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A common mechanism of action of the selective serotonin reuptake inhibitors citalopram and fluoxetine: Reversal of chronic psychosocial stress-induced increase in CRE/CREB-directed gene transcription in transgenic reporter gene mice

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ABSTRACT

The transcription factor CREB regulates adaptive responses like memory consolidation, addiction, and synaptic refinement. Recently, chronic psychosocial stress as animal model of depression has been shown to stimulate CREB transcriptional activity in the brain; this stimulation was prevented by treatment with the antidepressant imipramine, which inhibits both noradrenaline and serotonin reuptake. However, it was unknown whether the selective inhibition of serotonin reuptake is sufficient for inhibition of stress-induced CREB activation, as it is for the clinical antidepressant effect. Therefore, the effect of two selective serotonin reuptake inhibitors (SSRIs), citalopram and fluoxetine, was examined in this study. Transgenic CRE-luciferase reporter gene mice were used to monitor gene transcription directed by the CREB DNA binding site (CRE) in vivo. Chronic psychosocial stress for 25 days stimulated CRE/CREB-directed luciferase expression in the hippocampus and other brain regions. When applied alone to non-stressed mice, citalopram caused a transient increase after 24 h that was lost after 21 days of treatment, whereas fluoxetine had no effect after 24 h and produced an inhibition in the pons and hypothalamus after 21 days of treatment. However, both citalopram and fluoxetine treatment completely abolished the increase in CRE/CREB-directed transcription induced by chronic psychosocial stress. As indicated by Western blots, the changes in CRE/CREB-directed transcription were accompanied by corresponding changes in the phosphorylation of CREB at serine-119. These results further emphasize the role of CREB in stress-induced gene expression and suggest furthermore that inhibition of stress-induced CREB activity may be a common mechanism of action of SSRIs underlying their antidepressive effect.

1. Introduction

The transcription factor CREB has been implicated in the mechanism of action of antidepressant drugs. CREB is thought to mediate some of the changes in gene expression that are induced by chronic treatment with these drugs and that may underlie their antidepressant effect, as chronic, not acute, treatment is required for their beneficial clinical effect in depressive patients (Tardito et al., 2006). Many investigations studied the effect of antidepressant drugs on CREB expression in the brain and the phosphorylation of CREB at serine-119 (in CREB-327, corresponding to serine-133 in CREB-341) (Kuipers et al., 2006; Tardito et al., 2006; Thome et al., 2000; Tiraboschi et al., 2004). While this phosphorylation allows the recruitment of the CREB coactivator CBP (Shaywitz and Greenberg, 1999) and is thus required for CREB activation, it is not sufficient for CREB transcriptional activity (Boer et al., 2007b; Ravnskjaer et al., 2007; Schwaninger et al., 1995a). Those investigations are therefore difficult to interpret with respect to gene activation by CREB. Furthermore, antidepressant drugs are well known to produce no mood-elevating or euphoric effect in healthy individuals but to correct the mood disorder in patients that suffer from depression (Nelson, 1999). This questions the significance of studies performed in normal subjects. Although the etiology of depression is still unknown, it is generally accepted that repeated stressful events can precipitate the disease (Nestler et al., 2002). Chronic psychosocial stress in mice and other species has been shown to produce symptoms typically found in depressive patients (Fuchs and Flugge, 2002; Kudryavtseva et al., 1991; Rygula et al., 2005). Noteworthy, a recent study used for the first time chronic psychosocial stress in mice as an animal model of depression and studied the effect of an antidepressant drug on CREB activity in the brain as revealed by gene expression directed by the CREB DNA binding site, the cAMP response element (CRE), in CRE-luciferase transgenic reporter gene mice (Boer et al., 2007a). It was found that chronic psychosocial stress stimulates CREB activity in the brain and that chronic treatment with imipramine prevented the stress-induced increase in CREB activity. This study further emphasizes the role of CREB in the mechanism of action of antidepressant drugs.

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Imipramine is a tricyclic antidepressant and was one of the first antidepressant drugs that was introduced into the clinic (Nestler et al., 2002). It inhibits the neuronal reuptake transporters of serotonin and noradrenaline with almost similar potency (Owens et al., 1997). The extraneuronal concentrations of serotonin and noradrenaline increase early after initiation of therapy and produce under continued treatment with a delay of several weeks a new pattern of gene expression and the clinical antidepressant effect (Tardito et al., 2006). Today, in the treatment of depression drugs that are increasingly used selectively block the neuronal reuptake of serotonin. These selective serotonin reuptake inhibitors (SSRIs) are as, or almost as, effective as the traditional tricyclic antidepressant drugs, but are, in general, better tolerated due to less severe side effects (Turner et al., 2008; Westenberg and Sandner, 2006). However, it was unknown whether the selective inhibition of the neuronal reuptake of serotonin is sufficient to prevent the stress-induced increase in CREB activity as does the nonselective inhibition of both serotonin and noradrenaline reuptake by imipramine. Therefore, the effect of two SSRIs, citalopram and fluoxetine, was investigated in the present study.

2. Materials and methods

2.1. Animal housing conditions

The generation and characterization of the transgenic CRE-Luc mice used in this study has been described previously. The transgene in these mice consists of the luciferase reporter gene under control of four copies of the CRE from the rat somatostatin promoter and a truncated thymidine kinase promoter. Animals were kept on a 12-h light–dark cycle. Food pellets and water were available ad libitum. For acute and chronic drug treatment mice were single-housed in plastic cages (28 cm $L \times 16$ cm $W \times 13$ cm H). All animal studies were conducted according to the National Institutes of Health's Guidelines for Care and Use of Experimental Animals and were approved by the Committee on Animal Care and Use of the local institution and state.

2.2. Stress model

The model of chronic psychosocial stress in mice has been described before. Briefly, two male transgenic mice (12–15 weeks old) were housed in a cage separated by a perforated plastic partition. During 25 days the partition was removed every day for 10 min resulting in daily social conflicts. The daily interactions were recorded by two independent observers. After 3–5 days one mouse developed a dominant aggressive behaviour whereas the other became subordinate. Chasing and biting behaviour was defined as dominant whereas flight, freezing and vocalization indicated the subordinate posture and thus social stress. Non-stressed mice from the same litter kept separately served as controls. On day 26, 14 h after the last stress exposure, stressed and control mice were decapitated and brains were dissected.

As repeated stress with social defeat has been implicated in the development of depression, the effect of chronic psychosocial stress with and without SSRI treatment on CRE/CREB-directed gene transcription was investigated. The effect of chronic psychosocial stress on CRE activity using the sensory contact model has been reported previously (Boer et al., 2007a). In this model, the subordinate mouse is chronically stressed by the unavoidable sensory contact with the dominant conspecific. The dominant and subordinate behaviours as well as stress-induced weight loss of the subordinate animal were similar as has been reported before (data not shown) (Boer et al., 2007a).

2.3. Citalopram and fluoxetine treatment

Acute treatment: 25 mg/kg citalopram (Lundbeck, Denmark) was applied i.p. at 0900 and 1800. 10 mg/kg fluoxetine was applied i.p. at 0900. Mice were decapitated the following day at 0900 and brains were taken out for luciferase assay. Chronic treatment: 25 mg/kg citalopram was applied i.p. for 21 days at 0900 and 1800. 10 mg/kg fluoxetine was applied for 21 days at 0900. The mice were decapitated on day 22 of the treatment at 0900 and brains were dissected. Chronic treatment in stressed mice: mice were exposed to chronic psychosocial stress. The submissive animal was treated from day 5 to day 25 with citalopram or fluoxetine (as described above) while the daily stress procedure continued. On day 26, 14 h after the last stress exposure, the mice were decapitated and brains were taken out. Within all treatments, controls from the same litter received the solvent only. Serum concentrations of the drugs were determined in trunk blood.

2.4. Dissection of brain regions and luciferase assay

Transgenic mice were decapitated, the brains were removed and rinsed with ice-cold PBS. Defined regions were dissected in the following order: Olfactory bulb, cerebellum, pons, frontal part of the brain anterior to the optic chiasm containing parts of the prefrontal cortex and the striatum (PFC), colliculi, hypothalamus, cortex, and hippocampus. Pieces of tissue were frozen in liquid nitrogen and stored at −80 °C. The rationale for examining these regions was to study whether effects of psychosocial stress and/or antidepressant treatment are similar or different in diverse brain regions.

For the luciferase assay, the tissue was homogenized in potassium phosphate buffer (0.1 M K_2HPO_4, 0.1 M KH_2PO_4, pH 7.8) supplemented with 1 mM DTT, 4 mM EGTA, 4 mM EDTA, 0.7 mM PMSF, 5 µg/ml leupeptin, 5 µg/ml pepstatin and 5 µg/ml aprotinin (all from Sigma), and subjected to three cycles of freeze–thawing. After centrifugation, 50 µl of supernatants was mixed with 370 µl assay buffer containing 16.5 mM potassium phosphate, 20 mM glycylglycine, 12 mM $MgSO_4$, 3.2 mM EDTA, 1 mM DTT, and 2.2 mM ATP (Sigma), and luciferase activity was measured by adding the substrate luciferin (Promega, Mannheim, Germany) in glycylglycine buffer (25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA, 10 mM DTT) with an automatic dispenser (Autolumat 953, Berthold, Bad Wildbach, Germany). Measured relative light units were normalized to protein content determined by Bradford (Boer et al., 2007a).

2.5. Western blot in brain homogenates

Transgenic mice were decapitated, the brains were removed and immediately frozen in liquid nitrogen. Frozen tissue was pulverized under liquid nitrogen. Powder was transferred to boiling SDS sample buffer and boiled for 5 min. Tissue lysate proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane by electroblotting. CREB and phospho-CREB (phospho-Ser-119 in CREB-327 / phospho-Ser-133 in CREB-341) were immunostained with specific antibodies (NewEngland Biolabs, Frankfurt a.M., Germany) and respective peroxidase-labeled secondary antibodies. Immunoreactivity was detected using the ECL reaction (Amersham Pharmacia, Germany).

Specific bands were quantified densitometrically and the ratio between the intensity of phospho-CREB and CREB from the same homogenate was calculated.

2.6. Determination of citalopram and fluoxetine

Citalopram, fluoxetine and their pharmacologically active metabolites norfluoxetine and demethylcitalopram were determined in blood plasma using a high-performance liquid chromatography (HPLC) method with column switching and spectrophotometric detection, as described previously for citalopram (Rygula et al., 2005). Serum (0.1 ml) was injected into the HPLC system. For online sample clean-up on a column (10 × 4.0 mm i.d.) filled with LiChrospher CN material of 20 µm particle size (MZ-Analysentechnik, Mainz, Germany), the column was washed with deionized water containing 8% (v/v) acetonitrile to remove