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Region-specific bioconversion of dynorphin neuropeptide detected by *in situ* histochemistry and MALDI imaging mass spectrometry

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ABSTRACT

Brain region-specific expression of proteolytic enzymes can control the biological activity of endogenous neuropeptides and has recently been targeted for the development of novel drugs, for neuropathic pain, cancer, and Parkinson's disease. Rapid and sensitive analytical methods to profile modulators of enzymatic activity are important for finding effective inhibitors with high therapeutic value.

Combination of *in situ* enzyme histochemistry with MALDI imaging mass spectrometry allowed developing a highly sensitive method for analysis of brain-area specific neuropeptide conversion of synthetic and endogenous neuropeptides, and for selection of peptidase inhibitors that differentially target conversion enzymes at specific anatomical sites. Conversion and degradation products of Dynorphin B as model neuropeptide and effects of peptidase inhibitors applied to native brain tissue sections were analyzed at different brain locations. Synthetic dynorphin B (2 pmol) was found to be converted to the N-terminal fragments on brain sections whereas fewer C-terminal fragments were detected. *N*-ethylmaleimide (NEM), a non-selective inhibitor of cysteine peptidases, almost completely blocked the conversion of dynorphin B to dynorphin B(1–6; Leu-Enk-Arg), (1–9), (2–13), and (7–13). Proteinase inhibitor cocktail, and also incubation with acetic acid displayed similar results.

Bioconversion of synthetic dynorphin B was region-specific producing dynorphin B(1–7) in the cortex and dynorphin B (2–13) in the striatum. Enzyme inhibitors showed region- and enzyme-specific inhibition of dynorphin bioconversion. Both phosphoramidon (inhibitor of the known dynorphin converting enzyme neprilysin) and opiorphin (inhibitor of neprilysin and aminopeptidase N) blocked cortical bioconversion to dynorphin B(1–7), wheras only opiorphin blocked striatal bioconversion to dynorphin B(2–13).

This method may impact the development of novel therapies with aim to strengthen the effects of endogenous neuropeptides under pathological conditions such as chronic pain. Combining histochemistry and MALDI imaging MS is a powerful and sensitive tool for the study of inhibition of enzyme activity directly in native tissue sections.

1. Introduction

It is often desirable to screen enzyme activity in complex sample matrices, such as tissues, as this can give biologically more relevant data than *in vitro* assays. Neuropeptides are often involved in several different processes engaging different brain regions (example opioid neuropeptides: reward, pain, movement control, *etc.*); thus agonist/antagonist have often displayed unwanted side-effects as other areas than intended are affected. For example, for opioid peptides the need to alleviate pain is also associated with a risk of developing drug dependence. Therefore, it is highly desirable to find region-specific targets whenever this is possible. In particular brain enzymes often display characteristic expression patterns confined to distinct areas, for example the mouse angiotensin-converting enzyme is strongly localized to the striatum, globus pallidus, and substantia nigra of the basal ganglia [1]. The lateral striatum has been shown to express high levels of prodynorphin mRNA

Abbreviations: IMS, imaging mass spectrometry; DA, dopamine; PD, Parkinson's disease; LID, L-DOPA-induced dyskinesia; NEM, *N*-Ethylmaleimide; DynB, dynorphin B.

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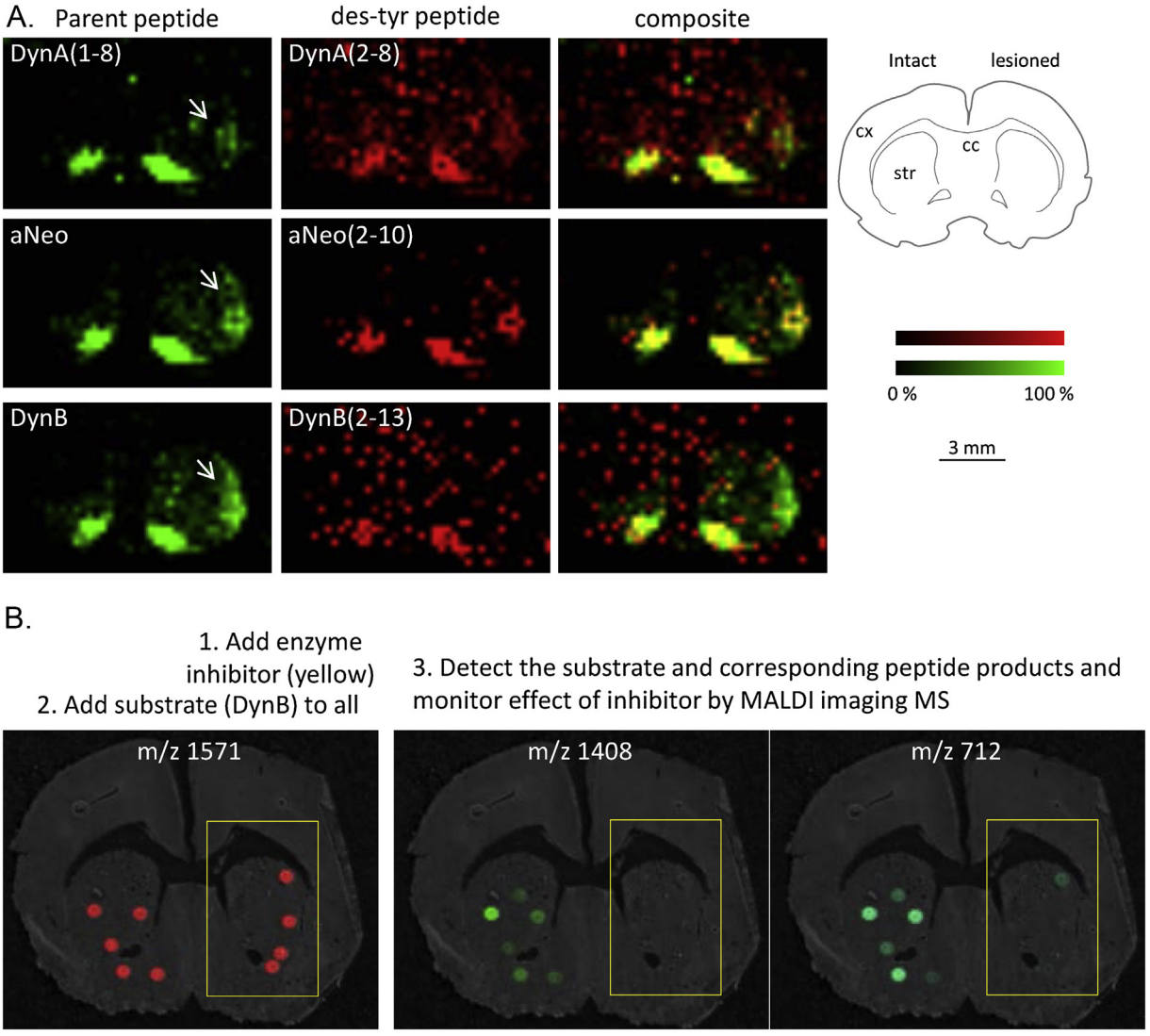

Fig. 1. Localized bioconversion in disease model and experimental set-up. (A) MALDI imaging MS of endogenous dynorphins in experimental Parkinson's disease (PD). The rat model of PD use a unilateral injection of 6-hydroxydopamine to produce a dopamine denervated striatum (to the right side in images), whereas the contralateral striatum is left intact. After L-DOPA-treatment some subjects develop abnormal involuntary movements that are similar to dyskinesia in PD patients. The severity of dyskinesia is positively correlates to the levels of dynorphin neuropeptides, predominantly dynorphin B (DynB), alpha-neoendorphin (aNeo), and aNeo(2–8)(arrows). The des-tyrosinated dynorphins were only up-regulated in the striatum, but not in other areas such as the substantia nigra (not shown) [4,5]. Modified from Hanrieder et al. [4] (B) Experimental design combining in situ histochemistry with MALDI imaging MS, where the enzyme inhibitor is first added to one side of a normal control brain section, here at the level of the striatum. A substrate, dynorphin B (DynB), dissolved in ammonium acetate is added to all spots on both sides of the brain section before applying the MALDI matrix. Using MALDI imaging MS it is possible to detect the levels of substrate and peptide bioconversion products simultaneously, such as dynorphin B(2–13) at *m/z* 1408 and Leu-Enk-Arg (*m/z*712). The intensity of color reflects peak intensity in mass spectra. The enzyme inhibitor NEM (67 nanomol) almost completely blocked the des-tyrosination of dynorphin B and Leu-Enk-Arg (yellow box). Corpus callosum, cc; cortex, cx; striatum, str. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and dynorphin neuropeptides after the development of L-DOPA-induced adverse drug effects (dyskinesia) in Parkinson's disease [2–5]. In previous studies we found that the levels of small dynorphins (dynorphin B and alpha-neoendorphin) in the parkinsonian striatum and substantia nigra were positively correlated with the severity of dyskinesia [4,5]. Although all dynorphins can bind and activate kappa opioid receptors, both dynorphin B and alpha-neoendorphin can interact with the other opiate receptors and in particular alpha-neoendorphin displays the least selectivity [6,7]. After release dynorphin peptides are bioconverted into shorter neuroactive fragments, sometimes targeting other receptors than the original parent peptide. In the rat model of Parkinson's disease, we have previously noted that in animals with a severe degree of motor adverse drug effects (dyskinesias) the des-tyrosination of the dynorphin peptides dynorphin A(1–8), dynorphin B, and alpha-neoendorphin is rapidly occurring in the striatum, but not the target structure substantia nigra (Fig. 1A) [4,5]. However, not much is known about the bioconversion of dynorphins *in vivo* and therefor dynorphin B was selected as the model neuropeptide in the current study [8,9].

Area-specific expression of CNS enzymes is known to modify the biological activity of several endogenous neuropeptides [8,10] and has recently been targeted for the development of novel drugs, for example for use in neuropathic pain [11–13]. Unbiased, rapid, and sensitive analytical tools to screen new drugs targeting enzymatic activity are important for finding drugs with high efficacy and therapeutic value.

This study focuses on establishing a fast and sensitive method with high-throughput capacity for screening enzyme inhibitors and their effects in different parts of the brain by combining *in situ*