BIOCHEMICAL CORRELATES OF LOCOMOTOR ACTIVITY PRODUCED BY EPIDURAL SPINAL CORD STIMULATION

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Abstract – *Obtained results shows that the peptide spectra in CSF before, during low and high frequency SCS are different. It was found that there is correlation between producing of neuropeptids with molecular weight (15-12 kDa) and appearance of stepping movements under high frequency SCS. One of neuropetids which could be produced in this conditions is the P substance. The experiments on rat olfactory cortex slices have shown the difference of molecular-cellular mechanisms under low and high frequency epidural stimulation.*

Keywords: spinal cord, locomotion, CPG, electrophoretical analysis, cerebral spinal fluid.

1. Introduction

It is know that in decerebrated cats the central pattern generator (CPG) can be activated by electrical stimulation of subthalamic and mesencephalic locomotor regions [1] as well as by stimulation of the locomotor strips in dorsolateral funiculi [2]. At the same time in acute spinal cats the locomotor activity can be induced by continuous electrical stimulation of dorsal roots, or dorsal columns only after the injection of the noradrenergic precursor L-dopa [3]. The aim of the study was to determine by means of epidural spinal cord stimulation (SCS) the locomotor ability of the spinal cord deprived of brain control and to evaluate is there the correlation between the locomotor activity produced by SCS and the appearance of specific neuropeptides in cerebral spinal fluid (CSF).

2. Function Types of Activities (Methods)

Epidural stimulation. SCS was performed both in chronic paraplegic patients and in acute experiments on spinal cats. The patients had complete transection of the cord at a level varying from the fourth to twelfth thoracic vertebra, as well as complete absence of voluntary motor control and sensation below the site of injury. In all patients, the proprioceptive and exteroceptive spinal reflex activity was retained. The stimulating electrode was penetrated into epidural space by lateral puncture method. The location of the electrode was verified by fluoroscopy. Recordings of the surface EMG activity in both proximal (*m. quadriceps, m. hamstring*) and distal (*m. soleus, m. tibialis*) muscles were performed in the supine position.

The cats were anaesthetized by injection of kalipsol (30 mg/kg) and following this an anemic or surgery decerebration was performed. The spinal cord was transected at Th10 level and then exposed by a laminectomy at L3- L7 vertebrae. The experiment was started 2-4 hours after spinalization. The EMG activity of *m. quadriceps* (Q), *m. semitendinosus* (St), m. *gastrocnemius* (G) and *m. tibialis anterior* (T) was recorded with bipolar electrodes inserted into these muscles on both sides. The strength of SCS was 10–400 mkA with pulse duration 0.2 ms. The frequency of SCS from 0,5 Hz to 50 Hz was used.

Biological assay method. In rat olfactory tangential cortex slices the focal potentials (FPs) were recorded in response to electrical stimulation of lateral olfactory tract (LOT). The slices were bathed in control medium. The composition of this artificial cerebrospinal fluid (ACSF) at 37.0±0.5 C was (mM): NaCl 124.0, KCl 5.0, $CaCl_2$ 2.6, $MgSO_4$ 1.2, KH_2PO_4 1.24, $NaHCO_3$ 3.0, Tris-HCl (buffer, pH 7.2) 23.0, d-glucose 10.0, pH 7,2-7,3; during the experiment O_2 was constantly fed in the ACSF. Single pulses with 5sec interval were applied to slices during the 15-20 min control period. Then the medium was switched to the testing CSF taken from patients: 1) before, 2) after high frequency (33 Hz) or low frequency (0.5 Hz) of spinal cord electrical stimulation. Parameters of stimulation were the same as in control. Every slice was used for testing of one ACSF type only.

3. Results

It was found that in paraplegic patients most frequently the stepping movements and locomotor- like EMG activity was observed, when the cathode was over the L2-L3 spinal cord segments with frequency of stimulation of 20-50 Hz, pulse duration of 0.3-1 ms and strength of 8-12 V. It is interesting that in spinalized cats the effective point for evoking of locomotor-like activity was located as in humans over preenlargement (L4-L5). As a rule, in the beginning, SCS evoked tonic activity in the hindlimb muscles, but then it was transformed to the burst activity. The optimal location for the electrodes was along the midline, overlying the dorsal columns.

The electroforetical analysis of CSF in patients have indicated that the peptide spectra in CSF before, during low and high frequency SCS are different. Data demonstrated that there is correlation between producing of neuropeptids with molecular weight (15-20 kDa) and appearance of stepping movements under SCS. One of

neuropetids which could be produced in this conditions is the P substance. According recent investigations, during fictive locomotion, this substance increase the frequency of locomotor activity and the duration of EMG bursts [4]. During low frequency SCS the peptides with molecular weight (30-40 kDa) were appeared in CSF.

In order to investigate the molecular-cellular mechanisms of cerebral spinal fluid effects we carry out experiments on the rat olfactory cortex slices. The application of CSF in olfactory cortex slices was 10-5 ml/l. It was revealed that perfusion of CSF taken before SCS induced significant reduction the amplitudes of *N* -methyl-*D*-aspartate (NMDA) acxitatory postsynaptic potential component of FPs in slices and increased of amplitude of slow inhibitory postsynaptic potential (n=4). After high frequency SCS an increase of presynaptic responses (action potential of LOT) but decreased the a-amino-3-hydroxy-5-methylisoxazole-4- propionic acid (AMPA) and NMDA postsynaptic components of FPs (n=4) was occured. The addition of CSF after low SCS resulted in the increase of amplitude presynaptic and postsynaptic components of FPs in tested slices (n=4).

During in wash the effects of different types of CSF were long (30-40 min). These data demonstrate that some specific substances are released into extracellular fluid in spinal patients. Data obtained indicate that these effects were caused more likely by polypeptides contained in CSF. At first, the ones evoked the long-term changes of excitability of slices. And secondly, these substances activated the different neurophysiological mechanisms: NMDA EPSP, fast IPSP.

Moreover, phasical reactions of Fps indicate the heterogeneity of peptides in CSF. Further experiments are in progress to investigate the nature and identification of the active substances in CSF.

For combined epidural electrical stimulation and epidural drug electrophoresis we have developed original device. The device used for transplantation was made from the compact titanium and consisted of a system of passages, the two electrodes and the microinjector. The device was implanted between bodies of the resected vertebrae on the final stage of the surgical anteriolateral decompression. The two electrodes implanted percutaneously in the posterior epidural space served as an anode, the device itself served as a cathode. We believe that the use of SCS and drug intervention simultaneously will effective in regulation of motor activity.

References

[1] Shik, M.L., (1994). Neurophysiology of mammalian locomotion. In Sovet scientific reviews: Section F. Physiology and general biology (Vol. 6, Part 5, pp. 1-49). Yverdon, Switzerland: Harwood Academic Publishers.

[2] Kazennikov, O.V. et al., (1983). Stepping elicited by stimulation of the dorsolateral funiculus in the cat spinal cord. Bulletin of Experimental Biology and Medicine, 96(8), 8-10. (In Russian).

[3] Grillner, Zangger, (1979). On the central generation of locomotion in the low spinal cat. Experimental Brain Research. 34, 241-261.

[4] Barthe and Clarac, (1997). Modulation of the spinal network for locomotion by substance P in the neonatal rat. Experimental Brain Research. 115(3): 485-92.

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