L-DOPA-induced Dyskinesia is Associated with Regional Increase of Striatal Dynorphin Peptides as Elucidated by Imaging Mass Spectrometry

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Introduction

The dopamine precursor L-DOPA (L-3,4-dihydroxy-phenylalanine) is still the most effective drug for symptomatic treatment of Parkinson’s disease (PD). However L-DOPA pharmacotherapy is accompanied by debilitating motor complications including L-DOPA-induced dyskinesia (LID). Opioid peptides are involved in various pathophysiological processes, including algesia, epilepsy, and drug dependence. A strong association between L-DOPA-induced dyskinesia (LID) and elevated prodynorphin mRNA levels has been established in both patients and in animal models of Parkinson’s disease [1]. However, to date the actual endogenous prodynorphin peptide products have not been characterized. In fact, little is known about which distinct opioid precursor-derived peptide products are involved in pathophysiologic mechanisms underlying LID. PPE mRNA can be processed into e.g. enkephalin, leu-enkephalin (Leu-Enk), met-enkephalin (Met-Enk), and enkephalin-containing peptide E and peptide F, whereas PDyn mRNA can be processed into several different dynorphins; including dynorphin A (DynA), dynorphin B (DynB), alpha-/beta-neoendorphin (a/bNeo), and Leu-Enk [2].

Imaging mass spectrometry (IMS) is an emerging technique of great potential for spatial profiling of molecular species in biological tissue samples [3] IMS has the unique advantage of high sensitivity and high molecular specificity, allowing comprehensive detection of multiple molecular species in a single tissue section [3, 4].

Methods

Here, matrix assisted laser desorption/ionization (MALDI) imaging mass spectrometry was used for characterization, localization, and relative quantification of striatal neuropeptides in a rat model of LID in Parkinson’s disease [1, 5].

A number of 30 animals received stereotaxic injections of 6-hydroxydopamine hydrochloride (6-OHDA–HCl) into the right ascending
DA fiber bundle [1]. Forelimb use asymmetry was assessed using the cylinder test screening at 3 weeks post 6-OHDA lesion [6]. L-DOPA treatment (single daily i.p. injections of 8 mg/kg) and recording of dyskinesia started the day after the limb-asymmetry test. L-DOPA-induced abnormal involuntary movements (AIMs) were recorded every other day as described previously [1]. Rats were sacrificed by decapitation 60 min after the last L-DOPA treatment. Snap frozen, dissected rat brain tissue sections were collected on a cryostate microtome and thaw-mounted onto conductive glass slides. The sections were subjected to matrix application using a chemical inkjet printer followed by MALDI MS analysis. MS data of assigned regions of interests (ROI; dorso-lateral, dorso-medial CPu) were extracted and subjected to comprehensive data analysis (baseline substraction, calibration, peak picking) and post analysis (binning, re-binning, statistical analysis).

All peptide masses were validated by means of LC-MSMS based peptidomic profiling of striatal peptide extracts. Furthermore double antigen immunohistochemistry was performed towards dynorphin B.

**Results and Discussion**

Using MALDI IMS, several opioid peptides could be detected in the present study, including dynorphins: dynorphin A(1–8), dynorphin B, alpha neoendorphin as well as enkephalins: MetEnkRF, MetEnkRGL, PEnk (198–209, 219–229). IMS analysis revealed elevated levels of dynorphin B, -neoendorphin, substance P, and PEnk (220–229) in the dorsolateral striatum of high-dyskinetic animals compared with low-dyskinetic and lesion-only control rats (Fig.1).

Furthermore, the peak-intensities of the prodynorphin-derived peptides, dynorphin B and alpha-neoendorphin, were strongly and positively correlated with LID severity. Interestingly, these LID associated dynorphin peptides are not those with high affinity to kappa opioid receptors, but are known to bind and activate also mu- and delta-opioid receptors. In addition, the peak intensities of a novel endogenous metabolite of alpha-neoendorphin lacking the N-terminal tyrosine correlated positively with dyskinesia severity. MALDI IMS of striatal sections from Pdyn knockout mice verified the identity of fully processed dynorphin peptides and the presence of endogenous des-tyrosine-neoendorphin.

Des-tyrosine dynorphins display reduced opioid receptor binding, which points to possible novel nonopioid receptor mediated changes in the striatum of dyskinetic rats. Since des-tyrosine dynorphins can only be detected by mass spectrometry, as no antibodies are available, these findings further highlight the importance of MALDI IMS analysis for the study of molecular dynamics in neurological diseases [4, 7-9].
Figure 1. Higher striatal neuropeptide levels in high dyskinetic animals. (A) Dynorphin B, α-neoendorphin and substance P are significantly increased in the DA denervated side in the striatum of high dyskinetic animals (HD) compared to the low dyskinetic (LD) and lesion control group (LC). (B) Peptide peak intensities (lesion/intact side) for DynB and aNeo, but not SP, are positively correlated with LID severity expressed as cumulative dyskinesia score. * p<0.05

References

