

Pharmacologically Diverse Antidepressants Rapidly Activate Brain-Derived Neurotrophic Factor Receptor TrkB and Induce Phospholipase-C γ Signaling Pathways in Mouse Brain

Tomi Rantamäki¹, Panu Hendolin², Aino Kankaanpää³, Jelena Mijatovic⁴, Petteri Piepponen⁴, Enrico Domenici⁵, Moses V Chao⁶, Pekka T Männistö⁴ and Eero Castrén^{*,1}

¹Neuroscience Center, University of Helsinki, Helsinki, Finland; ²Jurilab Ltd., Kuopio, Finland; ³Drug Research Unit, National Public Health Institute, Helsinki, Finland; ⁴Division of Pharmacology and Toxicology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland; ⁵Department of Molecular Psychiatry, Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline, Verona, Italy; ⁶Skirball Institute Program of Molecular Neurobiology NYU Medical Center, New York, NY, USA

Previous studies suggest that brain-derived neurotrophic factor and its receptor TrkB are critically involved in the therapeutic actions of antidepressant drugs. We have previously shown that the antidepressants imipramine and fluoxetine produce a rapid autophosphorylation of TrkB in the rodent brain. In the present study, we have further examined the biochemical and functional characteristics of antidepressant-induced TrkB activation *in vivo*. We show that all the antidepressants examined, including inhibitors of monoamine transporters and metabolism, activate TrkB rapidly in the rodent anterior cingulate cortex and hippocampus. Furthermore, the results indicate that acute and long-term antidepressant treatments induce TrkB-mediated activation of phospholipase-C γ 1 (PLC γ 1) and increase the phosphorylation of cAMP-related element binding protein, a major transcription factor mediating neuronal plasticity. In contrast, we have not observed any modulation of the phosphorylation of TrkB Shc binding site, phosphorylation of mitogen-activated protein kinase or AKT by antidepressants. We also show that in the forced swim test, the behavioral effects of specific serotonergic antidepressant citalopram, but not those of the specific noradrenergic antidepressant reboxetine, are crucially dependent on TrkB signaling. Finally, brain monoamines seem to be critical mediators of antidepressant-induced TrkB activation, as antidepressants reboxetine and citalopram do not produce TrkB activation in the brains of serotonin- or norepinephrine-depleted mice. In conclusion, our data suggest that rapid activation of the TrkB neurotrophin receptor and PLC γ 1 signaling is a common mechanism for all antidepressant drugs.

Neuropsychopharmacology (2007) **32**, 2152–2162; doi:10.1038/sj.npp.1301345; published online 21 February 2007

Keywords: TrkB tyrosine phosphorylation; cAMP response element binding protein; TrkB.T1 overexpression; norepinephrine; serotonin; forced swim test

INTRODUCTION

Current evidence suggests that antidepressant-induced neuroplastic changes, such as changes in synaptic plasticity, neurogenesis, and synaptogenesis, may at least partially explain the delayed-onset of action of antidepressants (Duman *et al*, 1997; Manji *et al*, 2001; Nestler *et al*, 2002; Castrén, 2005; Castrén *et al*, 2007). Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of neurotrophic factors, and its primary receptor TrkB seem to be particularly relevant factors involved in both the development of mood disorders and the action

of antidepressants (Altar, 1999; Nestler *et al*, 2002; Castrén, 2005; Castrén *et al*, 2007). BDNF levels are reduced in mood disorders and preclinical depression models, and long-term antidepressant treatment enhances brain BDNF gene expression and signaling (Nibuya *et al*, 1995, 1996; Smith *et al*, 1995; Russo-Neustadt *et al*, 1999; Chen *et al*, 2001b; Karege *et al*, 2002, 2005). A growing body of evidence suggests that BDNF-mediated TrkB signaling is both sufficient and necessary for antidepressant-like behaviors in rodents. Infusion of BDNF or overexpression of TrkB receptors produces antidepressant-like behavioral responses in the preclinical models of behavioral despair (Siuciak *et al*, 1997; Shirayama *et al*, 2002; Koponen *et al*, 2005). We recently demonstrated that the behavioral effects of two antidepressants, imipramine and fluoxetine (FLX) were abolished in transgenic mice with reduced BDNF levels or inhibited TrkB signaling in brain (Saarelainen *et al*, 2003) and this observation was

*Correspondence: Professor E. Castrén, Neuroscience Center, University of Helsinki, PO Box 56, 00014 Helsinki, Finland, Tel: +358 9 191 576 26, Fax: +358 9 191 576 20, E-mail: eero.castrén@helsinki.fi
Received 6 July 2006; revised 5 December 2006; accepted 19 December 2006

subsequently confirmed in forebrain-specific *BDNF* null mice by using desipramine (Monteggia *et al*, 2004, 2007), which shows a relatively high affinity to norepinephrine transporter (NET) when compared with imipramine and FLX. Furthermore, val/met polymorphism in *BDNF* gene, which influences the activity-dependent *BDNF* release (Egan *et al*, 2003) has recently been associated with mood disorders in humans (Sklar *et al*, 2002; Lang *et al*, 2005). Taken together, these observations suggest that activity-dependent and temporal activation of TrkB plays a critical role in the antidepressant response perhaps via the formation and stabilization of new functional synaptic connections.

Chronic but also acute antidepressant treatments produce alterations in *BDNF* mRNA levels in the rodent anterior cingulate cortex and hippocampus (Nibuya *et al*, 1995; Zetterström *et al*, 1999). In addition, antidepressants block and accelerate the reversion of stress-induced downregulation in *BDNF* mRNA and protein, respectively (Nibuya *et al*, 1995; Xu *et al*, 2004). Although some studies have reported upregulation of *BDNF* protein in the rodent hippocampus after prolonged antidepressant treatment (Xu *et al*, 2003; Wyneken *et al*, 2006), some studies have failed to see such significant alterations (Altar *et al*, 2003; Holoubek *et al*, 2004). These findings may reflect the fact that *BDNF* protein is quite stable inside the cell and undergoes both anterograde and retrograde transport in specific cellular compartments and only a limited amount of *BDNF* is released activity dependently in neurons (Balkowiec and Katz, 2002; Lu, 2003). Hence, only a small fraction of the total *BDNF* protein is extracellular and able to activate TrkB receptors, thus making it difficult to reliably measure extracellular *BDNF*. To circumvent this problem, we have used autophosphorylation of TrkB receptors as an indirect indicator of *BDNF* release from neurons. Using this assay, we previously demonstrated that the antidepressants FLX and imipramine, as well as the mood stabilizer, lithium, rapidly autophosphorylate TrkB in the rodent anterior cingulate cortex (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006). TrkB activation is also observed after prolonged antidepressant administration in the rodent brain further pointing out the role of this signaling event mediating the long-term changes of antidepressant treatment (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006).

The aims of the present study were to determine whether rapid induction of TrkB activity is a common factor for pharmacologically diverse antidepressants and to dissect the specific TrkB-mediated signaling events that are downstream of TrkB activity promoted by antidepressants. Furthermore, we extended the previous findings concerning the role of TrkB signaling in regulating the acute behavioral effects of antidepressants in the forced swim test (FST) (Saarelainen *et al*, 2003; Monteggia *et al*, 2004), a prototypic screening test of antidepressant action (Porsolt *et al*, 1977), by using selective noradrenergic and serotonergic antidepressants together with TrkB-deficient mice in this paradigm. Finally, given that all clinically used antidepressants regulate monoaminergic signaling, we sought to examine the role of serotonin and norepinephrine (NE) in antidepressant-induced brain TrkB activation in more detail.

MATERIALS AND METHODS

Animals

Adult male Balb/C and C57BL/6 mice were used in the biochemical experiments. In the behavioral studies, transgenic mice overexpressing the truncated form of TrkB receptor (TrkB.T1) and their wild-type (WT) littermates were employed and generated as previously described (Saarelainen *et al*, 2000). Animals were group-housed in standard laboratory conditions and food and water (or drug solution) were freely available. All animal experiments were carried out according to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Experimental Animal Ethical Committee of University of Helsinki.

Drug Treatments

For the acute antidepressant treatments, animals received a single i.p. injection of antidepressant or saline and tested in the FST (30 min following injection) (Porsolt *et al*, 1977; Koponen *et al*, 2005) or killed for biochemical assays (30, 60, or 120 min following drug administration). The following antidepressant drugs and doses were used: FLX-HCl (20–30 mg/kg, Sigma-Aldrich Finland Oy, Helsinki, Finland; OrionPharma Turku, Finland; or kindly provided by GlaxoSmithKline (GSK), Verona, Italy), citalopram-HBr (20 mg/kg, kindly provided by GSK), imipramine-HCl (30 mg/kg, Sigma-Aldrich), clomipramine-HCl (20 mg/kg, Sigma-Aldrich), reboxetine (free base) (20 mg/kg, kindly provided by GSK), and moclobemide (free base) (20 mg/kg, kindly provided by F Hoffmann-La Roche Ltd, Basel, Switzerland). Selected antidepressant doses have been routinely used in rodent models of behavioral despair (Porsolt *et al*, 1977; Wong *et al*, 2000; Drago *et al*, 2001; Weinstock *et al*, 2002; O'Leary *et al*, 2007). Moclobemide and reboxetine were dissolved in 50% DMSO/saline, citalopram in 50% ethanol/saline, others in saline. Saline was used as a control vehicle. For long-term treatments, mice had a free access to either tap water (controls) or 0.08 mg/ml FLX solution for 21 days (Santarelli *et al*, 2003). Mouse brain serotonin (5-HT) and NE contents were depleted using *p*CPA (4-chloro-*L*-phenylalanine, Sigma-Aldrich) and DSP-4-HCl (Sigma-Aldrich), respectively. *p*CPA (in 0.1 M NaOH, pH ~8) was injected twice a day (~0900 and ~1700 hours) at a dose of 100 mg/kg (i.p.) on 3 consecutive days before and DSP-4 (in saline) at a dose of 50 mg/kg (i.p.) at 7 days before treatment with antidepressants. Control animals received an equal volume saline.

Chemical Analyses

Plasma FLX levels were measured from trunk blood using gas chromatography-mass spectrometry (GC-MS). Briefly, FLX was extracted from 200 µl of plasma by mixing with 5 ml of toluene (+150 ng/ml of the internal standard flurazepam) and 200 µl of a buffer (0.5 M Na₂HCO₃ × 2H₂O). After centrifugation, the toluene layer was collected and 15 µl of the derivatization reagent (heptafluorobutyric anhydride, HFBA) was added together with 1 ml of saturated NaHCO₃. After centrifugation, the toluene layer was evaporated, the residue was dissolved into 50 µl of toluene,

and transferred into autosampler vials with inserts. An aliquot of 4 μ l of the toluene layer was injected into a GC-MS apparatus consisting of an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6890N gas chromatograph, an Agilent 5973 mass selective detector (EI, positive ions, 70 eV) and an Agilent ChemStation data system. The GC column was a DB-35MS (J&W Scientific Inc., Folsom, CA, USA, length 30 m, internal diameter 0.32 mm and film thickness 0.25 μ m). The column temperature was initially 150°C for 1 min, then increased 45°C/min to 330°C, with a final hold time of 2 min. MS detection was performed in selected ion monitoring mode. FLX was analyzed as its HFB-derivative. Plasma FLX levels after the acute (30 mg/kg, 60 min) and long-term (0.08 mg/ml, 21 days, *ad libitum*) FLX treatments were 511.4 ± 165.6 and 267.8 ± 100.7 ng/ml (average \pm standard deviation), respectively.

Brain serotonin, NE and dopamine levels were analyzed using high-performance liquid chromatography coupled with electrochemical detection (HPLC-EC) as recently described (Airavaara *et al*, 2006). Shortly, a piece of motor cortex was homogenized in a mixture of 0.2 M HClO₄ and antioxidant solution (Kankaanpää *et al*, 2001), centrifuged (20 800g, 35 min, 4°C), and the supernatants centrifugated again (8600g, 35 min, 4°C) through Vivaspinn filter concentrators (10 000 MWCO PES; Vivascience AG, Hannover, Germany). The column (Spherisorb[®] ODS2 3 μ m, 4.6 \times 100 mm; Waters, Milford, MA, USA) was kept at 50°C with a column heater (Croco-Cil, Bordeaux, France). The mobile phase consisted of 0.1 M NaH₂PO₄ buffer, 350 mg/l of octane sulfonic acid, 3.5–5% (v/v) CH₃OH and 450 mg/l EDTA (pH 2.7). The flow rate was 1 ml/min. Filtrate was injected into chromatographic system with a CMA/200 autoinjector (CMA, Stockholm, Sweden). Concentrations of amines were quantified using ESA[®] CoulArray Electrode Array Detector system (ESA Inc., Chelmsford, MA, USA).

Immunoprecipitation and Western Blot

Mice were killed with CO₂, brains quickly removed and bilateral anterior cingulate cortex and left hippocampus were dissected on a dish cooled on dry-ice. Samples were immediately homogenized in a lysis buffer (137 mM NaCl, 20 mM Tris, 1% NP-40, 10% glycerol, 48 mM NaF, H₂O, 2 \times Complete inhibitor mix- (Roche-Applied Science, Indianapolis, IN, USA) and 2 mM Na₂VO₃). After incubation on ice for 15 min, samples were centrifuged (16 100g, 15 min). Protein levels of the collected supernatants were then measured using the Lowry method (Biorad DC protein assay; Lowry *et al*, 1951). For Trk receptor immunoprecipitation, 500–600 μ g of protein was incubated overnight at +4°C with 5 μ l of Trk-antibody (sc-11, Santa Cruz Biotechnology (SCB), Santa Cruz, CA, USA) and immunocomplexes were precipitated with 35 μ l of 50% Protein-G Sepharose (in Triton + +: 137 mM NaCl, 20 mM Tris pH 8.0, 48 mM NaF, 0.5% TritonX + 2 \times Complete inhibitor mix (Roche-Applied) + 2 mM Na₃VO₂). Lectin precipitation was carried out essentially as described (Rantamäki *et al*, 2006) using Wheat Germ Lectin/NP + + (6MB or 4B Sepharose, Amersham Biosciences, Piscataway, NJ, USA or EY Laboratories, San Mateo, CA, USA). Proteins were separated in a SDS-PAGE under reducing conditions (Laemmli buffer: 20% glycerol, 4% SDS, 125 mM Tris (pH 6.8), 10%

β -mercaptoethanol, 0.02% bromophenol blue) and blotted to a PVDF membrane (300 mA, 1 h, +4°C). Membranes were incubated with the following primary antibodies: anti-pY674/5 of TrkA (corresponding to pY705/6 in TrkB, 1:1000–2000, Cell Signaling Technology (CST), Danvers, MA, USA), anti-pY705/6 (1:500–1000; Rantamäki *et al*, 2006), anti-pY816 of TrkB (1:5000), sc-11 (1:2000, SCB), anti-pCREB (1:1000, CST), anti-cAMP-related element binding protein (CREB) (1:1000, CST) anti-Shc (1:1000, Upstate, Charlottesville, VA, USA), anti-pAKT (1:1000, CST), antiphospholipase-C γ 1 (PLC γ 1) (1:1000, Upstate), and anti-p42/p44-mitogen-activated protein kinase (MAPK) (1:1000, CST). Further, the membranes were washed with TBS/0.1% Tween[®] (TBST) and incubated with horseradish peroxidase conjugated secondary antibody (1:10 000 in non-fat dry milk, 1 h, RT, BioRad Laboratories, Hercules, CA). After subsequent TBST washes, secondary antibodies were visualized using electrochemiluminescence kits (ECL; Amersham Biosciences) followed by an exposure to X-ray film and/or Fuji LAS-3000 camera (Tamro Medlabs, Vantaa, Finland) for ECL detection.

The Forced Swim Test

TrkB.TK– mice ($n = 35$) and their WT littermates ($n = 44$) were tested in the FST (Koponen *et al*, 2005) 30 min after the injection of citalopram (20 mg/kg), reboxetine (20 mg/kg), or saline without a pre-swim. The behavioral parameter recorded was latency to immobility. The test was carried out in a glass decanter (ϕ 18 cm, height 27 cm) half-filled with water ($23 \pm 1^\circ\text{C}$). During maximum of 6 min observation period, the first period of immobility over 2 s was recorded as the latency to immobility time.

Data Analysis

Immunoblot bands were quantitated using NIH ImageJ 1.32. Statistical analyses were carried out using two-sample two-tailed Student's *t*-test or with one- or two-way analysis of variance (ANOVA) with Newman–Keuls *post hoc* test. Criteria for statistical significance was $p < 0.05$. In the Western blot analyses, equal loading was confirmed using the Ponceau staining, which was routinely used for normalization, unless otherwise stated. Unless otherwise stated, all data are presented as mean \pm SEM (standard error of mean) and as percentage of control.

RESULTS

The Acute Effects of Diverse Antidepressants on TrkB Activation in Mouse Brain

Both acute and long-term treatment with the antidepressants imipramine and FLX or mood stabilizer lithium induces autophosphorylation of TrkB in the mouse anterior cingulate cortex (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006). In the present study, we examined whether TrkB activation is a response shared by pharmacologically diverse antidepressants or whether it is specific for antidepressants directed against certain pharmacological targets. TrkB autophosphorylation was measured by *Western blot* using our previously described antibody

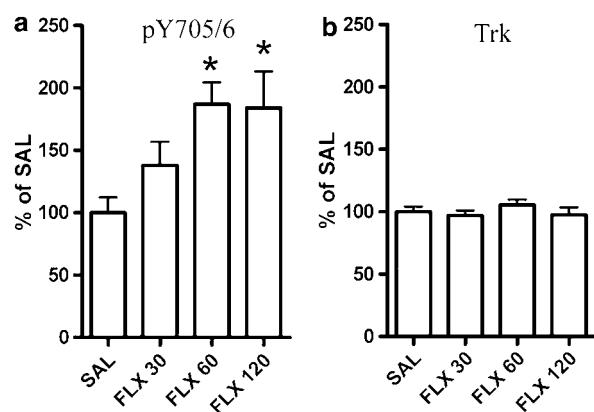


Figure 1 Time-dependent activation of TrkB receptors in the mouse hippocampus after acute FLX treatment (30 mg/kg, i.p.) (a) phosphorylation of TrkB catalytic domain (pY705/6) 30, 60, and 120 min following a single FLX injection (b) Trk protein levels in the hippocampus. Phospho-Trk values in (a) are normalized against total Trk levels. * $p < 0.05$, one-way ANOVA with Newman–Keuls *post hoc* test, $n = 5–7$ /group.

against the autophosphorylated catalytic domain of TrkB (pY705/6) (Rantamäki *et al*, 2006). Phosphorylation of the tyrosines 705/6 at the autophosphorylation loop is a critical step in the TrkB receptor activation (Huang and Reichardt, 2001). Moreover, pY705/6 levels are highly correlated with actual tyrosine kinase activity *in vitro* (Segal *et al*, 1996).

Preliminary studies were conducted to first determine the time course of the effects of FLX (30 mg/kg) on hippocampal pY705/6 levels ($n = 5–7$ /group). TrkB activation was increased 30 min after FLX administration and reached the maximal levels within 60 min (Figure 1a). In line with our previous observations (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006), the Trk protein levels were not altered after acute antidepressant treatment (Figure 1b). The ability of other antidepressants with different pharmacological profiles to induce TrkB autophosphorylation at the Y705/6 site was then examined. The following antidepressants were tested: the selective serotonin transporter (SERT) blocker citalopram (20 mg/kg), the NE transporter (NET) blocker reboxetine (20 mg/kg), the tricyclic antidepressants clomipramine (20 mg/kg), and imipramine (30 mg/kg) (a positive control; see Saarelainen *et al*, 2003), and the monoamine oxidase-A (MAO-A) inhibitor moclobemide (20 mg/kg) ($n = 4–7$ /group). Effects were first investigated at the 30 min time point, and drugs that did not alter pY705/6 levels at this time point were then re-evaluated at the 60 min time point. All the antidepressants tested significantly increased pY705/6 levels in the anterior cingulate cortex (Figure 2a) and hippocampus (Figure 2b) within 30–60 min of a single injection. None of the treatments influenced the expression levels of total TrkB protein (data not shown).

Antidepressants Increase the Activation of TrkB PLC γ 1 Site and the Phosphorylation of CREB

Phosphorylation of TrkB catalytic domain is considered as an initial step in TrkB receptor activation, which further regulates the phosphorylation and activation of other

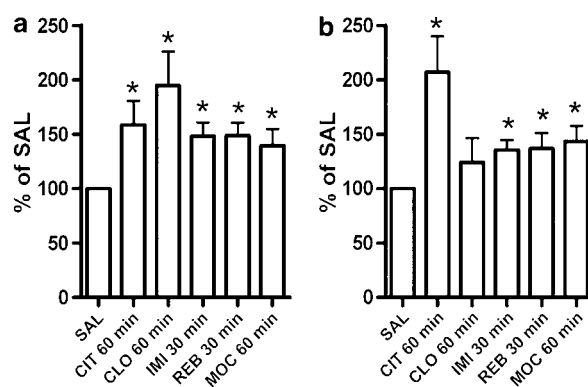


Figure 2 Acute effects (30, 60 min) of antidepressants belonging to different pharmacological groups on brain TrkB activation (measured as the phosphorylation of TrkB catalytic domain) (a) anterior cingulate cortex (b) hippocampus. Phospho-Trk values are normalized against total Trk levels. SAL: saline; CIT: citalopram (20 mg/kg); CLO: clomipramine (20 mg/kg); IMI: imipramine (30 mg/kg); REB: reboxetine (20 mg/kg); MOC: moclobemide (20 mg/kg). * $p < 0.05$, Student's *t*-test, $n = 4–7$ /group.

tyrosines of which Y515 and Y816 has been most extensively studied (Huang and Reichardt, 2001). Phosphorylation of Y515 leads to activation of the MAPK and AKT (protein kinase-B) signaling pathways, whereas phosphorylation of tyrosine 816 induces the association of the PLC γ 1 with this phosphotyrosine and the activation of PLC γ 1-mediated signaling pathway (Huang and Reichardt, 2001).

We tested the hypothesis that the TrkB-mediated PLC γ 1 pathway, a signaling event implicated in synaptic plasticity (Minichiello *et al*, 2002), is activated by antidepressant treatment. Activation of PLC γ 1 was measured by using a polyclonal antibody, which specifically recognizes the phosphorylation of TrkB receptor PLC γ 1 site (pY816) and by analyzing the interaction of PLC γ 1 with TrkB by measuring the co-immunoprecipitation of these proteins. Significant increase in pY816-band immunoreactivity was observed 60 min after a single 20 mg/kg FLX treatment (Figure 3a). Similar induction in pY816 levels was observed after the acute reboxetine (20 mg/kg, 30 min, 7), citalopram (20 mg/kg, 60 min, 7), and imipramine treatments (data not shown). Furthermore, we observed that acute FLX treatment tend to increase the association of PLC γ 1 with Trk receptors (Figure 3b), albeit the difference did not reach a statistically significant value. Given the importance of prolonged antidepressant treatment for clinical recovery from depression, association of PLC γ 1 with Trk was also analyzed after long-term FLX (0.08 mg/ml FLX in drinking solution, 21 days) and water administration ($n = 8–9$ /group). In the drug treatment group, average plasma FLX levels were clinically relevant being 268 ± 33 ng/ml (Altamura *et al*, 1994) at the time of brain tissue dissection. This FLX administration protocol has been previously shown to produce long-term alterations in neuroplasticity and animal behavior (Santarelli *et al*, 2003). Importantly, a significant increase in the association of PLC γ 1 with Trk was observed in the hippocampal samples when compared with control animals (Figure 3c) and this change coincided with increased autophosphorylation of TrkB receptor (see below, Figure 4f). Additional *Western blot* analyses revealed that PLC γ 1 or TrkB protein levels were not altered after

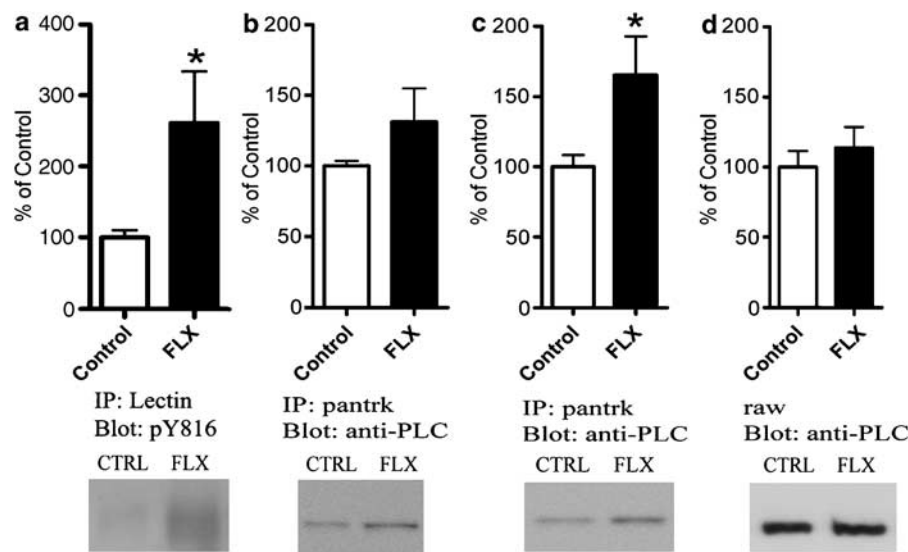


Figure 3 Effects of acute (20–30 mg/kg, i.p., 60 min, $n=5-6$) and long-term (0.08 mg/ml in drinking water, 3 weeks, $n=9$) FLX treatments on PLC γ 1 signaling in mouse hippocampus. (a) Effects of acute FLX (20 mg/kg) treatment on TrkB phosphorylation at TrkB PLC γ 1 site. (b) Effects of acute FLX (30 mg/kg) treatment on the interaction between TrkB and PLC γ 1. (c) Effects of long-term FLX treatment on interaction between TrkB and PLC γ 1. (d) PLC γ 1 protein levels after long-term FLX treatment. Phospho-Trk values are normalized against total Trk levels. Immunoprecipitation values are normalized against total Trk levels. * $p<0.05$, two-tailed Student's t -test.

prolonged antidepressant treatments when compared with the control group thus discounting the possibility that changes observed after prolonged antidepressant treatments were due to alterations in PLC γ 1 or TrkB protein levels (data shown partially in Figure 3d). Taken together, these data indicate that antidepressant-induced TrkB activation leads to increased PLC γ 1 signaling.

Although antidepressants and lithium readily induce autophosphorylation of the Y705/6 site, the phosphorylation of tyrosine 515 (pY515), the interaction site for the Shc adaptor protein is not altered by these drugs (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006). We have now confirmed that FLX fails to induce pY515 in the mouse hippocampus not only 30 min after administration, but also 60 and 120 min following FLX administration (30 mg/kg, $n=5-7$ /group) (Figure 4a). These observations, together with earlier findings (Lucas *et al*, 2003; Saarelainen *et al*, 2003; Rantamäki *et al*, 2006; Figure 3a), clearly indicate that the phosphorylation status of different tyrosine residues in TrkB are differentially and independently regulated *in vivo*. In order to investigate further the actions of antidepressants on Y515 signaling of TrkB, the phosphorylation status of AKT and MAPK from hippocampal samples obtained from mice acutely treated with FLX (30 mg/kg, 60 min) or saline were examined ($n=5-7$ /group). In agreement with lack of changes in pY515 levels after acute FLX treatment, no changes were observed in the tissue levels of either p42/p44-MAPK or pAKT when compared with saline treated animals (Figure 4b and c). Next, the phosphorylation of Shc-binding (Y515) site and the interaction of the three Shc adaptors with Trk receptors following 3 weeks of FLX (0.08 mg/ml FLX in drinking solution) or water was measured ($n=8-9$ /group). In Trk immunoprecipitated pool of proteins, no differences were observed in Shc interaction between treatment groups (Figure 4d). Similarly to acute antidepressant treatment, long-term FLX treatment did not

change the phosphorylation status of Y515 site in TrkB (Figure 4e). In the same samples, pY705/6 levels were significantly upregulated when compared with control mice (Figure 4f), thus discounting the possibility that chronic drug administration desensitizes the pY705/6 response (see Saarelainen *et al*, 2003). Together these findings indicate that acute and long-term antidepressant treatment increases TrkB receptor activity, which further leads to the activation of TrkB-PLC γ 1 signaling in the mouse brain without significantly altering the Shc-mediated signaling pathways of TrkB.

CREB signaling is a critical mediator of the long-term antidepressant response (Duman *et al*, 1997; Nestler *et al*, 2002; Carlezon *et al*, 2005). CREB and BDNF signaling are tightly coupled, as CREB phosphorylation increases BDNF expression and TrkB activation leads to the phosphorylation and activation of CREB. Minichiello *et al* (2002) have recently shown that the Y816 site of TrkB, in contrast to Y515 site, is an important regulator of CREB phosphorylation *in vitro*. Consequently, we asked whether the antidepressant-induced enhancement of TrkB-PLC γ 1 signaling results in increased phosphorylation of CREB *in vivo*. Both acute and long-term FLX treatments significantly increased the pCREB levels in the mouse hippocampus (Figure 5a and b). Similar induction in hippocampal pCREB levels were observed after the acute reboxetine (20 mg/kg, 30 min) and citalopram (20 mg/kg, 60 min) treatments (Reboxetine $187.04 \pm 17.98\%$, $p<0.01$; Citalopram $368.25 \pm 53.33\%$, $p<0.005$; % of Saline; $n=5$ /group; Student's t -test). These data are consistent with a model where antidepressants rapidly induce the autophosphorylation of TrkB, which through PLC γ 1 signaling, activates CREB. This model is supported by our previous observation that imipramine-induced increase in pCREB is blunted in the cingulate cortex of mice overexpressing the dominant-negative TrkB receptor in brain (Saarelainen *et al*, 2003).

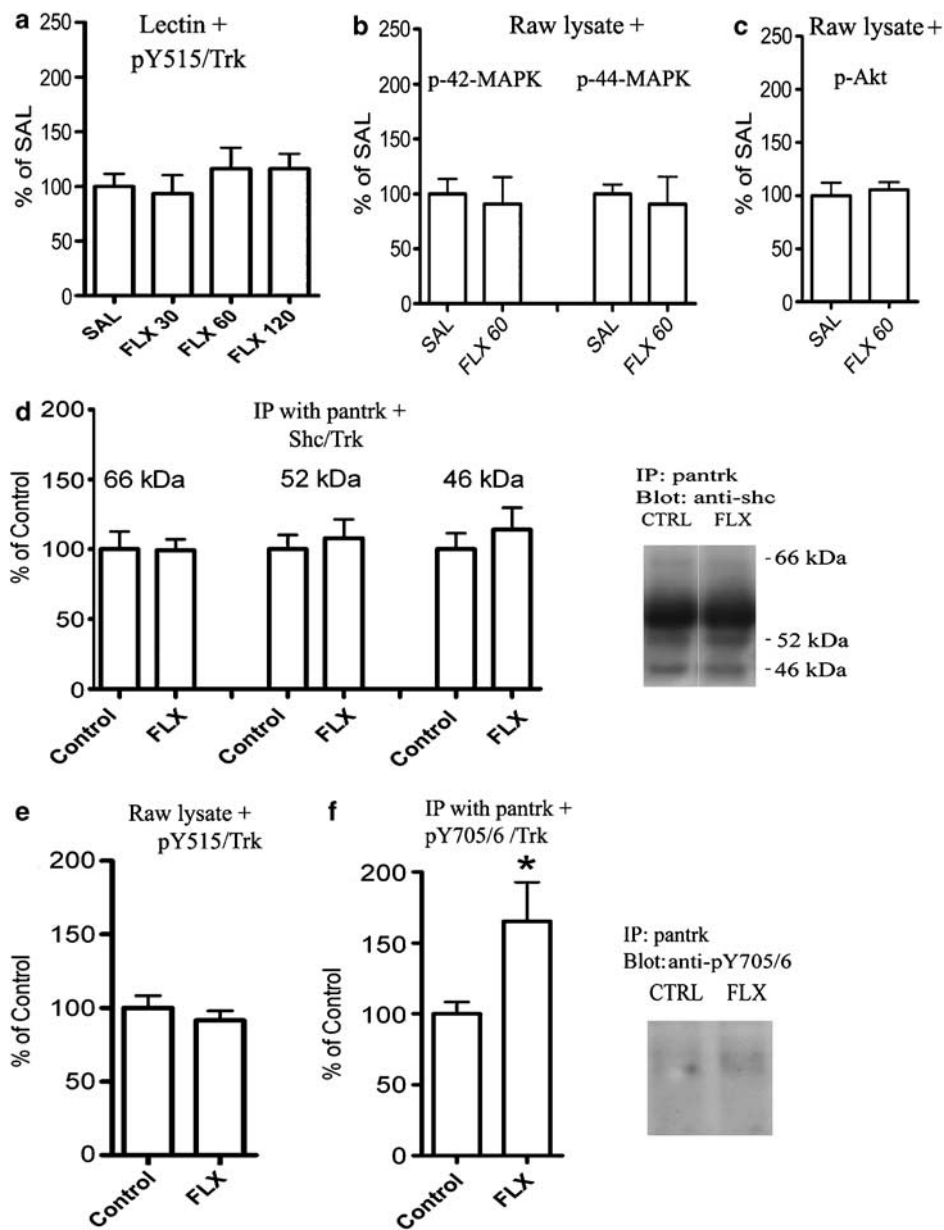


Figure 4 Effects of acute (30 mg/kg, i.p., $n = 5$; a–c) and long-term (0.08 mg/ml in drinking water, 3 weeks, $n = 9$; d–f) FLX treatments on the activation of Shc-mediated signaling pathways in mouse hippocampus. (a) Time-dependent (30, 60 and 120 min) phosphorylation of TrkB Shc binding site (pY515) after a single FLX treatment. (b) Phosphorylation of 42/44 MAPK 60 min following a single FLX treatment. (c) Phosphorylation of AKT 60 min following a single FLX treatment. (d) Interaction between TrkB and Shc adaptor proteins after prolonged FLX treatment. (e) Phosphorylation of the TrkB Shc binding site after prolonged FLX treatment. (f) Phosphorylation of the TrkB catalytic domain after prolonged FLX treatment. * $p < 0.05$, two-tailed Student's *t*-test.

The Behavioral Effects of Citalopram and Reboxetine in TrkB.T1 Overexpressing Mice

Recently, we and others have shown that the administration of BDNF or overexpression of TrkB produce antidepressant-like effects in rodent depression models (Siuciak *et al*, 1997; Shirayama *et al*, 2002; Koponen *et al*, 2005), whereas TrkB signaling is essential for the behavioral effects of noradrenergic and serotonergic antidepressants in the FST (Saarelainen *et al*, 2003; Monteggia *et al*, 2004). As the antidepressant treatments (imipramine, FLX, and desipramine) used in the previous experiments did not clearly differentiate between brain 5-HT and NE systems, we

examined the effects of acute treatment with the selective SERT blocker (citalopram, 20 mg/kg; Bymaster *et al*, 2002) and the selective NET blocker (reboxetine, 20 mg/kg; Page and Lucki, 2002) in WT and TrkB-deficient mice (TrkB.TK[−]) when tested in the FST. Both citalopram and reboxetine significantly increased latency to immobility in WT mice in the FST (Figure 6). As previously reported (Saarelainen *et al*, 2003), the behavior of saline-treated TrkB.TK[−] mice in FST was comparable to their WT littermates (Figure 6). In agreement with our previous findings with imipramine and FLX (Saarelainen *et al*, 2003), the acute behavioral effects of citalopram in the FST were essentially abolished in TrkB.TK[−] mice (Figure 6). In

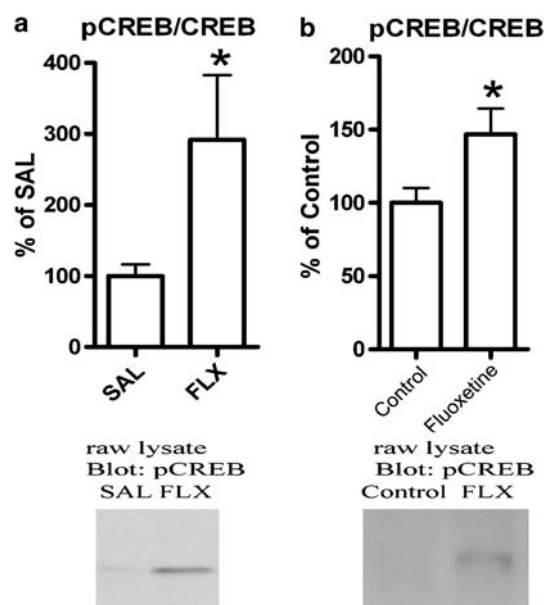


Figure 5 Effects of acute (30 mg/kg, 60 min, $n=5$; left) and long-term (0.08 mg/ml in drinking water, 3 weeks, $n=9$; right) FLX treatment on hippocampal CREB phosphorylation (shown as ratio of pCREB/CREB). * $p < 0.05$, two-tailed Student's *t*-test.

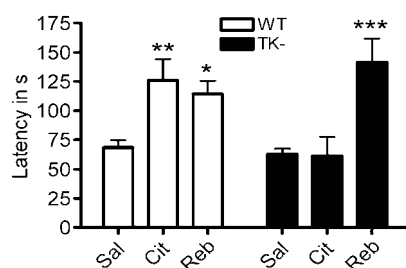


Figure 6 Behavioral effects of citalopram (Cit, 20 mg/kg, 30 min), but not of reboxetine (Reb, 20 mg/kg, 30 min), are diminished in mice with reduced TrkB signaling (TrkB.T1 overexpressing mice; TrkB.TK-) in the latency-to-immobility version of the FST. Data are presented as mean seconds \pm SEM to the first period of immobility longer than 2 s. ***, **, *, $p < 0.005$, < 0.01 , and < 0.05 , respectively, from the respective controls, two-way ANOVA with Newman-Keuls *post hoc* test, $n = 10-16$ /group.

striking contrast, however, acute reboxetine treatment significantly increased the latency to immobility time in TrkB.TK- mice in a manner similar to reboxetine-treated WT mice (Figure 6). Altogether, the present results suggest that the acute behavioral effects of serotonergic agent in the FST are crucially dependent on TrkB signaling. However, the acute behavioral effects of highly selective noradrenergic antidepressant reboxetine in the FST appear to be partially mediated through TrkB-independent neuronal circuits.

The Role of Monoamines in Antidepressant-Induced Brain TrkB Activation

Antidepressants that block monoamine transporters produce rapid elevation of extracellular monoamines, particularly 5-HT and NE, in the rodent forebrain. To investigate the role of these monoamines in antidepressant-induced brain TrkB activation, brain 5-HT or NE content was

Table 1 Cortical Tissue Levels of Monoamines in Drug-Naive Mice after DSP-4 and pCPA Treatments (for Administration Protocols, See Materials and Methods)

	SAL	DSP4	pCPA
DA	46.03 \pm 4.0	19.68 \pm 2.9**	48.48 \pm 9.8
5-HT	326.81 \pm 24.7	306.46 \pm 24.1	35.93 \pm 7.6***
NE	372.54 \pm 9.0	62.98 \pm 34.1***	319.8 \pm 32.5

Abbreviations: SAL, saline; pCPA, 4-chloro-L-phenylalanine; NE, norepinephrine; 5-HT, serotonin; DA, dopamine.

Data are presented as average ng/mg \pm SEM. **, ***, $p < 0.01$ and < 0.005 , respectively, Student's *t*-test.

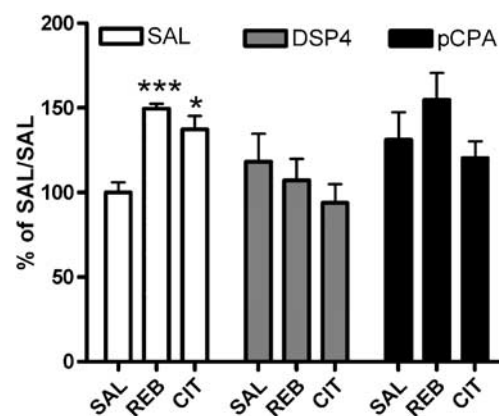


Figure 7 Role of serotonin and NE in antidepressant-induced TrkB activation (measured as phosphorylation of TrkB PLCγ1 site: pY816) in mouse anterior cingulate cortex. Phospho-Trk levels were analyzed from pool of glycosylated proteins as described in the Materials and methods and are normalized against total Trk levels. Data are presented as mean \pm SEM and as % of SAL/SAL values. *, ***, $p < 0.05$ and < 0.005 , respectively, as compared with the respective controls, two-way ANOVA with Newman-Keuls *post hoc* test, $n = 4-7$ /group.

depleted using pCPA (see Materials and methods) or DSP-4 (see Materials and methods), respectively, prior acute treatment with reboxetine (20 mg/kg, 30 min), citalopram (20 mg/kg, 60 min), and saline ($n = 4-7$ /group). Reboxetine and citalopram are among the most selective blockers for NET and SERT, respectively. As shown in Table 1, DSP-4 and pCPA robustly diminished tissue NE and 5-HT contents, respectively, in the mouse cortex. In sham animals, both citalopram and reboxetine induced significant activation of TrkB receptors, measured as phosphorylation of TrkB PLCγ1 site (Figure 7). Interestingly, in both DSP-4 and pCPA pre-treated mice, reboxetine and citalopram failed to alter the phosphorylation status of TrkB PLCγ1 site (Figure 7). These findings suggest that serotonergic and noradrenergic systems are critically involved in antidepressant-induced TrkB activation in the mouse brain.

DISCUSSION

Brain TrkB Activation is Induced by Different Antidepressants

The present study demonstrates that a variety of antidepressants irrespective of their pharmacological classifica-

tion activate in behaviorally effective doses (Porsolt *et al*, 1977; Wong *et al*, 2000; Drago *et al*, 2001; Weinstock *et al*, 2002; O'Leary *et al*, 2007) TrkB receptors in the rodent brain. Similar changes in TrkB activity have also been observed after acute lithium treatment (Rantamäki *et al*, 2006). These effects are rapid and observed within 1 h of administration and persist following long-term drug administration (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006). Importantly, in addition to the conventional antidepressants, the monoamine oxidase inhibitor moclobemide produced significant changes in brain TrkB activity.

Ligand-induced dimerization is the best characterized mechanism, which induces autophosphorylation and activation of TrkB and, therefore, our data suggest that antidepressants induce a rapid increase in BDNF release. However, as the majority of brain BDNF is intracellular, total brain BDNF does not reliably reflect its release and unfortunately, extracellular BDNF cannot yet be directly measured in a reliable manner. Indeed, we have failed to observe any changes in the levels of BDNF protein in the anterior cingulate cortex after imipramine and lithium treatment, two treatments that reliably induce TrkB autophosphorylation in the same brain regions (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006). Furthermore, although chronic treatment with a variety of antidepressants increases BDNF mRNA levels in brain (Nibuya *et al*, 1995), increases in BDNF protein levels by antidepressant treatments have not been consistently reported (Altar *et al*, 2003; Holoubek *et al*, 2004). These data suggest that antidepressants increase BDNF release and increased BDNF mRNA levels reflect a need to replenish stores of released BDNF. In agreement, a recent study demonstrated that chronic FLX treatment increased TrkB autophosphorylation in signaling endosomes but decreased TrkB protein in synaptic membranes (Wyneken *et al*, 2006), which is consistent with the BDNF-induced downregulation of synaptic TrkB (Carter *et al*, 1995; Haapasalo *et al*, 2002). However, we cannot rule out the possibility that antidepressants increase TrkB signaling without influencing BDNF release. Increased intracellular cAMP or membrane depolarization have been shown to increase the membrane localization of TrkB thus enhancing the ability of BDNF to induce TrkB autophosphorylation (Meyer-Franke *et al*, 1998; Haapasalo *et al*, 2002; Du *et al*, 2003; Ji *et al*, 2005). Furthermore, some G-protein-coupled receptors have been shown to transactivate TrkB receptors independently of BDNF (Lee *et al*, 2002a; Lee *et al*, 2002b; Berghuis *et al*, 2005). Finally, as NT-3 (and NT-4/5) is a low-affinity ligand for TrkB, antidepressants may induce TrkB activation by enhancing the effects of this neurotrophin (see Lewis *et al*, 2006). Whether these molecular mechanisms are involved in antidepressant-induced TrkB activation, remains to be investigated.

Acute and Long-Term FLX Treatments Induce CREB Phosphorylation and Activation of TrkB-Mediated PLC γ 1 Pathway

Signal transduction pathways activated by chronic antidepressant treatments have been intensively investigated (Duman *et al*, 1997; Manji *et al*, 2001; Coyle and Duman, 2003), but less is known about the pathways that are

activated acutely by antidepressants. TrkB receptors activate several intracellular signaling cascades that can be regulated independently of each other, among them, the MAPK, AKT, and PLC γ 1 pathways are best characterized (Huang and Reichardt, 2001). The present study revealed that acute and long-term antidepressant treatments increased the phosphorylation of TrkB PLC γ 1 site and its interaction with PLC γ 1. This finding is supported by the recent observation that pretreatment of primary cortical cultures with imipramine augment BDNF-induced association of TrkB with PLC γ 1 and thus increases the activation of PLC γ 1 (Yagasaki *et al*, 2006). Importantly, pY816 antibody used in the present study differentiates between different Trk family members, and thus, we were able to clearly demonstrate that antidepressants activate brain TrkB receptors. Interestingly, site-directed mutagenesis studies indicate that the TrkB-PLC γ 1 pathway is more important for the long-term potentiation than the Shc-mediated pathways (Minichiello *et al*, 2002). Furthermore, transgenic mice overexpressing TrkB receptors in brain show enhanced learning and increased PLC γ 1 pathway activity, but no change in the MAPK pathway (Koponen *et al*, 2004). Importantly, these mice also show an antidepressant-like behavioral response in the FST (Koponen *et al*, 2005), which further supports the association of the TrkB-PLC γ 1 pathway in antidepressant response. In conclusion, antidepressant-induced TrkB autophosphorylation appears to specifically activate the PLC γ 1 pathway in hippocampus, a pathway associated with synaptic plasticity in this brain region (Minichiello *et al*, 2002).

We did not find any evidence of increased or decreased activity of the MAPK or AKT pathways after acute or chronic FLX treatment in the hippocampus. This observation is consistent with the lack of alteration in the autophosphorylation of the TrkB Shc binding site, which mediates the activation of these signaling pathways (Saarelainen *et al*, 2003; Rantamäki *et al*, 2006). Previous studies on the role of MAPK pathway in the mechanism of antidepressant action have yielded conflicting results (Mercier *et al*, 2004; Tiraboschi *et al*, 2004; Fumagalli *et al*, 2005). The role of the pathways initiated by the TrkB Shc binding site have been extensively studied *in vitro* (Huang and Reichardt, 2001), but the replacement of the tyrosine 515 with a phenylalanine (which abolished the shc binding) in transgenic mice produced an unexpectedly mild phenotype (Minichiello *et al*, 2002), suggesting that other signaling pathways can largely compensate for the lack of MAPK pathway activation *in vivo*. Interestingly, whereas imipramine pretreatment augments BDNF-induced activation of TrkB-PLC γ 1 signaling *in vitro* no changes are observed in BDNF-induced activation of MAPK and AKT pathways (Yagasaki *et al*, 2006).

Concomitant with increased activity of TrkB and PLC γ 1, enhanced phosphorylation of CREB was also observed. This is a particularly interesting finding, as CREB plays an important role in antidepressant-induced plastic and behavioral responses. Overexpression of CREB in the hippocampus induces antidepressant-like behavioral effects in rodents (Chen *et al*, 2001a). Moreover, antidepressants induce the phosphorylation and activation of CREB in the rodent and human brain (Frechilla *et al*, 1998; Thome *et al*, 2000; Chen *et al*, 2001b; Conti *et al*, 2002; Saarelainen *et al*,

2003; Itoh *et al*, 2004; Tiraboschi *et al*, 2004; Laifenfeld *et al*, 2005). Moreover, Conti *et al* (2002) demonstrated that CREB is required for antidepressant-induced increases in BDNF mRNA levels. Although a variety of intracellular signaling events regulate CREB phosphorylation (Carlezon *et al*, 2005), there is a tight link between TrkB activity and CREB phosphorylation (Minichiello *et al*, 2002; Saarelainen *et al*, 2003; Rantamäki *et al*, 2006). Our data are consistent with a model whereby antidepressant-induced TrkB autophosphorylation activates CREB through the PLC γ 1-mediated pathway in rodent hippocampus. A report showing that PLC γ 1-Ca²⁺ pathway is the major signaling event mediating TrkB-induced CREB phosphorylation is consistent with this model (Minichiello *et al*, 2002).

The Behavioral Effects of Serotonergic Antidepressants are Mediated through TrkB-Dependent Neuronal Networks

BDNF-mediated TrkB signaling appears to be critically involved in the behavioral effects of antidepressants in the rodent models of behavioral despair (Hoshaw *et al*, 2005; Monteggia *et al*, 2004; Koponen *et al*, 2005; Saarelainen *et al*, 2003; Shirayama *et al*, 2002; Siuciak *et al*, 1997). However, majority of experiments have not observed depression-like behaviors in mice deficient of BDNF protein or TrkB signaling in the FST (MacQueen *et al*, 2001; Saarelainen *et al*, 2003; Figure 6). These findings may be however related to gender differences, as a recent study by Monteggia *et al* (2007) revealed a depressive-like behavior in female but not in male mice in the FST and in the sucrose preference test. Given that a variety of antidepressants increase brain TrkB signaling (Figure 2) and that TrkB signaling seem to have a key role in mediating the behavioral effects of antidepressants in the FST (Saarelainen *et al*, 2003; Monteggia *et al*, 2004), we examined the role of TrkB signaling in the behavioral effects of highly selective SERT and NET blocking antidepressants in this test. In agreement with our previous findings with FLX (Saarelainen *et al*, 2003), the behavioral effects of a specific serotonergic antidepressant citalopram were prevented in the dominant-negative TrkB.T1 overexpressing mice (which have stably reduced TrkB signaling) (Saarelainen *et al*, 2003). In striking contrast, acute treatment with a specific noradrenergic antidepressant reboxetine produced similar behavioral responses in both transgenic and WT mice, even though reboxetine acutely increased TrkB autophosphorylation. This is an interesting finding, as previous studies by Monteggia *et al* (2004, 2007) have shown that the behavioral effects of tricyclic antidepressant desipramine (shows more affinity against the NET compared with SERT) were absent in forebrain-specific conditional BDNF knockout mice. It is currently unclear whether the apparent discrepancy between the effects of reboxetine and desipramine in the FST are related to the higher selectivity of reboxetine towards NET, or whether this effect reflects differences in the transgenic mouse models used in the experimental procedures. Nevertheless, these results confirm the critical role of TrkB signaling in the mechanism of at least serotonergic antidepressants. In future studies, it would be informative to extend studies concerning the interactions between antidepressants and TrkB signaling to behavioral para-

digms, which more reliably measure therapeutic actions of antidepressant drugs.

The Role of Serotonin and NE in Antidepressant-Induced TrkB Activation

The antidepressants tested in the present study produce a significant and rapid enhancement of extracellular 5-HT and/or NE levels in rodent brain, albeit by different mechanisms, and these responses are considered central for the mechanism of antidepressant drugs (Pozzi *et al*, 1999; Bymaster *et al*, 2002; Page and Lucki, 2002). For example, antidepressant-induced acute behavioral effects in the tail suspension test and FST are diminished in monoamine-depleted mice (Page *et al*, 1999; Cryan *et al*, 2004). The present study investigated the role of brain monoamines in antidepressant-induced TrkB activation. We found that pretreatment with monoamine depleting agents pCPA and DSP4 abolished the ability of reboxetine and citalopram to induce rapid TrkB activation in the mouse anterior cingulate cortex. These results suggest that increased extracellular monoamine levels induced by antidepressants mediate the effects of these drugs on brain TrkB activation.

In conclusion, the results of the present study suggest that activation of TrkB signaling may be a common effect of all antidepressant drugs. Antidepressant-induced rapid changes in extracellular monoamine levels seem to be critical mediators of antidepressant-induced TrkB activity. Through the rapid activation of TrkB-PLC γ 1-CREB signaling, antidepressants could initiate plastic responses in critical neural circuits and these changes may be important for clinical antidepressant response.

ACKNOWLEDGEMENTS

We thank L Kaskela, O Nikkilä, M-E Hokkanen, J Knuuttila, and M Sairanen for excellent technical assistance; S Pastell and V Nousiainen for the animal care and OF O'Leary for critical reading of this paper and for her help in planning the pCPA administration. Fluoxetine for long-term treatments was kindly provided by Dr J Sirviö (OrionPharma, Turku, Finland). Moclobemide was obtained from Roche. This study was supported by grants from the Sigrid Juselius Foundation, the Academy of Finland, GlaxoSmithKline, the Research and Science Foundation of Farnos and the Finnish Cultural Foundation.

REFERENCES

- Airavaara M, Mijatovic J, Vihavainen T, Piepponen TP, Saarma M, Ahtee L (2006). In heterozygous GDNF knockout mice the response of striatal dopaminergic system to acute morphine is altered. *Synapse* 59: 321–329.
- Altamura AC, Moro AR, Percudani M (1994). Clinical pharmacokinetics of fluoxetine. *Clin Pharmacokinet* 26: 201–214.
- Altar CA (1999). Neurotrophins and depression. *Trends Pharmacol Sci* 20: 59–61.
- Altar CA, Whitehead RE, Chen R, Wortwein G, Madsen TM (2003). Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. *Biol Psychiatry* 54: 703–709.

- Balkowiec A, Katz DM (2002). Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons. *J Neurosci* 22: 10399–10407.
- Berghuis P, Dobszay MB, Wang X, Spano S, Ledda F, Sousa KM et al (2005). Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. *Proc Natl Acad Sci USA* 102: 19115–19120.
- Bymaster FP, Zhang W, Carter PA, Shaw J, Chernet E, Phebus L et al (2002). Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology (Berlin)* 160: 353–361.
- Carlezon WA, Duman RS, Nestler EJ (2005). The many faces of CREB. *Trends Neurosci* 28: 436–445.
- Carter BD, Zirngiebel U, Barde YA (1995). Differential regulation of p21ras activation in neurons by nerve growth factor and brain-derived neurotrophic factor. *J Biol Chem* 270: 21751–21757.
- Castrén E (2005). Is mood chemistry? *Nat Rev Neurosci* 6: 241–246.
- Castrén E, Vöikar V, Rantamäki T (2007). Role of neurotrophic factors in depression. *Curr Opin Pharmacol* 7: 18–21.
- Chen AC, Shirayama Y, Shin KH, Neve RL, Duman RS (2001a). Expression of the cAMP response element binding protein (CREB) in hippocampus produces an antidepressant effect. *Biol Psychiatry* 49: 753–762.
- Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001b). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry* 50: 260–265.
- Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA (2002). cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. *J Neurosci* 22: 3262–3268.
- Coyle JT, Duman RS (2003). Finding the intracellular signaling pathways affected by mood disorder treatments. *Neuron* 38: 157–160.
- Cryan JF, O'Leary OF, Jin SH, Friedland JC, Ouyang M, Hirsch BR et al (2004). Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors. *Proc Natl Acad Sci USA* 101: 8186–8191.
- Drago F, Nicolosi A, Micale V, Lo Menzo G (2001). Placebo affects the performance of rats in models of depression: is it a good control for behavioral experiments? *Eur Neuropsychopharmacol* 11: 209–213.
- Du J, Feng L, Zaitsev E, Je HS, Liu XW, Lu B (2003). Regulation of TrkB receptor tyrosine kinase and its internalization by neuronal activity and Ca²⁺ influx. *J Cell Biol* 163: 385–395.
- Duman RS, Heninger GR, Nestler EJ (1997). A molecular and cellular theory of depression. *Arch Gen Psychiatry* 54: 597–606.
- Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A et al (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112: 257–269.
- Frechilla D, Otano A, Del Rio J (1998). Effect of chronic antidepressant treatment on transcription factor binding activity in rat hippocampus and frontal cortex. *Prog Neuropsychopharmacol Biol Psychiatry* 22: 787–802.
- Fumagalli F, Molteni R, Calabrese F, Frasca A, Racagni G, Riva MA (2005). Chronic fluoxetine administration inhibits extracellular signal-regulated kinase 1/2 phosphorylation in rat brain. *J Neurochem* 93: 1551–1560.
- Haapasalo A, Sipilä I, Larsson K, Akerman KE, Stoilov P, Stamm S et al (2002). Regulation of TRKB surface expression by brain-derived neurotrophic factor and truncated TRKB isoforms. *J Biol Chem* 277: 43160–43167.
- Holoubek G, Noldner M, Treiber K, Müller WE (2004). Effect of chronic antidepressant treatment on beta-receptor coupled signal transduction cascade. Which effect matters most? *Pharmacopsychiatry* 37(Suppl 2): S113–S119.
- Hoshaw BA, Malberg JE, Lucki I (2005). Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. *Brain Res* 1037: 204–208.
- Huang EJ, Reichardt LF (2001). Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* 24: 677–736.
- Itoh T, Tokumura M, Abe K (2004). Effects of rolipram, a phosphodiesterase 4 inhibitor, in combination with imipramine on depressive behavior, CRE-binding activity and BDNF level in learned helplessness rats. *Eur J Pharmacol* 498: 135–142.
- Ji Y, Pang PT, Feng L, Lu B (2005). Cyclic AMP controls BDNF-induced TrkB phosphorylation and dendritic spine formation in mature hippocampal neurons. *Nat Neurosci* 8: 164–172.
- Kankaanpää A, Meririnne E, Ariniemi K, Seppälä T (2001). Oxalic acid stabilizes dopamine, serotonin, and their metabolites in automated liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Sci Appl* 753: 413–419.
- Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G (2005). Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biol Psychiatry* 57: 1068–1072.
- Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res* 109: 143–148.
- Koponen E, Rantamäki T, Vöikar V, Saarelainen T, Macdonald E, Castrén E (2005). Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. *Cell Mol Neurobiol* 25: 973–980.
- Koponen E, Vöikar V, Riekkari R, Saarelainen T, Rauramaa T, Rauvala H et al (2004). Transgenic mice overexpressing the full-length neurotrophin receptor trkB exhibit increased activation of the trkB-PLCgamma pathway, reduced anxiety, and facilitated learning. *Mol Cell Neurosci* 26: 166–181.
- Laifenfeld D, Karry R, Grauer E, Klein E, Ben-Shachar D (2005). Antidepressants and prolonged stress in rats modulate CAM-L1, laminin, and pCREB, implicated in neuronal plasticity. *Neurobiol Dis* 20: 432–441.
- Lang UE, Hellweg R, Kalus P, Bajbouj M, Lenzen KP, Sander T et al (2005). Association of a functional BDNF polymorphism and anxiety-related personality traits. *Psychopharmacology (Berlin)* 180: 95–99.
- Lee FS, Rajagopal R, Chao MV (2002a). Distinctive features of trk neurotrophin receptor transactivation by G protein-coupled receptors. *Cytokine Growth Factor Rev* 13: 11–17.
- Lee FS, Rajagopal R, Kim AH, Chang PC, Chao MV (2002b). Activation of trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. *J Biol Chem* 277: 9096–9102.
- Lewis MA, Hunihan L, Franco D, Robertson B, Palmer J, Laurent DR et al (2006). Identification and characterization of compounds that potentiate NT-3-mediated trk receptor activity. *Mol Pharmacol* 69: 1396–1404.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265–275.
- Lu B (2003). BDNF and activity-dependent synaptic modulation. *Learn Mem* 10: 86–98.
- Lucas G, Hendolin P, Harkany T, Agerman K, Paratcha G, Holmgren C et al (2003). Neurotrophin-4 mediated TrkB activation reinforces morphine-induced analgesia. *Nat Neurosci* 6: 221–222.
- MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT et al (2001). Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. *Behav Neurosci* 115: 1145–1153.

- Manji HK, Drevets WC, Charney DS (2001). The cellular neurobiology of depression. *Nat Med* 7: 541–547.
- Mercier G, Lennon AM, Renouf B, Dessouroux A, Ramaugé M, Courtin F et al (2004). MAP kinase activation by fluoxetine and its relation to gene expression in cultured rat astrocytes. *J Mol Neurosci* 24: 207–216.
- Meyer-Franke A, Wilkinson GA, Kruttgen A, Hu M, Munro E, Hanson MG et al (1998). Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. *Neuron* 21: 681–693.
- Minichiello L, Calella AM, Medina DL, Bonhoeffer T, Klein R, Korte M (2002). Mechanism of TrkB-mediated hippocampal long-term potentiation. *Neuron* 36: 121–137.
- Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T et al (2004). Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc Natl Acad Sci USA* 101: 10827–10832.
- Monteggia LM, Luikart B, Barrot M, Theobald D, Malkovska I, Nef S et al (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol Psychiatry* 61: 187–197.
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002). Neurobiology of depression. *Neuron* 34: 13–25.
- Nibuya M, Morinobu S, Duman RS (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 15: 7539–7547.
- Nibuya M, Nestler EJ, Duman RS (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* 16: 2365–2372.
- O'leary OF, Bechtholt AJ, Crowley JJ, Valentino RJ, Lucki I (2007). The role of noradrenergic tone in the dorsal raphe nucleus of the mouse in the acute behavioral effects of antidepressant drugs. *Eur Neuropsychopharmacol* 17: 215–226.
- Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I (1999). Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology (Berlin)* 147: 162–167.
- Page ME, Lucki I (2002). Effects of acute and chronic reboxetine treatment on stress-induced monoamine efflux in the rat frontal cortex. *Neuropsychopharmacology* 27: 237–247.
- Porsolt RD, Le Pichon M, Jalfre M (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266: 730–732.
- Pozzi L, Invernizzi R, Garavaglia C, Samanin R (1999). Fluoxetine increases extracellular dopamine in the prefrontal cortex by a mechanism not dependent on serotonin: a comparison with citalopram. *J Neurochem* 73: 1051–1057.
- Rantamäki T, Knuuttila JE, Hokkanen ME, Castrén E (2006). The effects of acute and long-term lithium treatments on trkB neurotrophin receptor activation in the mouse hippocampus and anterior cingulate cortex. *Neuropharmacology* 50: 421–427.
- Russo-Neustadt A, Beard RC, Cotman CW (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology* 21: 679–682.
- Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E et al (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J Neurosci* 23: 349–357.
- Saarelainen T, Lukkarinen JA, Koponen S, Gröhn OH, Jolkonen J, Koponen E et al (2000). Transgenic mice overexpressing truncated trkB neurotrophin receptors in neurons show increased susceptibility to cortical injury after focal cerebral ischemia. *Mol Cell Neurosci* 16: 87–96.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S et al (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301: 805–809.
- Segal RA, Bhattacharyya A, Rua LA, Alberta JA, Stephens RM, Kaplan DR et al (1996). Differential utilization of trk autophosphorylation sites. *J Biol Chem* 271: 20175–20181.
- Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* 22: 3251–3261.
- Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol Biochem Behav* 56: 131–137.
- Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G et al (2002). Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. brain-derived neurotrophic factor. *Mol Psychiatry* 7: 579–593.
- Smith MA, Makino S, Kvetnansky R, Post RM (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 15: 1768–1777.
- Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S et al (2000). cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J Neurosci* 20: 4030–4036.
- Tiraboschi E, Tardito D, Kasahara J, Moraschi S, Pruneri P, Gennarelli M et al (2004). Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. *Neuropsychopharmacology* 29: 1831–1840.
- Weinstock M, Poltyrev T, Bejar C, Youdim MB (2002). Effect of TV3326, a novel monoamine-oxidase cholinesterase inhibitor, in rat models of anxiety and depression. *Psychopharmacology* 160: 318–324.
- Yagasaki Y, Numakawa T, Kumamaru E, Hayashi T, Su T-P, Kunugi H (2006). Chronic antidepressants potentiate via sigma-1 receptors the brain-derived neurotrophic factor-induced signaling for glutamate release. *J Biol Chem* 281: 12941–12949.
- Wong EH, Sonders MS, Amara SG, Tinholt PM, Piercey MF, Hoffmann WP et al (2000). Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biol Psychiatry* 47: 818–829.
- Wyneken U, Sandoval M, Sandoval S, Jorquera F, Gonzalez I, Vargas F et al (2006). Clinically relevant doses of fluoxetine and reboxetine induce changes in the TrkB content of central excitatory synapses. *Neuropsychopharmacology* 31: 2415–2423.
- Xu H, Luo C, Richardson JS, Li XM (2004). Recovery of hippocampal cell proliferation and BDNF levels, both of which are reduced by repeated restraint stress, is accelerated by chronic venlafaxine. *Pharmacogenom J* 4: 322–331.
- Xu H, Richardson JS, Li XM (2003). Dose-related effects of chronic antidepressants on neuroprotective proteins BDNF, bcl-2 and Cu/Zn-SOD in rat hippocampus. *Neuropsychopharmacology* 28: 53–62.
- Zetterström TS, Pei Q, Madhav TR, Coppell AL, Lewis L, Grahame-Smith DG (1999). Manipulations of brain 5-HT levels affect gene expression for BDNF in rat brain. *Neuropharmacology* 38: 1063–1073.