available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Substance P induces expression of the corticotropin-releasing factor receptor 1 by activation of the neurokinin-1 receptor

Maike Hamke, Inga Herpfer, Klaus Lieb, Carolin Wandelt, Bernd L. Fiebich*

Department of Psychiatry and Psychotherapy, University of Freiburg Medical School, Hauptstr.5, D-79104 Freiburg, Germany

ARTICLE INFO

Article history:
Accepted 13 March 2006
Available online 23 June 2006

Keywords:
Affective disorder
Depression
HPA axis
Somatostatin receptor type 4
Therapy
Etiology
Signal transduction

Abbreviations:
CRF1 receptor, corticotropin-releasing factor receptor
MAPK, mitogen-activated protein kinase
NK, neurokinin
NK-1-R, neurokinin-1 receptor
PKC, protein kinase C
SP, substance P

ABSTRACT

The neuropeptide substance P (SP) has been found to be possibly involved in the etiology of affective and anxiety disorders. However, the molecular mechanisms underlying this involvement are still poorly understood. In this study, we used macroarrays to investigate the differential gene expression profile induced by SP, particularly of genes which have been shown to be involved in the pathophysiology of affective disorders. As a model system, we used the human astrocytoma cell line U373 MG as well as primary rat astroglial cells, which both are known to express functional neurokinin-1 receptors (NK-1-R) and to secret various cytokines upon stimulation with SP. Among several regulated genes, we found that SP (100 and 1000 nM) induced the expression of the corticotropin-releasing factor receptor 1 (CRF1 receptor). Further analyses revealed that this induction was mediated (a) via NK-1-R, as the selective NK-1-R-antagonist L-733,060 (1 μM) strongly inhibited SP-induced CRF1 receptor expression, and (b) intracellularly, by protein kinase C, p42/44 and p38 mitogen-activated protein kinases (MAPK), as shown by using specific inhibitors of signal transduction pathways. In conclusion, this study demonstrates that SP induces CRF1 receptor expression in cells of the CNS, which may be of potential interest for a better understanding of the interplay between SP and the stress hormone axis and, thus, diseases like affective or anxiety disorders. Further studies are needed to substantiate this link in vivo.

1. Introduction

The neuropeptide substance P (SP) is a member of a family of structurally related peptides, called tachykinins (also known as neurokinins, NK), which are involved in the regulation of several biological processes (Pennefather et al., 2004; Pioro et al., 1990; Quartara and Maggi, 1997; Severini et al., 2002). Among these peptides, SP is by far the most abundant tachykinin in the CNS (Otsuka and Yoshioka, 1993; Pioro et al., 1990). For example, SP-containing neurons are found in the midbrain and basal ganglia, the hypothalamus, the limbic system including the hippocampus, the amygdala, and the spinal cord (Pioro et al., 1990). SP is co-localized with other neurotransmitters, e.g. with serotonin

* *Corresponding author*. Fax: +49 761/270 6667.
E-mail address: klaus_lieb@psyallg.ukl.uni-freiburg.de (K. Lieb).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.03.026

in the raphe nucleus (Sergeyev et al., 1999), with dopamine in the midbrain and striatum, and with GABA and acetylcholine in the cortex (Otsuka and Yoshioka, 1993).

The biological actions of SP are mediated via the activation of G-protein-coupled seven-transmembrane domain receptors of the NK-receptor family, with SP having a preferential affinity to the NK-1 receptor subtype (NK-1-R) (Otsuka and Yoshioka, 1993). Intracellularly, NK-1-R binding is followed by activation of several second messenger systems, namely protein kinases and transcription factors including calcium, inositol triphosphate, p42/44 and p38 mitogen-activated protein kinases (MAPK), protein kinase C (PKC) (Fiebich et al., 2000; Jeurissen et al., 1994; Lieb et al., 1998; Lieb et al., 2003; Luo et al., 1996; Martin et al., 1992) as well as the transcription factors nuclear factor kappa B (NF-κB) and nuclear factor IL-6 (NF-IL6) (Azzolina et al., 2002; Guo et al., 2002; Lieb et al., 1997, 1998, Otsuka and Yoshioka, 1993).

Because of its distribution pattern and its neuromodulatory effects, SP has been supposed to be involved in the etiology of certain psychiatric disorders such as anxiety and affective disorders including major depression and bipolar disorders (Herpfer and Lieb, 2005; Quartara and Maggi, 1997; Rupniak and Kramer, 1999; Rupniak, 2002; Stout et al., 2001). Evidence for such an involvement arises from animal as well as human studies. The behavior of mice, in which expression of the NK-1-R gene has been disrupted (NK1–/–), like that of rodents given an NK-1-R-antagonist, resembles the behavior seen in animals after administration of classical antidepressants (Rupniak et al., 2001). Moreover, genetic or pharmacological inactivation of the NK-1-R results in modulation of the serotonergic and noradrenergic neurotransmitter system, which may explain in part the observed behavioral effects (for a review, see Herpfer and Lieb, 2005; Rupniak, 2002). In humans, most evidence for an involvement of SP and its receptor in the etiology of affective disorders comes from studies evaluating the usefulness of NK-1-R-antagonists in the treatment of affective disorders. Two published studies found a superior effect of the NK-1-R-antagonists MK-869 and L-759274, respectively, over placebo in the treatment of major depressive disorder (Kramer et al., 1998, 2004). The role of SP in depressive disorders is supported by recent studies, in which we found that SP given intravenously to healthy male subjects leads to a significant worsening of mood (Lieb et al., 2002) and that SP serum levels might be a possible predictor of antidepressant response (Lieb et al., 2004).

So far, little is known about the gene expression patterns induced by SP. Thus, an investigation of these gene expression patterns enables us to identify new gene targets of SP and may in the long run lead to a better understanding of the involvement of SP in the etiology and treatment of affective disorders. Therefore, we used the macroarray technology to identify new molecular targets of SP with a special focus on the induction of genes which have already been shown to be involved in the pathophysiology of affective disorders, such as genes relevant for the regulation of the activity of the hypothalamus–pituitary–adrenal (HPA) axis.

2. Results

2.1. *SP-regulated genes in U373 MG human astrocytoma cells and selection of genes to be verified on the mRNA and protein level*

We stimulated U373 MG cells with 100 nM SP for 1, 4, and 24 h. Based on previously published findings, 100 nM is the dose where in U373 MG cells the most pronounced SP-induced gene expression would be expected (Lieb et al., 1998). However, in a preliminary study, neither 1 h nor 24 h stimulation led to any significant alterations in gene expression patterns (data not shown), and these times were therefore not further investigated. Only a stimulation for 4 h led to significant changes. This result is in line with previously published findings, demonstrating that SP-induced gene expression (e.g. of IL-6 and IL-8) in U373 MG cells peaks after 4 to 8 h (Lieb et al., 1997, 1998). Table 1 provides a summary of the induced gene expression pattern in U373 MG cells after 4 h of SP stimulation. Fig. 1A shows an example of a macroarray hybridized with labeled cDNA of cells treated with 100 nM SP for 4 h.

Among these regulated genes, only one gene was identified which is supposed to be directly involved in the pathophysiology of affective disorders: the corticotropin-releasing factor receptor 1 (CRF1 receptor). We found its expression to be 10.5-fold up-regulated by SP in two independent macroarray experiments (Fig. 1B).

Another gene which was also regulated by SP and which might interact with the CRF system was the somatostatin receptor type 4 (SS4R). We found its expression to be 8.5-fold up-regulated in two independent macroarray experiments.

Table 1 – Relative increase of gene expression after stimulation of U373 MG human astrocytoma cells with 100 nM SP for 4 h as compared to unstimulated control cultures and normalized to housekeeping genes

Protein/gene	GenBank accession	Mean ratio $n = 2$
Corticotropin-releasing factor receptor I (CRF1 receptor)	X72304	10.5
Somatostatin receptor type 4 (SS4R)	D16826	8.5
Histidine decarboxylase (HDC)	X54297	5.7
Inward rectifier potassium channel 4; hippocampal inward rectifier (HIR); HRK1; KIR23	U07364	5.4
Calpain 1 large (catalytic) subunit; mu-type calcium-activated neutral proteinase (muCANP)	X04366	5.4
Brain-specific homeobox/POU domain protein 5 (brn-5)	Z21966	≥
Serine/threonine protein phosphatase 2B catalytic subunit beta isoform; calmodulin-dependent calcineurin A subunit beta isoform; CAM-PRP catalytic subunit	M29551	≥

≥: undefined induction: control cells show no signal higher than background, making it impossible to calculate the ratio.