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Antidepressants differentially affect expression of complexin I and II RNA in rat hippocampus

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Abstract Disturbance of synaptic transmission is currently viewed as an important pathophysiological mechanism and therapeutic target of mood disorders. Amongst other lines of evidence this theory is based on human post-mortem investigations showing differential expression of complexins. In order to discriminate between molecular correlates of the disease itself and effects of psychotropic drugs given to patients, we performed an animal trial using subchronic antidepressant treatment. Cohorts of adult male Sprague–Dawley rats were treated over a period of 14 days with intraperitoneal injections of either saline (0.9%, $n=8$), desipramine (15 mg/kg, $n=7$), fluoxetine (10 mg/kg, $n=8$), or tranylcypromine (10 mg/kg, $n=5$). Brain slices were used for in situ hybridizations with ^{35}S labelled RNA probes of the genes complexin I, complexin II and syntaxin 1 A, the SNARE complex protein interacting with the complexins, and assessed semi-quantitatively for region-specific expression levels. Expression of complexin I was induced only in habenular nuclei after treatment with fluoxetine. In contrast, complexin II was significantly induced by desipramine and tranylcypromine, but not fluoxetine, in several brain regions. All treatment groups, but most significantly fluoxetine-treated animals, showed higher expression levels of syntaxin 1A. Antidepressants differentially affect expression levels of complexin I and more prominently complexin II and syntaxin 1A. The induction of complexin II and syntaxin 1A might strengthen the synaptic transmission at axo-dendritic or axo-axonal synapses. Previous post-mortem findings reporting on downregulation of complexins cannot be explained as mere effects of psychotropic drug treatment.

Keywords Animal model · Antidepressants · Complexin · Depression · Desipramine · Fluoxetine · Synapse · Syntaxin 1A · Tranylcypromine

Introduction

Disturbances of synaptic transmission are considered to be one of the major pathomechanisms of mood disorders and antidepressant drugs exert at least a part of their effects by modulating synthesis and clearance of neurotransmitters or ligand–receptor interactions (Garcia 2002; Nestler et al. 2002). In animal models of stress syndromes, anxiety and depression, electrophysiological investigations have reported on altered synaptic function (Alvarez et al. 2003; Maroun and Richter-Levin 2003), and molecular studies have shown altered expression patterns of synaptic vesicle proteins (SVP) (Kinnunen et al. 2003).

As parts of the synaptic release mechanism (Sudhof 2004), complexin I and II, also known as synaphin 2 and 1, coregulate assembly and function of the SNARE complex in the cytosol (McMahon et al. 1995) [synaptosomal associated protein of 25 kDa (SNAP 25) receptor (SNARE)—alternatively called soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptor]. The complexins, highly conserved hydrophilic proteins, co-localize with the SVPs syntaxin 1A and SNAP 25, interact with syntaxin 1A (Hu et al. 2002) binding along the groove between the syntaxin and synaptobrevin coils (Marz and Hanson 2002). They are crucial for a late step of Ca^{2+} -dependent neurotransmitter release (Reim et al. 2001; Tokumaru et al. 2001). Expression studies revealed differential localization at axospinal and axodendritic (complexin II) versus axosomatic (complexin I) synapses (Takahashi et al. 1995; Ishizuka et al. 1999; Yamada et al. 1999). Whereas complexin I knockout mice exhibit severe neurological symptoms, complexin II was found to be essential for cognitive function and learning behavior, as revealed by intensive behavioral testing (Glynn et al. 2003).

Due to their regulatory function in the SNARE complex, the complexins have been considered as candidate genes

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causing altered synaptic function in mood disorders. Therefore, post-mortem studies were performed on samples of patients with bipolar disorder or major depression to investigate the expression of complexin I and II. Complexin I protein turned out to be significantly reduced in brain of patients with schizophrenia and major depression (Sawada et al. 2002); meanwhile, reduced levels of complexin II were found in the anterior cingulate cortex and hippocampus of patients with major depression and more consistently in bipolar disorder (Eastwood and Harrison 2000, 2001), indicating a particular vulnerability of excitatory connections. Complexin expression, however, might also be influenced by long-term psychopharmacological treatments, as animal trials using antipsychotic substances showed an induction of complexin I after treatment with olanzapine (striatum and frontoparietal cortex) and of complexin II after treatment with chlorpromazine (frontoparietal cortex) (Nakahara et al. 2000). In contrast, application of haloperidol over a period of 4 weeks resulted in reduced complexin expression (Eastwood et al. 2000), and Sawada et al. (2002) was unable to detect any influence of antipsychotic treatment on complexin expression. However, to the best of our knowledge, it is an open question, whether or not antidepressants per se alter the expression of complexin I and II and their main interactor in the SNARE complex, syntaxin 1A.

This question is even more important, since antidepressants are known to affect gene transcription (Coyle and Duman 2003), for example via activation of the cyclic AMP response element binding protein (CREB) (Thome et al. 2000; D'Sa and Duman 2002). In addition, antidepressive treatment has been shown to affect the expression of the synaptic vesicle proteins such as synaptophysin, synaptotagmin III, VAMP 5 and synapsin 1 (Rapp et al. 2004) and VAMP 2 (Yamada et al. 2002).

The present study therefore, is aimed to assess the influences of antidepressants on the expression levels of complexin I, complexin II and syntaxin 1A in "healthy" rats after 2 weeks of application. We used three antidepressants with distinct target features: desipramine, a reuptake inhibitor of noradrenaline; fluoxetine, a selective serotonin reuptake inhibitor; and tranylcypromine, a non-selective inhibitor of monoamine oxidase type A and type B.

Material and methods

Animal trials: Male Sprague–Dawley rats were housed in standard rodent cages (Ehret, Emmendingen, Germany) on a 12-h light–dark cycle with lights on at 07:00 A.M. Animals were provided with food (Sniff, Soest, Germany) and water ad libitum. All experimental procedures were approved by the respective Committee on Animal Care and Use and carried out following the local Animal Welfare Acts and in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). At the age of 6 months, the rats were given daily intraperitoneal injections of either saline (0.9%, $n=8$), desipramine (15 mg/kg, $n=7$), fluoxetine (10 mg/kg, $n=8$), or tranylcypromine (10 mg/kg, $n=5$) for 2 weeks. Rats were sacrificed 3 h after the last injection, and their brains were removed and shock-frozen on dry ice before being stored at -80°C . Coronal sections (20 μm) at the level of the dorsal hippocampus (distance to the bregma, -3.80 mm; plate 33 in the rat brain atlas of Paxinos and Watson 1986) were cut in a cryostat and thaw-mounted on superfrost plus microscopic slides, fixed in 4% paraformaldehyde, dehydrated in ethanol and stored at -20°C .

In situ hybridizations (Zink et al. 2004) were performed with ^{35}S -UTP labelled cRNA probes of complexin I [GenBank accession number: NM_022864, nucleotides (nts) 295–719], complexin II (NM_053878, nts 319–720), and syntaxin1A (NM_053788, nts 871–1283). We used two sections per animal in the case of each gene of interest. These sections did not differ between the animals with respect to the distance to the bregma. cDNAs were in vitro transcribed using Sp6 or T7 RNA Polymerase (MBI Fermentas, St Leon Roth, Germany) to obtain sense and antisense probes. Efficiency of ^{35}S -UTP incorporation was measured and hybridizations with antisense and sense probes at concentrations of 10^7 cpm/ml were carried out under high stringency conditions (50% formamide, 55°C) for 16 h. After several washing steps including RNase A digest, slices were dehydrated and exposed to X-ray films (Biomax MR1 18×24 cm).

Autoradiographic films were analyzed with a Sony video camera XC ST 70, and the AIS software (Applied Information Systems, Chapel Hill, USA) at the levels of the hippocampal regions dentate gyrus (DG), CA1 and CA3, the amygdala (AM) representing the anterolateral amygdala area (AHIAL) and the posteromedial cortical amygdala nucleus (PMCO), medial habenula (MH), and parietal cortex (PC). Gray value images of the co-exposed ^{14}C -plastic standards (Amersham Perkin Elmer, Wellesley, USA) were used to compute a calibration curve by non-linear, least squares fitting, which defined the relationship between gray values and concentration of radioactivity. Non-specific binding was assessed separately for each section in the white matter separating hippocampal CA1 and cerebral cortex. These readings were subtracted from gray values in the regions of interest (total binding) resulting in a semi-quantitative determination of mRNA abundance.

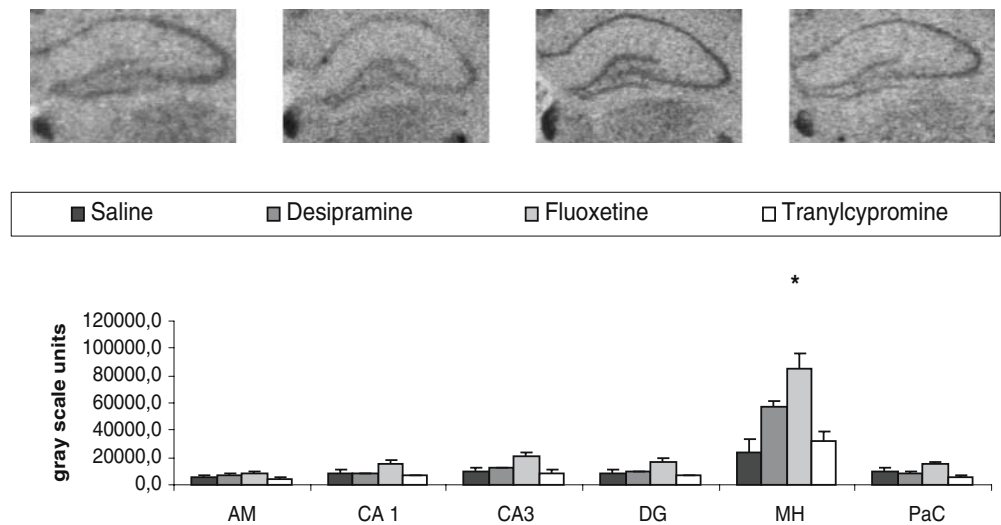
For statistical evaluation, we applied non-parametric methods and compared each treatment group pairwise with the saline-injected group using the Mann–Whitney *U*-test. In order to adjust the resulting levels of significance for multiple testing, we performed a Bonferroni correction. A level of $p<0.05$ was accepted as significant (*).

Results

Results

Expression of complexin I was found to be most prominent in medial habenula and low to moderate in other brain regions, in accordance with a previous report (Eastwood et al. 2000). In contrast, complexin II and syntaxin 1A revealed a more widely distributed expression pattern with especially abundant signals in dentate gyrus and CA3. The

Fig. 1 Expression of complexin I in several brain regions (*AM* amygdala, *CA1* cornu ammonis region 1, *CA3* cornu ammonis region 3, *DG* dentate gyrus, *MH* medial habenular nuclei, *PaC* parietal cortex) after subchronic intraperitoneal treatment with saline, desipramine, fluoxetine, and tranylcypromine. Representative in situ hybridizations are given in the upper panel, comparison of means in the diagram. The level of significance is given as follows: * $p \leq 0.05$



semi-quantitative evaluation of the in situ hybridizations in comparison to saline-treated animals revealed induced expression of complexin I (see Fig. 1) only in medial habenula nuclei after treatment with fluoxetine (+260%, $p = 0.027$). Additionally, a trend to higher complexin I expression in this region was detected in desipramine-treated animals. In contrast, treatment with tranylcypromine did not show significant differences. Complexin II (see Fig. 2) was found to be induced by desipramine in amygdala (+225%, $p = 0.048$), CA1 (+262%, $p = 0.024$), medial habenula (+218%, $p = 0.048$) and parietal cortex (+193%, $p = 0.036$), and by tranylcypromine in CA1 (+466%, $p = 0.021$). Moreover, tranylcypromine increased complexin II at the level of a statistical trend in amygdala, CA3, dentate gyrus

and medial habenula. In contrast, fluoxetine did not have significant effects on complexin II expression.

Antidepressant treatment altered the relative levels of complexin I and II expression (cpxI/cpxII) in comparison to saline injection (100%). Fluoxetine increased the quotient in the amygdala (+13%), CA1 (+5%), CA3 (+44%), dentate gyrus (+33%), medial habenula (+125%) and parietal cortex (+13%). In contrast, desipramine and tranylcypromine reduced the ratio cpxI/cpxII in the amygdala (-66 and -78%), CA1 (-74 and -86%), CA3 (-51 and -80%), dentate gyrus (-53 and -82%), medial habenula (-26 and -77%) and parietal cortex (-67 and -80%).

In order to assess the expression of syntaxin 1A, the main protein-protein interactor of the complexins in the

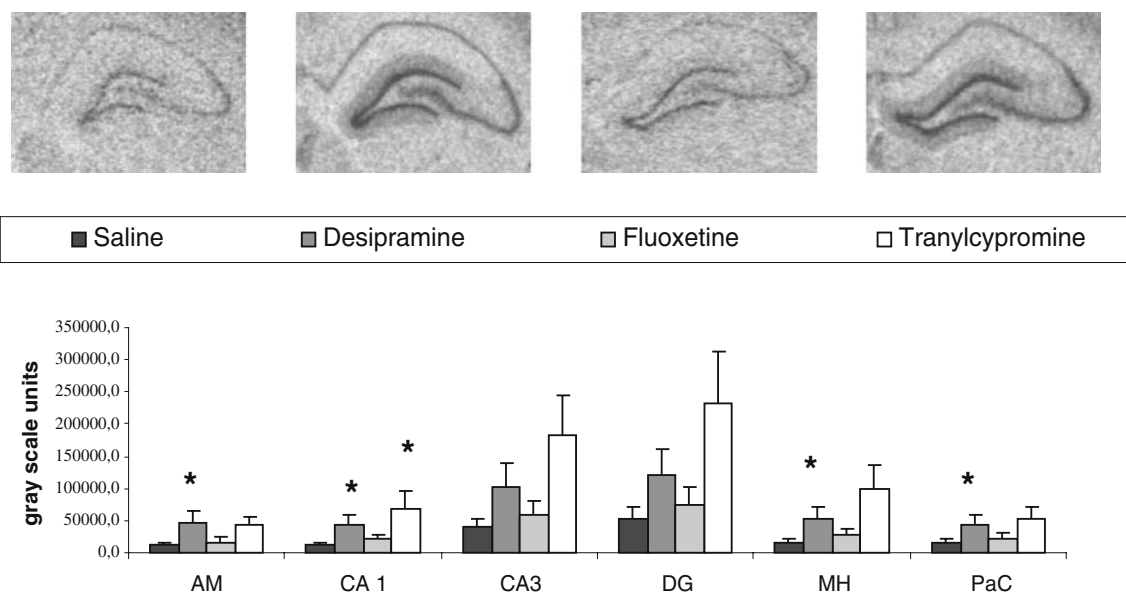


Fig. 2 Expression of complexin II in several brain regions (*AM* amygdala, *CA1* cornu ammonis region 1, *CA3* cornu ammonis region 3, *DG* dentate gyrus, *MH* medial habenular nuclei, *PaC* parietal cortex) after subchronic intraperitoneal treatment with saline, desipramine, fluoxetine, and tranylcypromine. Representative in situ hybridizations are given in the upper panel, comparison of means in the diagram. The level of significance is given as follows: * $p \leq 0.05$

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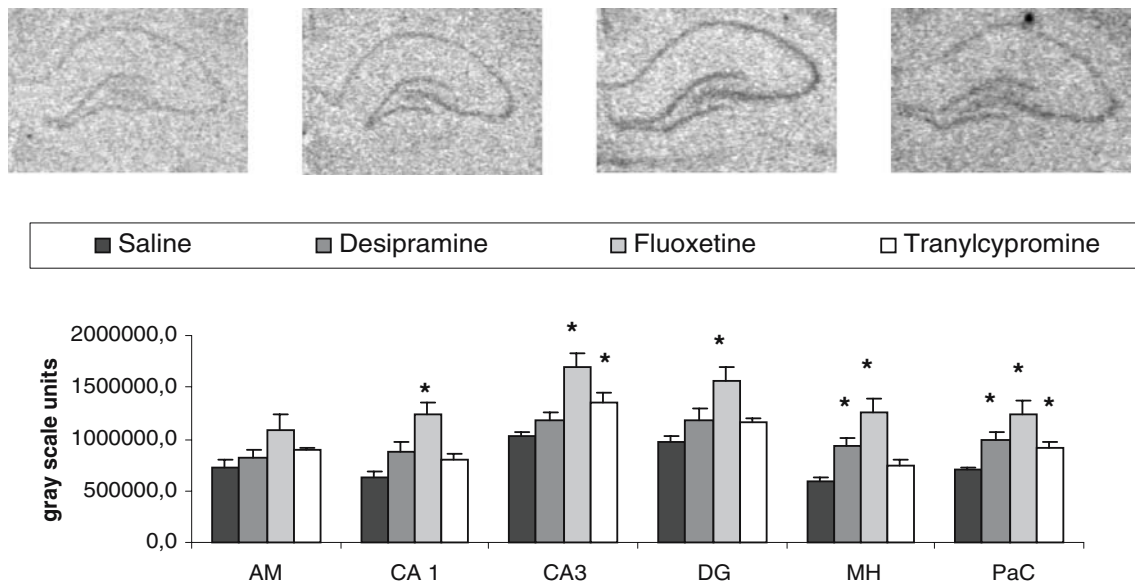


Fig. 3 Expression of syntaxin 1A in several brain regions (*AM* amygdala, *CA1* cornu ammonis region 1, *CA3* cornu ammonis region 3, *DG* dentate gyrus, *MH* medial habenular nuclei, *PaC* parietal cortex) after subchronic intraperitoneal treatment with saline, desip-

ramine, fluoxetine and tranylcypromine. Representative in situ hybridizations are given in the *upper panel*, comparison of means in the diagram. The level of significance is given as follows: * $p \leq 0.05$

SNARE complex, we also hybridized a labelled probe of this gene (see Fig. 3). All treatment groups showed elevated expression levels in parietal cortex (desipramine +40%, $p=0.003$; fluoxetine +76%, $p=0.003$, tranylcypromine +28%, $p=0.039$). Furthermore, fluoxetine treatment led to induction in CA1 (+94%, $p=0.003$), CA3 (+66%, $p=0.003$), dentate gyrus (+62%, $p=0.006$) and medial habenula (+113%, $p=0.001$), whereas desipramine induced syntaxin 1A in medial habenula (+58%, $p=0.003$). After tranylcypromine treatment the expression rose in CA3 (+33%, $p=0.024$). None of the treatment with antidepressant drugs induced changes of syntaxin 1A expression in the amygdala.

Discussion

The present study was undertaken to obtain insights into antidepressant effects on transcriptional changes of synaptic vesicle proteins (SVPs). We describe differential influences of treatment with desipramine, fluoxetine and tranylcypromine on the expression of complexin I and II, as well as of syntaxin 1A in comparison to a saline-treated cohort of control animals.

SVPs have been implicated in the pathophysiology of mood disorders (Garcia 2002; Nestler et al. 2002), and human post mortem studies have described reduced expression of the complexins as potential marker genes of major depression and bipolar disorders (Eastwood and Harrison 2000, 2001; Sawada et al. 2002). These results raise the important question, whether or not the reported transcriptional changes were due to the psychopathological condition itself or to antidepressant medication taken by the patients. In contrast to animal trials with antipsychotic drugs (Eastwood et al. 2000; Nakahara et al. 2000; Sawada

et al. 2002), experiments with antidepressant drugs have not been published as far as we know.

This study looks at the effect of three classes of antidepressants, with primary actions on serotonin (fluoxetine), norepinephrine (NE) (desipramine) and both of them (tranylcypromine). In assessing changes of mRNA abundance, we are restricted to the levels of transcription or mRNA stability. Subsequent processing may affect the levels of the active proteins. Nonetheless, these data provide a clue as to the effects of antidepressants on synaptic physiology.

Admittedly, so far no attempt has been made to identify the specific synaptic localizations of the described changes. Further limitations are due to the animal model of subchronic and intraperitoneal application to healthy rats, which make any direct comparison to antidepressant therapy in human beings difficult.

The treatments tested in our animal trial had only limited effects on the expression of complexin I. After treatment with the SSRI fluoxetine, increased expression was detected in medial habenula, a brain region involved in limbic circuits. Overall, the quotient $cpxI/cpxII$ increased in several brain regions. Complexin I is mainly located at axosomal synapses most likely belonging to inhibitory interneurons (Takahashi et al. 1995; Ishizuka et al. 1999; Yamada et al. 1999). However, human post-mortem studies with brain samples of depressed or bipolar patients did not reveal consistent differential effects in major depression, indications for reduced hippocampal and parahippocampal expression in bipolar disorder (Eastwood and Harrison 2000, 2001; Sawada et al. 2002). Alterations in the expression of complexin I, II or syntaxin 1A mRNAs in the medial habenula have not been reported in human post-mortem samples. In summary, we would hypothesize that differential expression of complexin I is not a major target

of antidepressants or involved in important pathophysiological processes in depression.

Excitatory neurons are assumed to express complexin II, which is located in axospinal and axodendritic synapses (Takahashi et al. 1995; Ishizuka et al. 1999; Yamada et al. 1999). A number of post-mortem studies have shown reduced expression in brain samples of patients with bipolar disorder (Eastwood and Harrison 2000, 2001) and major depression (Eastwood and Harrison 2001), contrasting to unchanged expression in another sample of brains with major depression (Sawada et al. 2002). Treatment of our animals with the tricyclic substance desipramine and the MAO-A/ Mao-B inhibitor tranylcypromine resulted in increased expression of complexin II and a reduction of the quotient $cpxI/cpxII$ in several brain regions. Improved availability of complexin II might facilitate SNARE complex function and synaptic transmission at excitatory synapses. Hence, complexin II might be considered as both a candidate gene of the pathophysiological mechanism of affective psychoses, and as a target gene of noradrenergic antidepressant substances—a functional hypothesis that is in line with the cognitive and learning deficits detected in complexin II knockout mice (Glynn et al. 2003).

Since both complexin I and II interact with syntaxin 1A, a constitutive component of the core SNARE complex, we were additionally interested in the expression of this gene. Antidepressants used in this animal study, most significantly fluoxetine, induced syntaxin 1A expression and might promote the SNARE complex formation by increased availability of this protein. One might be tempted to speculate that syntaxin 1A expression in brains of depressive patients might be down-regulated as well. However, post-mortem data on syntaxin 1A expression are not available so far.

The data obtained here may bring us closer to answering a long-standing, more general question in antidepressive therapy: why does it take approximately 3 weeks until antidepressants show an appreciable effect? Inhibition of neurotransmitter uptake cannot be the only process. Our findings fit well into recent concepts about antidepressants inducing changes of gene expression and thereby modulating neural and synaptic plasticity (Coyle and Duman 2003). This holds especially true for the altered expression of SVPs after antidepressive treatment (Yamada et al. 2002; Rapp et al. 2004) and after application of stress, a major risk factor of depression (Thome et al. 2001). A complete understanding of both altered SVP expression during disease and of transcriptional regulation of SVPs through antidepressants seems to be highly desirable.

In order to further analyze this molecular network, we are planning investigations on complexin expression in animal models of affective disorders and expression studies on other SVPs after antidepressant treatment. Furthermore, studies on the protein level are required.

In conclusion, the data of this study provide strong evidence that treatment with antidepressant drugs differentially affects complexin and syntaxin 1A gene expression.

These results support the notion that these molecules represent attractive candidate genes involved in fundamental processes of affective disorders and, as such, important target genes of antidepressive therapy.

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