Journal of Neuroscience Research 87:2551–2560 (2009)

Effects of Escitalopram on the Regulation of Brain-Derived Neurotrophic Factor and Nerve Growth Factor Protein Levels in a Rat Model of Chronic Stress

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Escitalopram (ES-CIT) is a widely used, highly specific antidepressant. Until now there has been very little evidence on how this drug under pathological conditions affects an important feature within the pathophysiology of stress-related disorders such as depression: the endogenous neurotrophins. By using a well-characterized rat model in which chronic stress induces depressive-like behavior, the levels of neurotrophins brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) were determined in representative brain regions and serum using a highly sensitive improved fluorometric two-site ELISA system. There was a significant increase of BDNF in the left and right cortices after stress treatment (twofold increase) that was reversed by application of ES-CIT. An ES-CIT-dependent NGF reduction in stressed rats was detectable in the right cortex only ($P = 0.027$). The left hippocampus revealed significantly higher amounts of BDNF (2.5-fold increase) protein than the right hippocampus. These interhemispheric differences were unrelated to stress or ES-CIT treatment in all animals. BDNF and NGF of the frontal cortex, cerebellum, and serum did not change between the study groups. There was a negative correlation between body weight and serum BDNF, independent of stress or ES-CIT treatment. In conclusion, BDNF and NGF show substantial changes in this rodent model of chronic social stress, which is susceptible to antidepressant treatment with ES-CIT and therefore may constitute a neurobiological correlate for the disease.

Key words: BDNF; depression; escitalopram; NGF; serum, body weight

Escitalopram (ES-CIT), the active S-enantiomer of the racemic selective serotonin reuptake inhibitor (SSRI) citalopram (CIT), is a widely used antidepressant drug. Aside from up-regulation of cerebral serotonin by ES-CIT, little is known about how other important neurobiological features of depressive disorders are affected by this drug. For chronically stressed rats, a recent study provides evidence of a CIT-dependent alteration of stress-regulated genes such as cyclic adenosine monophosphate binding protein (CREB) and neuron-specific enolase in the dorsal raphe nucleus (Abumaria et al., 2007). Under physiological conditions, ES-CIT was shown to decrease frontal cortex and hippocampus brain-derived neurotrophic factor (BDNF) protein in the rat, whereas lithium led to an increase of this factor in these brain regions (Jacobsen and Mork, 2004). So far, few data exist on the influence of ES-CIT on cerebral or serum levels of neurotrophins such as nerve growth factor (NGF) and BDNF under pathological conditions. Both neurotrophins have repeatedly been postulated to be involved in the pathophysiology of stress-related behavior and depression (Aloe et al., 1994; von Richthofen et al., 2003; Lang et al., 2004b,c, 2005; Dwivedi et al., 2005; Duman and Monteggia, 2006; Schulte-Herbrüggen et al., 2006, 2007). As essential modulators of neuronal activity and synaptic plasticity in the central and peripheral nervous systems (Lewin and Barde, 1996;

Contract grant sponsor: Deutsche Forschungsgemeinschaft (DFG); Contract grant number: HE 1392/3-1.

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Received 22 August 2008; Revised 15 January 2009; Accepted 12 February 2009

Published online 9 April 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.22080

Kerr et al., 1999; McAllister et al., 1999), neurotrophins have received increasing attention, insofar as their dysregulation might be responsible for the inappropriate adaptive neuronal response to stress with pathological consequences, such as diminished dendritic branching and hippocampal volume reduction (for review see Hayley et al., 2005).

In well-characterized rat models of mild (Sanchez et al., 2003) and chronic stress, using a resident–intruder paradigm (Rygula et al., 2005), the application of selective serotonin reuptake inhibitors (SSRIs) such as CIT and ES-CIT has been shown to exert anxiolytic effects and to reverse upcoming depression-associated behaviors such as anhedonia and motivational deficits (Sanchez et al., 2003). Here we focus on the endogenous content of the neurotrophins BDNF and NGF in a well-characterized rat model of chronic psychosocial stress. In recent studies, this chronic stress model has undergone pharmacological validation as a model of depressive-like symptoms, showing a selective reversal of stress-induced behavioral disturbances after treatment with fluoxetine, citalopram, and reboxetine, whereas diazepam and haloperidol were not effective (Rygula et al., 2008). Therefore, rats were subjected to chronic social defeat, as described previously (Rygula et al., 2005, 2006a), and in parallel were treated with the antidepressant ES-CIT for a period of 4 weeks. In our study, ES-CIT was given orally in drinking water to minimize physical stress from injections.

In the present study, the effects of chronically applied ES-CIT on NGF and BDNF protein levels in the serum and candidate brain regions mediating depressive behavior were investigated. In addition, interhemispheric differences in neurotrophin content were investigated, because BDNF and structural hemispheric lateralization have recently been described in rodents that were partially susceptible to antidepressive treatment (Schulte-Herbrüggen et al., 2006; Czeh et al., 2007).

MATERIALS AND METHODS

Experimental Animals

Male Wistar rats (Harlan-Winkelmann, Borchen, Germany) weighing 180–200 g at the time of arrival were used for the study. They were housed individually in type III macrolon cages with rat chow and water available ad libitum. The animal facility was maintained at 21°C with a reversed 12 hr:12 hr light/dark cycle (lights off at 10:00 AM). After arrival, animals were habituated to the conditions for 2 weeks and handled daily (control phase). Lister hooded rats weighing 300–350 g (Harlan-Winkelmann) were used as residents. The resident males were paired with sterilized females and housed in large plastic cages (60 × 40 × 40 cm = l × w × h) located in a separate room. All experimental manipulations were performed during the dark phase of the light/dark cycle under dim red light. Animal experiments were conducted according to the European Council Directive of November 24, 1986 (86/609/ECC), and were approved by the Lower Saxony Federal State Office for Consumer Protection and Food Safety.

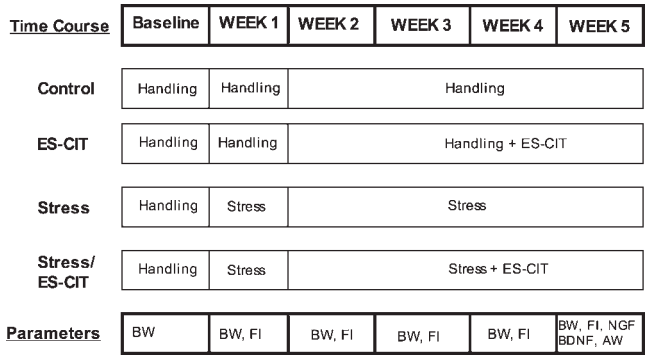


Fig. 1. Experimental study design of chronic stress and escitalopram treatment. Animals in the control group were handled without being exposed to stress or drug treatment. Animals in the stress group were exposed daily to chronic social stress for 5 weeks and received no drug treatment. Animals of the ES-CIT group were subjected to chronic stress for 5 weeks, and chronic escitalopram (ES-CIT) treatment via drinking water was started 1 week after the beginning of the stress period and continued for the following 4 weeks. The ES-CIT group includes animals that received chronic ES-CIT treatment for 4 weeks but no stress. All animals were individually housed during the whole experimental period. Group size was n = 8 animals. BW, body weight; FI, fluid intake; AW, adrenal weight.

Social Stress

Four groups of male Wistar rats were analyzed: control, stress, stress ES-CIT, and control ES-CIT (n = 8 per group, Fig. 1). Chronic social stress was induced by a resident–intruder paradigm as described recently (Rygula et al., 2005). Before starting the daily social defeat procedure, the female rat was removed from the cage of the resident male. Thereafter, the experimental (Wistar) rat was introduced into that home cage of the unfamiliar aggressive male for 1 hr. Usually within 1–3 min, the intruder was attacked and defeated by the resident, as shown by freezing behavior and submissive posture, whereupon intruder and resident were separated. For the rest of the hour, the intruder was kept in a small wire-mesh compartment (25 × 15 × 15 cm) within the resident's cage to be protected from direct physical contact but remaining in olfactory, visual, and auditory contact with the opponent. Thereafter, the intruder was returned to its home cage. Animals from the stress and the stress CIT groups were subjected to social defeat daily for 5 weeks. To avoid individual differences in intensity of the defeat, intruders were confronted each day with a different resident. Control animals were handled daily throughout the entire experiment. Handling consisted of picking up each rat, transferring it to the experimental room, and returning it to its home cage. Body weight of all experimental animals was determined weekly.

Administration of Escitalopram

Escitalopram hydrochloride (ES-CIT) solution (10 mg/ml) was kindly donated by Lundbeck A/S (Copenhagen, Denmark). Animals were treated chronically with ES-CIT (2.5 mg/100 mg body weight/day) for 4 weeks starting at week 3, 1 week after the beginning of the social stress (Fig. 1). To attain ES-CIT serum levels in the range of those

observed in human patients treated with this drug (Rao, 2007), ES-CIT was administered orally via drinking water as previously described for the antidepressant drug moclobemide (Montkowski et al., 1995). This route of administration minimizes potential stress effects that might be induced by injections, and it mimics the clinical situation. The ES-CIT solution was freshly prepared every day and poured into opaque light-protected water bottles. The dose of dissolved ES-CIT (stock solution provided by Lundbeck was 10 mg/ml) was adjusted according to individual water consumption (average drinking volume per rat was 30 ml/day) and to body weight of each animal. Therefore, the amount of consumed water was measured daily by weighing the bottles at 12:00. Animals of the control and the stress group received tap water.

Drug Monitoring

Drug monitoring was performed in a pilot study using separate groups of animals. Male Wistar rats (n = 15) were given ES-CIT orally once per day. Repeated blood analyses revealed ES-CIT levels under the therapeutic concentration (data not shown). Therefore, the drug was administered via drinking water during the whole day, as described above. The amount of consumed water was measured daily by weighing the bottles, commencing 3 days before starting the treatment and continuing for the following 5 days. This procedure allowed us to adjust the applied dose of dissolved ES-CIT to the individual water consumption and body weight of each animal. Animals were weighed daily. On the fifth day, at 12:00, animals were decapitated, and trunk blood samples were taken. Blood samples were centrifuged (10 min, 10,000*g* at 4°C) for the preparation of serum, which was stored frozen (–20°C) until assayed for drug concentrations.

Dissection of the Brains and Adrenals and Homogenization Procedure

At the end of week 5, all animals were sacrified. After decapitation, trunk blood samples were taken. Blood was centrifuged (10 min, 10 min, 10,000*g* at 4°C) for the preparation of serum, which was stored frozen (–20°C). Cerebellum, frontal cortex and remaining neocortex, and hippocampus were dissected from both brain hemispheres separately. The tissue pieces were weighed, immediately frozen on dry ice, and stored at –80°C until use. The adrenals were dissected, cleaned and weighed, and the organ weights were expressed as percentages of body weights.

Each brain sample was homogenized by ultrasonication (Sonifier B 12; Branson Sonic Power Co., Schwäbisch Gmünd, Germany) in 1 ml of lysing buffer containing 0.1 M Tris-HCl, pH 7.0, 0.4 M NaCl, 0.1% NaN^3, and a variety of protease inhibitors as contained in Protease Inhibitor Cocktail tablets (Complete; purchased from Roche Diagnostics GmbH, Penzberg, Germany) for 5–10 minutes. The resulting homogenates were stored at –80°C until further processing.

Determination of NGF and BDNF Protein Levels

Each brain region was consecutively processed for quantification of NGF and BDNF. Determinations of recovery and specific and nonspecific neurotrophin binding (the latter against mouse IgG1 obtained from MOPC 21; Sigma Chemicals, Deisenhofen, Germany) involved quadruplicate fluorescence determinations for each tissue sample in each neurotrophin assay. The neurotrophin levels were expressed as picograms per milligram of tissue (wet weight). To minimize the influence of unavoidable variances between experiments (Hellweg et al., 1989, 1998, 2003, 2007), neurotrophin levels from corresponding controls and differentially treated animals were always measured in the same assay. Endogenous NGF levels in the rethawed homogenates (again diluted 1:1 with lysing buffer) were determined by a fluorometric two-site enzyme immunoassay (ELISA) that has been described in detail elsewhere (Hellweg et al., 1989, 1998). NGF content was expressed as equivalents of mouse 2.5S NGF. The detection limit of the assay was 0.25 pg/ml. Endogenous levels of BDNF were measured in the rethawed homogenates using commercial ELISA kits in principle according to the manufacturer's instructions (Promega, Madison, WI) but improved and adapted to the fluorometric technique used also for NGF determination as described in detail previously (Hellweg et al., 2003, 2006). Differing from commercial neurotrophin ELISAs, the mean recovery of exogenous neurotrophin (NGF, BDNF 125 pg/ml each) added to the total homogenate ranged from 60% to 90% in each assay. With these improved fluorometric ELISAs, it is feasible to quantify the endogenous neurotrophins NGF and BDNF in the same brain tissue with a minimal wet weight of 5 pg as described in detail elsewhere (Hellweg et al., 2006). Serum concentrations of BDNF and NGF were determined by these ELISAs as described in detail previously (Ziegenhorn et al., 2007).

Statistical Analysis

Because sample sizes were small, nonparametric tests were used to test hypotheses. Interhemispherical differences in neurotrophin concentrations and tissue weights were tested with Wilcoxon's matched-pairs signed rank test with a two-tailed $\alpha < 0.05$. Differences in neurotrophin concentrations between experimental groups were tested with a Kruskal-Wallis test with a two-tailed $\alpha < 0.05$. Spearman's rank correlations were calculated to describe associations between serum levels of neurotrophins and body weights at different ages. For adrenal weight data, group means were analyzed by two-factorial ANOVA (stress vs. treatment). To detect significant differences among the experimental groups, a Bonferroni post hoc test was performed in Graph Pad Prism 4.0. Results are presented as mean ± SEM. A probability level of 95% was used to determine statistical significance ($P < 0.05$).

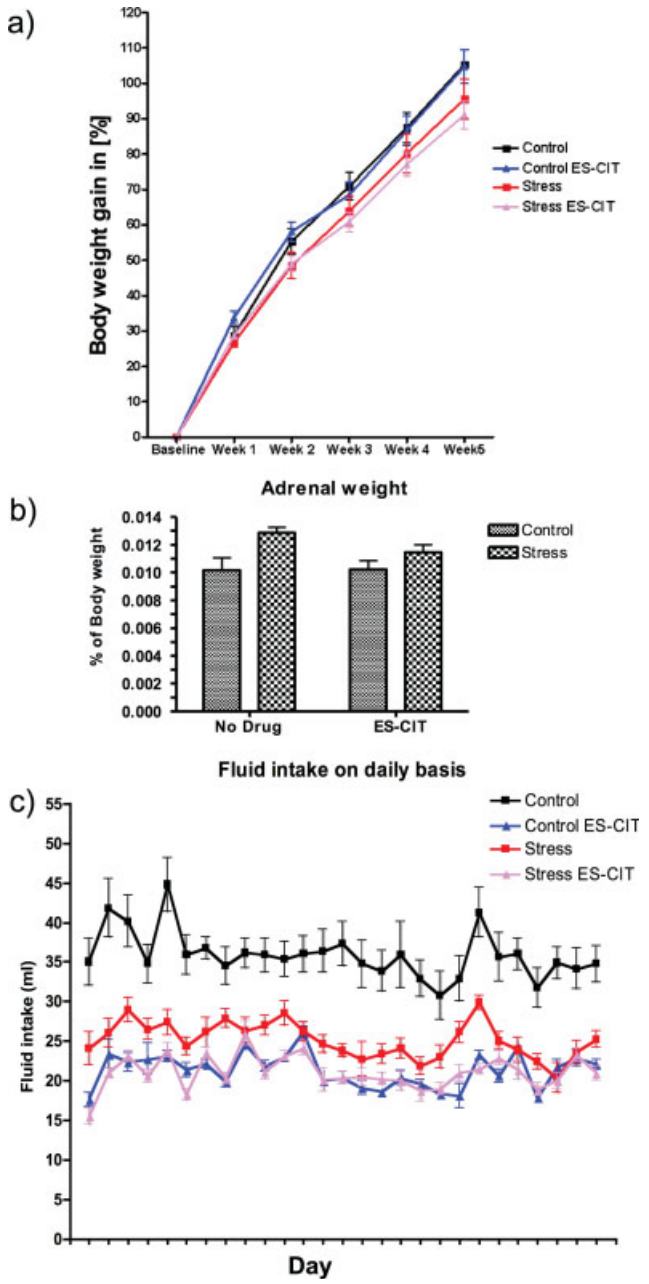
RESULTS

Body Weight Gain, Weight of Adrenal Glands, and Fluid Intake

In rats, reduced body weight (BW), altered fluid intake, and increased adrenal relative weight have been shown to be reliable indices of stress experience (Muscat and Willner, 1992; Sapolsky et al., 2000). In the present study, stressed rats gained less body weight, reaching statistical significance in week 5 ($P < 0.05$) compared with untreated controls (Fig. 2a). ES-CIT had no significant effect on BW.

Daily social stress for 5 weeks resulted in increased adrenal weight ($P < 0.05$; Fig. 2b), which is in line with previous studies using this model and revealing a depressive-like phenotype. ES-CIT treatment had no significant influence on adrenal weight.

Daily fluid intake of control animals was significantly higher compared with controls receiving ES-CIT in all cases ($P < 0.001$), compared with chronically stressed animals in most cases ($P < 0.001$), and compared with stressed animals receiving additional ES-CIT treatment in all cases ($P < 0.001$). Study groups other than controls did not differ significantly in their drinking behavior (Fig. 2c).



Interhemispherical Differences

To investigate possible differences between the right and the left hemispheres, all brain regions analyzed were compared with respect to interhemispherical differences of their neurotrophin content. In the left hippocampus, BDNF was significantly higher, with a mean difference of 29.89 pg/mg (SD 22.09; $P < 0.0001$) compared with the right side (Fig. 3; note the different scaling of the y-axes). A similar effect could be seen for the left hippocampal NGF levels, revealing a mean difference of only 0.75 pg/mg (SD 1.74; $P < 0.03$). Even after correction for multiple testing, the BDNF effect remained statistically significant for each separate study group ($P < 0.01$). For NGF, the interhemispherical difference reached significance only in pooled samples of all study groups, whereas a trend toward enhanced left hippocampus NF was detectable in every treatment group. Because the difference between left and right hippocampus is quite small, and a former study in rats did not reveal these differences (Korsching et al., 1985), the biological relevance of this result is questionable. Moreover, the differences were independent of stress or pharmacological treatment; they occurred in untreated control animals as well. To exclude this effect being dependent on tissue weight differences as a result of brain preparation, we tested sample weight differences in treated and control animals. No significant sample weight differences were detectable. Thus, according to these data, the detected side differences of BDNF concentrations in the hippocampus are real. Further analyses of BNDF and NGF in investigated brain regions did not reveal any differences between the left and the right hemispheres (Figs. 3, 4).

Cerebral BDNF Protein Levels

The influence of chronic stress and subsequent antidepressant medication on BDNF protein levels was analyzed in different brain regions in rats by a highly sensitive and specific fluorometric ELISA. Chronic stress

Fig. 2. **a:** Body weight gain in different treatment groups. Effects of social stress and escitalopram treatment on body weight gain. Body weight gain was calculated as percentage of the initial (baseline) body weight. Data are shown as mean ± SEM values of control, control + escitalopram, stress, and stress + escitalopram (n = 8 for each group). Statistical analyses are corrected for multiple testing (Bonferroni test). **b:** Effect of 5-week chronic stress and escitalopram on adrenal weight. Weight of adrenal glands was calculated as percentage of body weight at the end of the experiment. Data are mean ± SEM of control animals and stress animals with and without treatment with escitalopram (n = 8 for each group). Asterisks indicate a significant difference (*$P < 0.05$). **c:** Effects of social stress and escitalopram treatment on daily fluid intake. Graphs show the daily fluid intake in milliliters in controls, controls + escitalopram, stress, and stress + escitalopram during the last 4 weeks of the study. Data are presented as mean ± SEM (n = 8 for each group). Controls show an increased daily fluid intake ($P < 0.001$) compared with the other three study groups. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

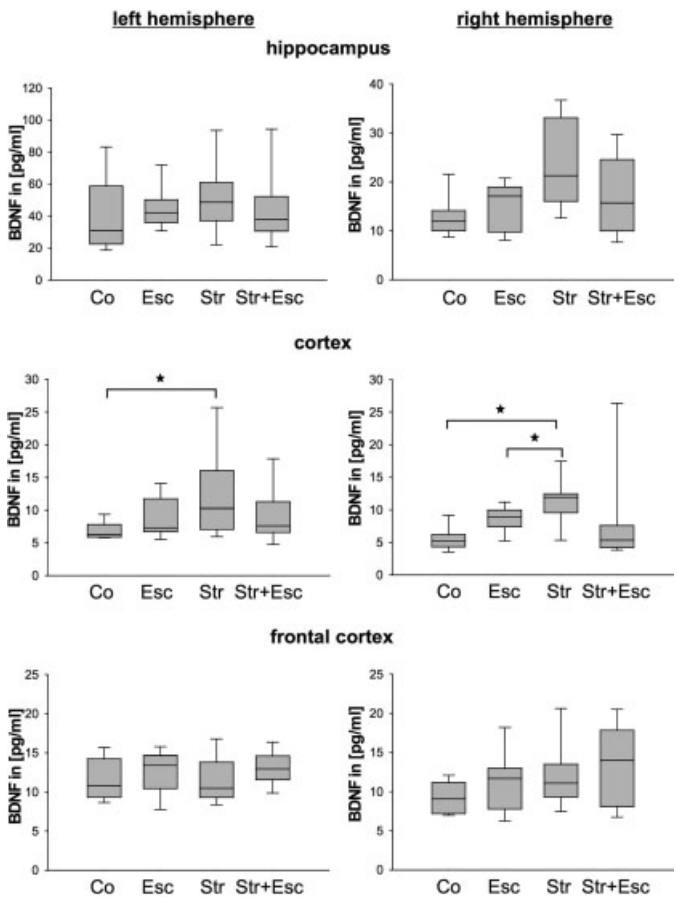


Fig. 3. Changes in cerebral BDNF levels with chronic stress and escitalopram (ES-CIT) treatment. The figure shows bilateral BDNF protein concentrations of the hippocampus, frontal cortex, and residual neocortex in the four study groups (controls, controls + ES-CIT, stress, and stress + ES-CIT). Data are separately given for the right and left hemisphere and are presented as boxplots displaying the median, quartiles, and extremes (n = 8 for each group). Asterisks indicate a significant difference ($\star P < 0.05$) corrected for multiple testing (Bonferroni test).

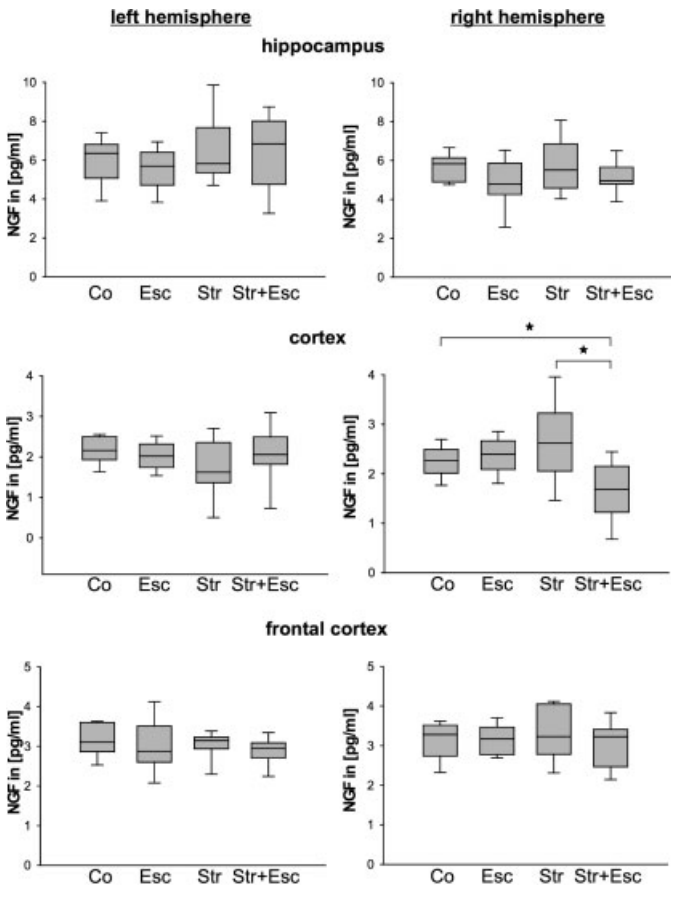


Fig. 4. Changes in cerebral NGF levels with chronic stress and escitalopram (ES-CIT) treatment. The figure shows NGF protein concentrations of the hippocampus, frontal cortex, and residual neocortex in the four study groups (controls, controls + ES-CIT, stress, and stress + ES-CIT). Data are separately given for the right and left hemisphere and are presented as boxplots displaying the median, quartiles, and extremes (n = 8 for each group). Asterisks indicate a significant difference ($\star P < 0.05$) corrected for multiple testing (Bonferroni test).

treatment led to a significant increase of BDNF content in the right (twofold increase) and left (1.5-fold increase) neocortex compared with unstressed controls. Treatment with ES-CIT prevented this BDNF protein level increase in stressed animals. Also, data suggest a 1.5-fold increase of BDNF in the hippocampus of both hemispheres. However, this does not reach statistical significance (Fig. 3). BDNF content of the cerebellum (data not shown) and frontal cortex of both hemispheres remained unchanged after chronic stress and pharmacological intervention with ES-CIT (Fig. 3).

Cerebral NGF Protein Levels

NGF regulation under the stress procedure and serotonergic treatment with ES-CIT was investigated by NGF protein quantification by a highly selective fluorometric ELISA. Animals receiving stress and antidepressive treatment with ES-CIT showed significantly lower NGF levels in the right neocortex compared with animals receiving stress only ($P = 0.037$) and with untreated controls ($P = 0.027$). This effect did not appear in the left neocortex. There was no significant difference in NGF content in the frontal cortex, hippocampus, or cerebellum between stressed, ES-CIT-treated animals and controls (Fig. 4).

Serum Neurotrophin Protein Levels and Correlation Analyses

Because alteration of NGF and BDNF blood levels after stress have been controversial (Aloe et al., 1994; Karege et al., 2002; Lang et al., 2004a; Lommatzsch et al., 2006; Ziegenhorn et al., 2007), serum NGF and BDNF concentrations were determinated by ELISA. There was no significant difference in any of the neuro-

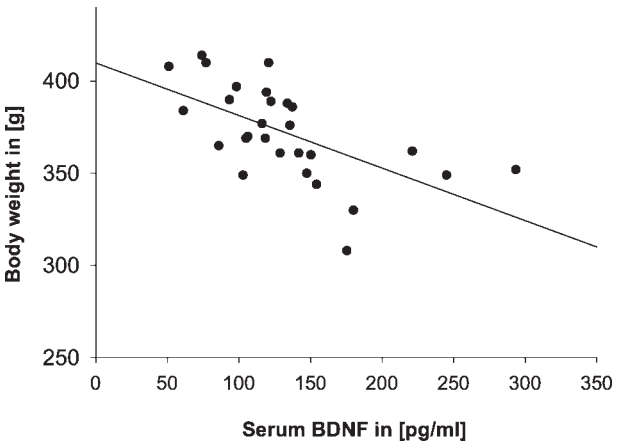


Fig. 5. Correlation of serum BDNF and body weight. This graph represents the correlation (including regression line) of serum BDNF and body weight in the fifth week, including all different study groups (control, stress, control + escitalopram, stress + escitalopram). Each dot represents one individual (n = 28). Correlation was calculated with SPSS using Spearman's correlation coefficient (r = 0.69, $P < 0.0001$).

trophins investigated between any treatment groups (data not shown). In addition to statistical analyses of differences between the chronic stress and ES-CIT-treated groups, correlation analyses between serum neurotrophin levels and body weight were performed. There was a negative, statistically significant correlation between the serum BDNF level and the body weight in every week (first week: r = –0.62, $P = 0.0005$; second week: r = –0.62, $P = 0.0016$; third week: r = –0.62, $P = 0.0005$; fourth week: r = –0.64, $P = 0.0002$; fifth week: r = –0.69, $P < 0.0001$; Fig. 5). Subgroup correlation showed that neither chronic stress nor treatment with ES-CIT significantly altered the negative correlation between serum BDNF and body weight (data not shown). By contrast, serum NGF did not show a significant correlation with body weight (data not shown).

DISCUSSION

This study is the first to investigate systematically the effect of ES-CIT, the SSRI with the highest selectivity for the 5-HT transporter (Owens et al., 1997, 2001), on cerebral neurotrophin levels in a model of chronic stress. The administered chronic social stress has been repeatedly shown to induce depression-associated behavior in rats that can be reversed by treatment with SSRIs such as CIT and fluoxetine (Rygula et al., 2005, 2006a,b). In this experiment, changes in adrenal weight and body weight gain in stressed animals are in line with previous studies using this model and revealing a depressive-like phenotype under stress (Rygula et al., 2005, 2006a). Thus, it can be assumed that the model has provided the same behavioral changes as shown before. Here we abstained from performing additional behavioral testing in order not to interfere with stress-induced neurochemical features. Removing the animals from their home cages, handling by the experimenter, and transfer in the test apparatus, which is a novel, unfamiliar environment, cause a more or less extended series of changes in the stress response pattern. Because of the disturbances, these operations preceding behavioral tests must be considered as possible interfering factors in animal experimentation (Claasen, 1994).

There has been thorough behavioral validation of this rat model (Rygula et al., 2005). In the present study, stress treatment leads to a significant increase of neocortical BDNF protein levels, which can be seen in the hippocampus as a tendency only. The missing statistical significance in the latter case might be due to the small sample size of the treatment groups. In addition, there was a significantly higher BDNF and NGF content in the left hippocampus compared with the right side independent of treatment. For mice we recently reported an enhanced BDNF concentration in the right frontal cortex compared with the left side that was not influenced by stress treatment (Schulte-Herbrüggen et al., 2006). Similarly, in the present study, interhemispheric differences appeared in all treatment groups in the same way. With our model of chronic social stress, a previous study reported a stress-induced hemispheric difference in medial prefrontal proliferation that could be reversed by fluoxetine treatment (Czeh et al., 2007). In this study, these underlying structural differences are not reflected by corresponding neurotrophin contents. The interhemispheric differences, which may differ with strain and species, show the necessity to look carefully after each side separately to avoid misinterpretation of the data. Hemispheric lateralization and specialization of mood are well-described findings. Beyond a lateralization under physiological conditions, a hemispheric assymmetry has been demonstrated under several psychopathological conditions (Bench et al., 1992; Phillips et al., 2003; Coan and Allen, 2004). There are current brain models of emotional processing suggesting that positive emotions are lateralized toward the left hemisphere, whereas negative emotions show a lateralization to the right hemisphere (Rotenberg, 2004). To our knowledge, a study focussing on the relation between structural interhemispheric diferences and changes in BDNF and NGF protein levels has not ben performed yet and is needed to understand better whether there is a morphological correlate of the revealed changes in hippocampal BDNF.

An increase of cerebral BDNF concentration in response to stress seems to be counterintuitive, in that the traditional ''neurotrophin hypothesis of depression'' assumes a stress-related hippocampal decrease of BDNF expression (for recent review see Martinowich et al., 2007). Recently, the scientific debate on BDNF regulation in depression has proposed a more differentiated analysis of this neurotrophic factor in stress-related diseases. Even though there are great numbers of preclinical studies revealing decreased cerebral BDNF expression that is associated with a depressive phenotype (Nibuya et al., 1995; Smith et al., 1995b; Ueyama et al., 1997;

Barrientos et al., 2003; Pizarro et al., 2004; Roceri et al., 2004), there is a considerable amount of research that denies (Schulte-Herbrüggen et al., 2006) or even opposes (Branchi et al., 2006) the above-mentioned stress- and treatment-related BDNF regulation (for a balanced review see Groves, 2007). Different study outcomes may be due to different stress models, application of acute or chronic stress, dose and time of medication, strain differences, or finally a focus on either BDNF mRNA or BDNF protein levels. Owing to the fact that BDNF exerts a significant axonal transport, levels of BDNF mRNA and its encoding protein often do not correlate at all (Conner et al., 1997; Jacobsen and Mork, 2004). As a consequence of partially opposing data on this issue, a differentiated view on BDNF regulation in different brain areas has to be adopted, insofar as this factor can be down-regulated in one area and up-regulated in another (Smith et al., 1995a,b; Schulte-Herbrüggen et al., 2007).

In our chronically stressed rats, a 4-week ES-CIT treatment was able to prevent stress-induced BDNF increase in the right and left neocortex. Former studies concentrated on effects of ES-CIT on BDNF under physiological conditions. A 3-week administration of ES-CIT at a dose of 10 mg/kg/day via subcutaneously implanted minipump lowered BDNF protein levels in the frontal cortex and hippocampus, whereas BDNF mRNA remained unchanged compared with control animals receiving saline via minipump only (Jacobsen and Mork, 2004). Frontal cortex BDNF concentrations did not change at all in the present study, in which we used an oral application of the drug. No changes in cerebral BDNF protein were seen also in rats treated with another SSRI (fluoxetine) over 21 days, whereas the serotonin-norepinephrine reuptake inhibitor duloxetine led to a differential regulation in frontal cortex, with reduced mature BDNF in the cytosol but markedly increased levels in the synaptosomal fraction (Calabrese et al., 2007). In our model of chronic stress administration, it can therefore be assumed that the antidepressive action of the SSRI ES-CIT is at least partially mediated by altered BDNF regulation. A reciprocal interaction of the serotonergic system and BDNF has recently been reviewed (Martinowich and Lu, 2007). Even though the mechanisms have remained elusive until now, there is some evidence showing SSRI alter hippocampal levels of CREB in the rat. CREB is known to regulate the expression of BDNF (Nibuya et al., 1996). Repeatedly, studies have shown that CREB can be activated by phosphorylation at serine 133 by different signaling pathways: MAPK pathways, cAMP-PKA, and Ca^{2+}-CaM-KIV (Shaywitz and Greenberg, 1999). It is plausible that antidepressant medication could enhance BDNF gene expression by activating CREB through one of these pathways. This hypothesis is supported by the fact that acute viral vector-mediated overexpression of CREB in the hippocampus resulted in decreased depressive-like behaviors in rats in different behavioral tasks (Chen et al., 2001). Several other SSRIs and also noradrenalin reuptake inhibitors and monoamine oxidase inhibitors have been shown mostly to up-regulate BDNF mRNA and protein (Nibuya et al., 1995, 1996; Russo-Neustadt et al., 1999; Coppell et al., 2003; Dias et al., 2003; Xu et al., 2003; De et al., 2004; Vinet et al., 2004). In the present study, ES-CIT also showed a tendency to increase cortical BDNF compared with control without pharmacological treatment, whereas, compared with stressed rats, ES-CIT is lower in that it prevents the stress-induced up-regulation of BDNF. Further studies using quantitative receptor autoradiography to correlate stress-induced neurotrophin changes with SSRI sites or other related serotonin receptors constitute promising strategies for identifying additional pathways through which antidepressants work.

In addition, the present study revealed an ES-CIT-dependent decrease of right cortical NGF in chronically stressed rats showing the NGF content even under the untreated control conditions. In rats, antidepressant treatment with lithium at various dosages increased NGF in the hippocampus, amygdala, frontal cortex, and limbic forebrain, whereas NGF in the striatum, midbrain, and hypothalamus was unchanged (Hellweg et al., 2002). In a mouse model of induced learned helplessness after administration of a defined series of foot shocks, stress treatment led to a transient NGF decrease in the frontal cortex after 6 hr that was absent in control mice (Schulte-Herbrüggen et al., 2006). A similar observation was made in rats after threatening treatment with or without painful stimuli, which was followed by a significant reduction of NGF protein in the amygdala and the frontal cortex after 2 hr (von Richthofen et al., 2003). In both studies, NGF returned to control levels after a stress-dependent decrease. Therefore, it is not surprising that in the present study cerebral NGF concentrations of most brain areas investigated correspond to untreated control, insofar as our study design includes one late time of NGF determination. On the other hand, the chronic stress administered in this study differs considerably from the previous models using a relatively short period of stress induction, and further investigation of NGF in this model will be needed (cf. Foreman et al., 1993).

The hippocampus, amygdala, and NGF are involved in processing of fear and are known to prompt behavioral responses to stress (Hecker and Mesulam, 1994; Levi-Montalcini et al., 1996; Herman and Cullinan, 1997). Hippocampal NGF content did not change after electric foot shocks, whereas other studies showed NGF decreases after forced motor activity (Scaccianoce et al., 2000; von Richthofen et al., 2003). In the present study, hippocampal NGF levels did not change compared with untreated controls and under SSRI treatment.

There are also inconsistent data on stress-dependent alterations of serum NGF in humans, which has been revealed to be increased (Aloe et al., 1994) or unchanged (Lang et al., 2004a). Both of those studies studies investigated NGF serum levels in different forms of acute stress, whereas the present study uses a chronic stress treatment

in which no changes in NGF serum concentrations can be seen. With regard to serum BDNF, there are increasinging data focusing on its relation to depressive symptoms, especially in humans. Although there are considerable differences in the BDNF levels between studies, they all point to decreased serum BDNF in depression (Karege et al., 2002; Aydemir et al., 2006; Sen et al., 2008) that were reversed to healthy control levels after treatment with various SSRIs (Gonul et al., 2005; Aydemir et al., 2006; Huang et al., 2007), such as venlafaxin (Aydemir et al., 2005) and ES-CIT (Aydemir et al., 2006). In the present study, rats do not reflect this aspect of human depression; BDNF serum levels remained unchanged irrespective of stress or pharmacological treatment. Recent epidemiological studies have shown that the risk of gastrointestinal bleeding is increased in patients receiving SSRIs (de Abajo et al., 1999, 2006; Dalton et al., 2003, 2006; Halperin and Reber, 2007). Additional studies indicate that bleeding episodes requiring hospital admission are more frequent and that transfusion requirements during orthopedic surgery are increased in patients taking SSRIs (Movig et al., 2003; Meijer et al., 2004). The bleeding diathesis that is seen after SSRI administration is linked to a decrease in platelet function that occurs when serotonin reuptake into platelets is inhibited. One may only hypothesize that in rats serotonergic function in platelets is regulated differently and that a complex homeostasis of BDNF uptake and platelet degranulation leads to equilibrated BDNF levels that do not reflect the stress-induced changes. In contrast, another feature of human serum BDNF regulation appeared in the rodents. Serum BDNF levels have repeatedly been shown to be lower in overweight children and adolescents than in corresponding controls of normal weight (El-Gharbawy et al., 2006; Han et al., 2008). Lommatzsch and colleagues (2005) showed a significant negative correlation between plasma BDNF and weight and a trend toward a negative correlation between serum BDNF and weight in a cohort of middle aged (20–60 years old) healthy subjects. This effect may disappear with age, insofar as serum BDNF and body weight were not correlated in a large cohort of elderly individuals (70–103 years old; Ziegenhorn et al., 2007). Thus, an important role of BDNF in energy homeostasis has been discussed. Therefore, our young to middle-aged rats revealing a significant negative correlation between serum BDNF and body weight correspond well to the human situation and thus may serve as a tool of investigation with which to understand this phenomenon better.

In summary, our observations suggest that BDNF is up-regulated with chronic social stress in rats. Treatment with the SSRI ES-CIT that has been shown before to cure sufficiently anhedonia and depressive-related behavior, to reverse the stress effect, and to restore BDNF levels to the level of healthy controls. Moreover, body weight and BDNF serum levels show a negative correlation, which has also been demonstrated in humans of comparable age. Therefore, rats may present a valuable tool for investigating the underlying mechanisms of this feature. Finally, a limitation of the study is the focus on neurotrophin protein levels in brain tissue homogenates. A combined approach using, e.g., in situ hybridization or immunohistochemistry may help in further understanding changes in neurotrophin levels and the corresponding cellular sources.

ACKNOWLEDGMENTS

Part of this work was performed as a doctoral thesis for A.Z.

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