythmic heart effects	storage of LE in 80-120 nm large dense-core vesicles
vasotonic in femtomolar concentration	long stimulus for release of LE in synaptic cleft

Summary of Case Report

This is the first case report describing a treatment with leucine enkephalin for an up to now 14 years long-lasting persistent endotoxemia causing chronically systemic and neuronal inflammation with septic polyneuropathy, encephalopathy and parkinsonism after one single accidental contamination with 10 µg highly purified Salmonella minnesota S-LPS in 1995. The LPS has not been detoxified by the body proven by a 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 100% identified Salmonella minnesota S-LPS. Positron emission tomography (PET) with Fluoro-Dopa showed ca. 70% loss of dopaminergic function in the striatum in 2001. Cerebral glucose metabolism was determined with [Fluorine-18] fluoro-2-deoxy-D-glucose using PET in 1998. In summary the usually normal 100% 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. In 2006 electroneurography conduction studies showed conduction blocks of sensory nerves and missing F-waves in the N. peronaeus, which is the most sensible nerve to endotoxins in the blood of septic patients (Fig. 3).[3,4] Treatment with 1 µmol LE started in December 2008.

Results of Electroneurography: LPS-induced Polyneuropathy





	distal latency	amplitude	nerve conduction velocity
N. tibialis dexter:			
ankle-knee	7.2 ms (delayed)	8.7 mV	45.3 m/s
knee-AHB	15.8 ms (strong delayed)	5.3 mV (reduced)	
N. peronaeus dexter:			
ankle – Be knee	3.8 ms	5.5 mV	43.7 m/s
Be knee – EDB	12.6 ms	5.0 mV	

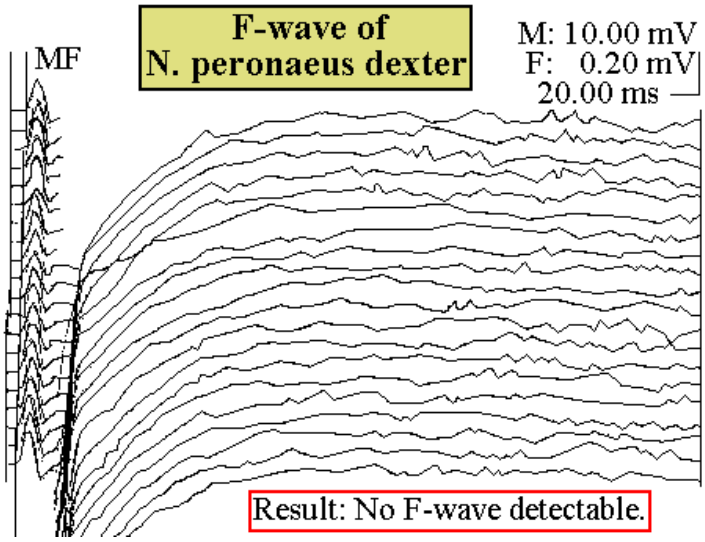


Fig. 3: Results of electrophysiological examinations of the 34 years old female patient 11 years after sepsis in 2006.

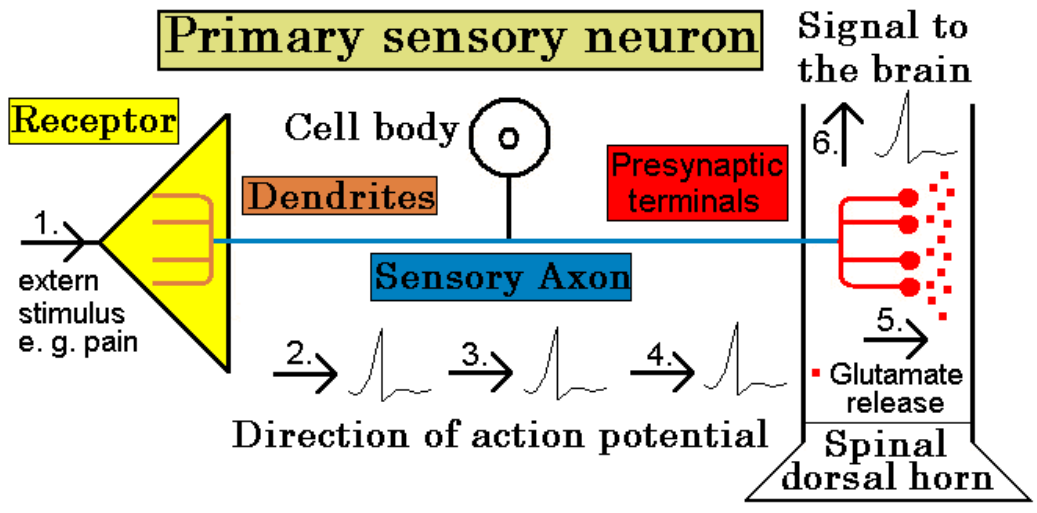
Results of Treatment with Leucine Enkephalin in the Case Report

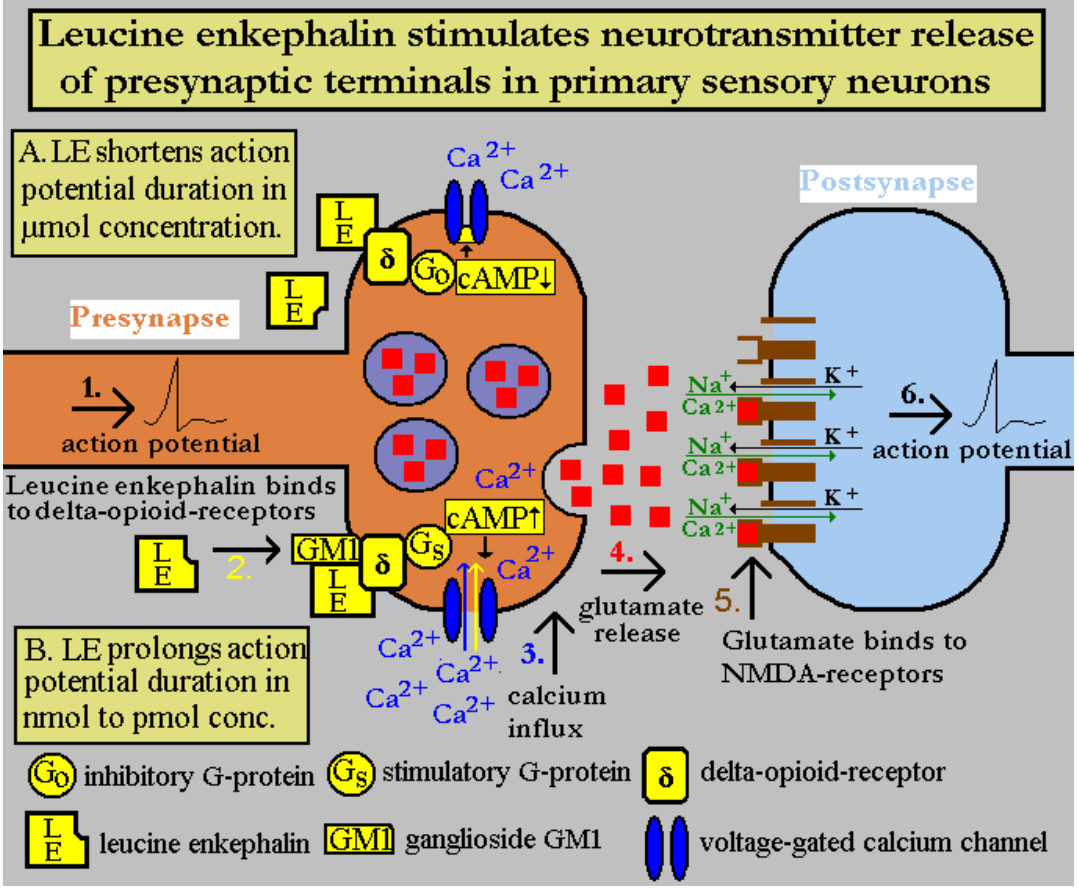
Tab.2 : Effects of Leucine Enkephalin Treatment on different Symptoms

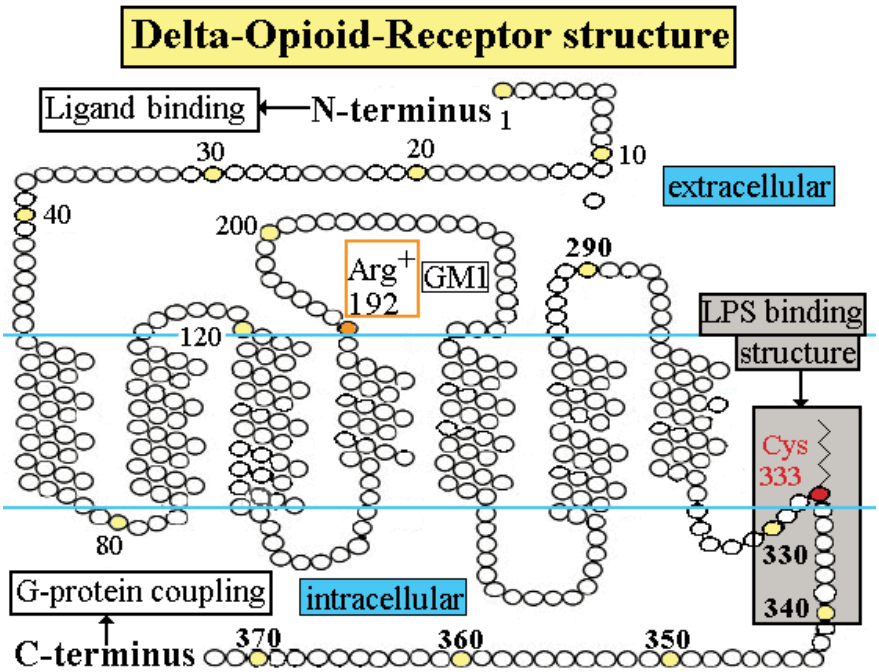
Polyneuropathy			**Encephalopathy**			**Parkinsonism**			**Others**		
Pain	+	++	Concentration	++	++	Rigidity	++	+	Myocloni	++	+
Paraesthesia	+	+	Tiredness	0	+	Stiffness	++	+	Seizures	+	+
Numbness	++	+	Short time memory	++	+	Tremor	0	+	Dysphagia	+	+
Reduced sensibility	+++	+++	Long time memory	0	+	Bradykinesia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

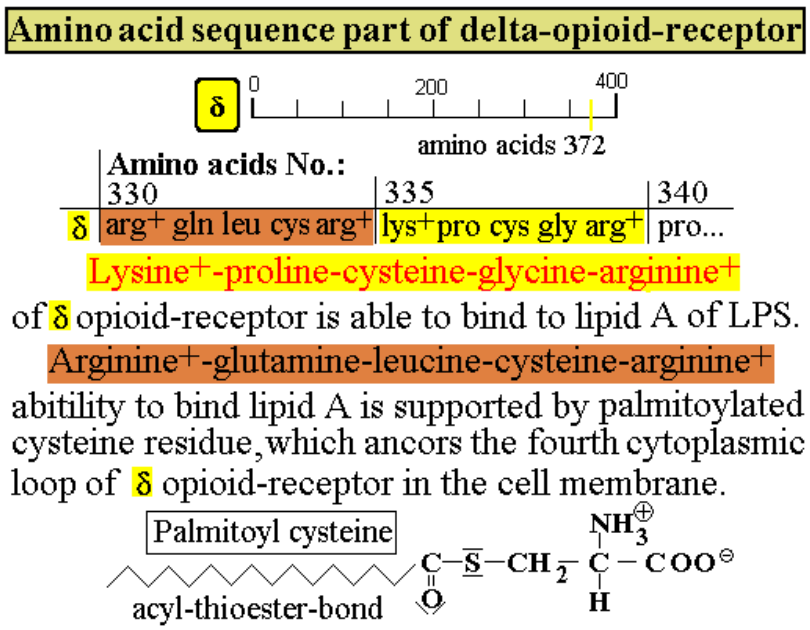
in grey colour: effects 5 hours after 1 x 1 µmol LE		in white colour: long-term effects of LE	
0	no effect	++	strong improvement of symptoms
+	improvement of symptoms	+++	very strong improvement of symptoms

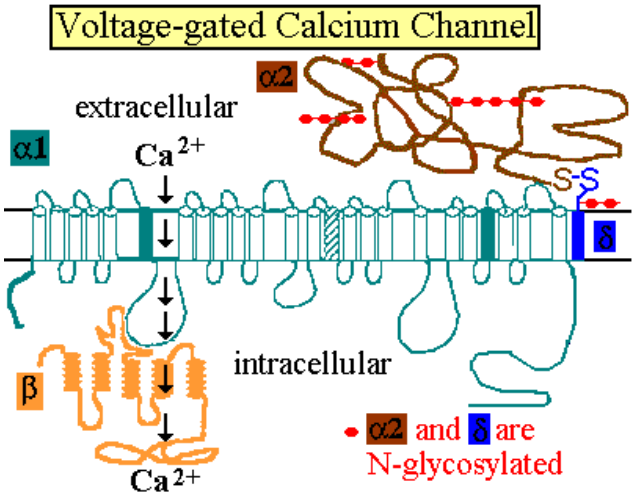
Effects of Leucine Enkephalin in primary Sensory Neurons




Leucine enkephalin stimulates neurotransmitter release of presynaptic terminals in primary sensory neurons
A. LE shortens action potential duration in µmol concentration.
Presynapse
Postsynapse
Ca 2+
cAMP↓
1.
action potential
Leucine enkephalin binds to delta-opioid-receptors
2.
GM1
cAMP↑
Gs
Go
δ
LE
3.
calcium influx
4.
glutamate release
5.
Glutamate binds to NMDA-receptors
6.
action potential
Na+
K+
B. LE prolongs action potential duration in nmol to pmol conc.
Go inhibitory G-protein
Gs stimulatory G-protein
δ delta-opioid-receptor
LE leucine enkephalin
GM1 ganglioside GM1
voltage-gated calcium channel


Delta-Opioid-Receptor structure
Ligand binding
N-terminus
1
10
20
30
40
extracellular
200
290
Arg+
192
GM1
120
LPS binding
structure
Cys
333
330
340
80
G-protein coupling
intracellular
C-terminus
370
360
350





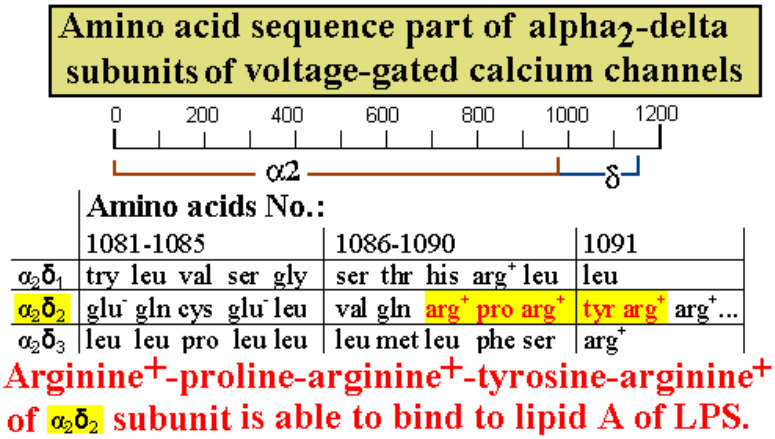


Fig. 4: LPS blocks the LE stimulatory effect in primary sensory neurons by:
1. Binding to d-opioid-receptors with steric hindering of G-protein coupling
2. Binding to alpha$_2$-delta$_2$-subunits of voltage-gated calcium channels reducing calcium influx into the cell
3. Binding to ganglioside GM1 linked to delta-opioid-receptors by arg 192

Discussion and Conclusions:

Salmonella minnesota S-LPS was uptaken by e. g. macrophages, microglia and astrocytes, being chemically unmodified intercalated in 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).[1] The intracellular C-terminal loop of delta-opioid-receptors is blocked by lipid A bound to lys+-pro-cys-gly-arg+ (Fig. 4) resulting in inability to couple to G-proteins with stopping of LE signals. Stimulatory Gs-protein coupled delta-opioid-receptors bind to ganglioside GM1 with arg 192. Cells without GM1 or antibody blocked GM1 like in Campylobacter jejuni infections (Guillain-Barré-Syndrome) react to LE by Go-protein coupled inhibitory signals.

How is orally administered LE effective in LPS-induced septic polyneuropathy and encephalopathy?

Polyneuropathy: LPS-blocked delta opioid receptors downregulate preproenkephalin mRNA expression with reduced LE levels. Polyneuropathy patients have very low blood LE levels. Increased levels of LE by oral uptake are able to activate blocked delta-opioid receptors [5] by causing a new palmitoylation signal to cysteine 333 (Fig. 4) 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 LE stimulatory signal causes expression of GM1. As the treatment with LE in the patient have long-term effects even after stopping the uptake of LE for months LE seems to start a self-propagating mechanism with reactivation of LPS-blocked delta-opioid receptors, upregulated preproenkephalin mRNA and GM1 expression leading to normalized LE levels in the nervous and immune system of the patient.

Encephalopathy: Neuroinflammation with memory impairment induced by chronic intraventricular LPS infusion was marked by completely block of voltage-gated calcium channel dependent long-term potentiation in rats' hippocampus,[8] probably caused by binding of LPS to pentapeptide arg+-pro-arg+-tyr-arg+ of alpha$_2$-delta$_2$-subunits of calcium channels (Fig. 4). LE increases the influx of calcium into cells and improves memory function. Dorsal horn ganglions are switching in inflammatory status the expression of alpha$_2$-delta$_2$-subunits to alpha$_2$-delta$_1$-subunits without LPS-binding pentapeptide. LE stimulates the cerebral blood flow [13] with improved dopamine and glucose metabolism of the patient. Ultralow fmol concentration of LE are delta-opioid-receptor independent neuroprotective by reduction of the production of reactive oxygen species in LPS-stimulated mixed microglia dopaminergic cell cultures.[9]

Conclusions: This is the first case report reporting about an effective treatment of septic polyneuropathy and encephalopathy with parkinsonism with µmol doses of LE every third day. Dalargin (Russian synthetic LE) is useful in treating patients with postoperative multiorgan failures. Clinical trials with septic patients suffering from neurological sequelaes should be considered.

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