Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1041 (2005) 167–180
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Detection of β-endorphin in the cerebrospinal fluid after intrastriatal microinjection into the rat brain

Malin Höistad[a,*], Jenny Samskog[b], Kirsten X. Jacobsen[c], Annika Olsson[d], Hans-Arne Hansson[e], Ernst Brodin[d], Kjell Fuxe[a]

[a]Department of Neuroscience, Karolinska Institutet, Retziusv. 8, 17177 Stockholm, Sweden
[b]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Scheeles väg 2, 17177 Stockholm, Sweden
[c]Department of Cellular and Molecular Medicine, University of Ottawa, 451 Smythe Road, Ottawa, Canada K1H8M5
[d]Department of Physiology and Pharmacology, Karolinska Institutet, Nanna Svartz väg, 17177 Stockholm, Sweden
[e]Department of Anatomy and Cell Biology, University of Göteborg, 40530 Göteborg, Sweden

Accepted 1 February 2005
Available online 13 March 2005

Abstract

We have investigated to what extent microinjected β-endorphin could migrate from the rat brain parenchyma into the CSF compartment. Exogenous rat β-endorphin (0.1 nmol) was microinjected into the left striatum 1 mm from the lateral ventricle in anesthetized male rats. CSF samples were collected at different time points up to 2 h post-injection from a catheter affixed to the atlanto-occipital membrane of the cisterna magna. Radioimmunoassay and mass spectrometry were performed on the CSF samples, and brain sections were immunostained for β-endorphin and μ-opioid receptors. The β-endorphin injected rats showed a marked increase in β-endorphin immunoreactive (IR) material in the CSF, with a peak at 30–45 min post-injection, and this β-endorphin-IR material existed mainly as the intact β-endorphin peptide. The immunohistochemistry results revealed the appearance of distinct β-endorphin-IR cell bodies in the globus pallidus and the bed nucleus of stria terminalis supracapsular part, regions distant from the injection site, at 2 h post-injection of exogenous β-endorphin. The β-endorphin-IR in several of the globus pallidus cell bodies colocalized with the μ-opioid receptor-IR at the cell surface. These findings show that upon delivery of synthetic β-endorphin, there is a significant intracerebral spread of the injected peptide, reaching regions far from the site of injection via diffusion in the extracellular space and flow in the cerebrospinal fluid. This may be of relevance when interpreting studies based on intracerebral injections of peptides, and advances our knowledge regarding the migration of compounds within the brain.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Peptides: anatomy and physiology

Keywords: Intraparenchymal injections; Radioimmunoassay; Mass spectrometry; Migration; Diffusion; Volume transmission

1. Introduction

Upon intracerebral injections of peptides or other compounds, it is rarely determined where the injected compound itself appears, and/or to what extent it migrates away from the site of injection. In studies employing such microinjections, several assumptions are tacitly made, the fundamental one being that the injected peptide or ligand reaches its respective receptors, see [13]. A number of important issues may be raised regarding this topic, dealing with the intracerebral migration of compounds: (i) intraparenchymal injections assume that the injected compound stays in the restricted area and exerts its function in that region; (ii) intracerebroventricular (i.c.v.) injections assume

Abbreviations: CSF, cerebrospinal fluid; ECF, extracellular fluid; IR, immunoreactivity; IRM, immunoreactive material; i.c.v., intracerebroventricular; BSTs, bed nucleus of the stria terminalis, supracapsular part; GP, globus pallidus; MOR1, μ-opioid receptor-1; VT, volume transmission

* Corresponding author.
E-mail address: Malin.Hoistad@neuro.ki.se (M. Höistad).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.02.014

that the injected peptides reach their targets located beyond the ventricular lining; (iii) assessing the central effects after intraparenchymal or i.c.v. injections of compounds do generally not verify the true morphological site of action of the compound.

Further aspects of this topic involves the existence of endogenous neuroactive signals that may use intraventricular and/or intraparenchymal migration as a complementary mode of communication, namely: (I) compounds which exhibit a mismatch morphology between its release sites and its receptor sites [2], and (II) endogenous signals in the cerebrospinal fluid (CSF) that may employ the CSF as a mode of communication to reach distant targets [84].

To address the issue of intracerebral migration of compounds, it is thus relevant to assess to what degree an injected peptide or compound may spread from its site of injection. For instance, different types of peptide and/or protein migration studies have been performed previously, employing intracerebral administration of ligands [13,64,74] or tracers [37,90,91] with subsequent morphological analysis. However, few peptide migration studies have been performed in vivo. One attempt was made by Duggan et al., using an antibody microprobe approach to assess the possibility of long distance migration of β-endorphin [32,73].

Different investigations have been performed in order to elucidate potential endogenous signals employing intraparenchymal or intraventricular migration as means of accessing its receptors. For instance, several ligand-receptor mismatches between certain peptides and their receptors have been shown (see [57]), including β-endorphin [2]. Such morphologic findings have generated the hypothesis of Volume Transmission (VT), in which ligands may diffuse and flow in the extracellular space and the cerebrospinal fluid (CSF) to reach distant target areas [2,40]. Regarding the CSF, it was suggested already in the 1970s and 1980s that the CSF may serve as an alternative communication channel for hormones and neuropeptides [16,54,63,94]. Although the presence of many transmitters and neuropeptides has been confirmed in the CSF, few in vivo studies have been performed to address the issue of the CSF as a transport medium for neuropeptides.

The neuropeptide β-endorphin is involved in behaviors essential for self and species survival, such as responses to painful stimuli, stress, reward and motivation, memory and control of autonomic functions, see [4,20,60,72]. Beta-endorphin is a 31 amino acid peptide produced from the precursor pro-opiomelanocortin and β-endorphin immunoreactive cell bodies are mainly found in the arcuate nucleus and in periarcuate regions of the hypothalamus, but also in the nucleus of the solitary tract in the brainstem and in the pituitary gland [19,20,109]. Beta-endorphin acts on μ- and δ-opioid receptors, which are G_i-coupled receptors distributed over the entire somatic-dendritic membrane of neurons in a large number of CNS regions [25,30,71].

A mismatch between the distribution of β-endorphin terminals and their respective opioid receptor sites in specific brain areas has been reported [2,33,44,75,77]. There is for instance a significant density of μ- and/or δ-opioid receptors in the frontal, piriform and entorhinal cortices, as well as in the hippocampus, in the absence of any detectable β-endorphin-immunoreactive (IR) terminals [77]. It can however not be excluded that in these regions, enkephalin may instead be the primary ligand for the present μ- and/or δ-opioid receptors. In other regions, there exists a very high density of β-endorphin-IR terminals, for instance in the periaqueductal grey, the periventricular thalamic nucleus and in several hypothalamic nuclei, but with only a minor-to-modest occurrence of μ- and/or δ-opioid receptors [42,76,77]. Similar mismatches have also been found for other opioid peptides, such as dynorphin [26]. From the observed transmitter-receptor mismatch data, it has been hypothesized that β-endorphin may operate as a volume transmission signal [2]. Furthermore, Duggan et al. have found that β-endorphin can diffuse within the brain and exert actions at distant target sites after arcuate nucleus stimulation in the rat brain [32,73].

The goal of this paper was two-fold: to assess whether microinjected β-endorphin could migrate from the striatum to the CSF compartment as an intact peptide, and to assess the possible role of the CSF as a complementary communication pathway for neuroactive peptides to reach distant target sites in the brain. In order to address these questions, we investigated whether β-endorphin could migrate from the brain parenchyma into the CSF, using intrastriatal microinjections of exogenous rat β-endorphin in conjunction with continuous CSF sampling from the cisterna magna. This methodological setup may thus be viewed as a model system for neuropeptide migration in vivo within the rat brain.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (200 g; B&K Universal, Sollentuna, Sweden) were kept in a 12:12 hour night/day cycle (lights on 07:00 and off 19:00) in a temperature- and humidity-controlled environment, housed in groups of 3–5, with food and water ad libitum. Experiments were carried out with permission of the Local ethical committee for research on laboratory animals (CFN, Dnr. 152/01 and 333/02).

2.2. Cisterna magna catheterization

The CSF was sampled from the cisterna magna (cerebello-medullary cistern) [49]. The rats (n = 28) were anaesthetized in halothane and mounted in a stereotactic