Synergistic neurochemical and behavioural effects of acute intrahippocampal injection of brain-derived neurotrophic factor and antidepressants in adult mice

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Abstract

Preclinical data support the view that brain-derived neurotrophic factor (BDNF) and serotonergic systems regulate circuits involved in affective disorders. The present study examined neurochemical and behavioural consequences of an acute intrahippocampal injection of BDNF combined with an antidepressant by using in-vivo intracerebral microdialysis in the ventral hippocampus (vHi) in conscious mice and behavioural paradigms predictive of antidepressant and anxiolytic-like effects [the mouse forced swim test (FST), the open-field (OF) paradigm and the elevated plus maze (EPM)]. Neurochemical data revealed that BDNF (100 ng) potentiated the effects of the systemic administration of a serotonin selective reuptake inhibitor (SSRI; paroxetine 4 mg/kg i.p.) and that of a locally applied citalopram perfusion on dialysate 5-HT levels in the vHi. These neurochemical changes correlated with behavioural data since, in the FST, antidepressant-like activity of paroxetine as measured on swimming behaviour was potentiated by BDNF. These data suggest an interesting synergy between BDNF and SSRI on antidepressant-like activity. Furthermore, in both the OF and EPM paradigms BDNF induced an anxiogenic-like activity, whereas paroxetine prevented this effect. Finally, the neurochemical and behavioural effects of BDNF on the serotonergic system might occur at both pre- and post-synaptic levels since by using in-situ hybridization, we showed that TrkB-R mRNA was expressed in the hippocampus and the dorsal raphe nucleus in adult mice. Taken together the neurochemical and behavioural effects of BDNF suggest that these behavioural changes were mediated by increases in 5-HT neurotransmission in vHi. Thus a BDNF + SSRI combination may offer new alternatives to treat mood disorders.

Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays a key role in the regulation of both depression and anxiety disorders and their treatment (Martinovich & Lu, 2008). In humans, various clinical studies reported reduced brain BDNF levels in unmedicated depressed patients (Chen et al. 2001; Dwivedi et al. 2003). In rodents, acute and chronic stress decreased BDNF expression in the hippocampus and other brain regions (Duman et al. 1997). This reduction in BDNF expression was counterbalanced by chronic antidepressant treatments (Duman et al. 1997). Indeed, Nibuya and colleagues reported that chronic selective serotonin reuptake inhibitors (SSRIs), increased BDNF protein levels and expression (mRNA) in rat adult hippocampus by enhancing 5-HT neurotransmission (Calabrese et al. 2007; Nibuya et al. 1995). BDNF requires activation of the high-affinity protein kinase receptor family, TrkB (tropomyosine-related kinase B) to exert its biological effects. It was shown that both acute and chronic SSRI
treatment increased TrkB receptor (TrkB-R) phosphorylation in mouse hippocampus (Rantamaki et al. 2007; Saarelainen et al. 2003). Antidepressants activate TrkB-R quite rapidly indicating a fast increase in BDNF release following antidepressant treatment. These studies suggest that BDNF up-regulation could be an essential component of antidepressant activity. Interestingly, results obtained after acute intrahippocampal BDNF injection in rats (Hoshaw et al. 2005, Shirayama et al. 2002) or transgenic mice overexpressing BDNF in forebrain (Govindarajan et al. 2006) displayed a paradoxical antidepressant/anxiogenic-like activity. In this latter work, anxiety was measured in the open-field (OF) paradigm and the elevated plus maze (EPM), and a 2-d paradigm of the Porsolt forced swim test (FST) was used to evaluate antidepressant efficacy. A reciprocal interaction between BDNF and serotonin (5-HT) in the central nervous system was suggested by Altar (1999). In line with this hypothesis, we recently showed that the constitutive decrease in brain BDNF levels in BDNF+/− mice abolished paroxetine-induced increase in [5-HT]_{ext} in the ventral hippocampus (vHi) but not the frontal cortex, suggesting the region-specific alteration of serotonin transporter (SERT) function (Guiard et al. 2008).

In the present study, we investigated the neurochemical and behavioural effects of acute co-administration of BDNF+ antidepressant since this combination has been poorly studied. By using intracerebral in-vivo microdialysis, we first assessed the neurochemical effects of BDNF combined with a SSRI (paroxetine or citalopram) on extracellular (5-HT) levels ([5-HT]_{ext}). Moreover, since many studies support the view that increased BDNF levels improve performance on the Porsolt FST, we investigated the behavioural consequences of the co-administration of paroxetine/BDNF in this model. The hippocampus is endowed with a dense serotonergic and noradrenergic innervation (Gobbi & Blier, 2005) that may participate in the regulation of swimming and climbing behaviours, respectively (Dulawa et al. 2004; Holick et al. 2008). For this reason, we scored these parameters in the mouse FST. Furthermore, as it was suggested that the hippocampus might be involved in the origin of anxiety-like behaviours (Bannerman et al. 2004; Leonardo & Hen, 2006), we used the OF and EPM paradigms to assess the anxiety index of the BDNF+ antidepressant combination. The OF test is a classical approach/avoidance paradigm in which the novel environment concurrently evokes both anxiety and exploration (Dulawa et al. 2004). The EPM is an anxiety-related paradigm based on exploration of a maze that evokes either anxiety (open arms) or safety (closed arms) (Crawley, 1999). Several hypotheses can be considered regarding the mechanisms by which BDNF induces marked modifications in 5-HT homeostasis. Since the hippocampus and dorsal raphe nuclei (DRN) are considered to be critical sites for antidepressant therapy (El Mansari et al. 2005), in-situ hybridization (ISH) experiments were carried out to localize TrkB-R mRNA in these brain regions in order to assess the physiological relevance of neurochemical and behavioural experiments.

Materials and methods

Animals

Young adult male Swiss mice (Janvier, France) aged 7–8 wk, weighing 23–25 g, were used across the studies. All animals were housed in groups of five mice per cage under standard conditions. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive no. 87-848, October 19, 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 92-196 to A.M.G.).

Drug treatment

Intrahippocampal BDNF injection in awake, freely moving mice (Abcys, France) was dissolved in 0.1% BSA and administered bilaterally into the vHi (0.2 μl/min for 2 min using a Picoplus microinjector; Harvard Apparatus, France), at a dose of 100 ng, according to Benmansour et al. (2008). The stereotaxic coordinates from Bregma (mm) were: A −3.4, L ±3.4, V −4.0. We applied this procedure for both neurochemical and behavioural experiments.

For microdialysis experiment, BDNF (100 ng) was locally perfused 60 min after paroxetine administration (Fig. 1a) or during citalopram perfusion (Fig. 1b) via a silica catheter glued to the microdialysis probe (Guiard et al. 2008). BDNF injection was performed 60 min after paroxetine administration, i.e. when the blockade of SERT was allowed to reach the plateau of [5-HT]_{ext}. BDNF injection was carried out in awake, freely moving animals, 20 h after the end of the surgery. First, paroxetine was administered 30 min prior to the test as previously described (Guilloux et al. 2006), then BDNF injection was carried out 15 min after paroxetine administration, since the peak of [5-HT]_{ext} for the antidepressant/BDNF combination was ob-
served 15 min after BDNF injection in the microdialysis experiment. For all behavioural studies, the mice were placed in the test 15 min after the BDNF or NGF intrahippocampal injection.

**Antidepressant drug administration**

The SSRIs, paroxetine hydrochloride and citalopram hydrobromide were generous gifts from GlaxoSmithKline laboratory (Harlow, UK) and Lundbeck laboratory (Copenhagen, Denmark), respectively. Paroxetine was dissolved in distilled water and administered intraperitoneally (i.p.) at a dose of 4 mg/kg (Guilloux et al. 2006). Citalopram was dissolved (final concentration 1 \( \mu \)M) in artificial cerebrospinal fluid (aCSF; Romero & Artigas, 1997) [composition (mM): 147 NaCl, 3.5 KCl, 1.26 CaCl\(_2\), 1.2 mgCl\(_2\), 1.0 NaH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\) (pH 7.4 ± 0.2)] locally perfused by ‘reverse microdialysis’ at a flow rate of 1.5 \( \mu \)l/min.

**Intracerebral microdialysis procedure**

Concentric dialysis probes (0.30 mm outer diameter) were constructed of cuprophane as previously described (Guiard et al. 2008). All microdialysis probes had an active length of 2 mm and were implanted in the vHi of anaesthetized mice (chloral hydrate, 400 mg/kg i.p.) according to the mouse brain atlas of Paxinos & Franklin (2001). The stereotaxic coordinates from Bregma (mm) were: A = −3.4, L = ±3.4, V = −4.0.
Animals were allowed to recover from the surgery overnight. On the next day, ~20 h after surgery, the probes were continuously perfused with aCSF at a flow rate of 1.5 μl/min using a CMA/100 pump (Carnegie Medicin, Sweden). Dialysate samples were collected every 15 min in tubes and were analysed for 5-HT by high-performance liquid chromatography apparatus (XL-ODS, 4.6 x 7.0 mm, particle size 3 μm; Beckman) coupled to an amperometric detector (1049A, Hewlett-Packard, France; Guiard et al. 2008) [limit of sensitivity for 5-HT ~0.5 fmol/sample (signal-to-noise ratio = 2)]. The placement of microdialysis probes was verified as previously described (Bert et al. 2004).

**Behavioural procedure**

**Mouse FST**

We used the FST procedure previously described (Dulawa et al. 2004; Holick et al. 2008). Mice were placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 18 cm water, maintained at 23–25 °C and videotaped for 6 min with a camera. The predominant behaviour (swimming, immobility, or climbing) was scored by an experimenter blind to treatment during the last 4 min of the 6-min testing period.

**OF paradigm**

Motor activity was quantified in two Plexiglas OF boxes 43 × 43 cm² (Med Associates, USA) during a 10-min session. Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls 2.5 cm apart to record x–y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100 ms resolution. The computer defined gridlines that divided each OF into centre and margin regions, with each of four lines being 11 cm from each wall. Dependent measures were the following parameters in the centre: total time, numbers of entries, and distance divided by total distance travelled.

**EPM paradigm**

The EPM was constructed of wood painted black and consisted of four arms, each 7.6 cm wide and 28 cm long, elevated 31 cm above the floor. Two of the arms were enclosed by 15-cm-high walls, whereas two arms were open. The behaviour of mice was scored for 5 min. Number of entries and time spent in open and closed arms were scored by an experimenter blind to treatment.

**General histology**

Mice were perfused with 4% paraformaldehyde (PFA). Brain was immersed in the same fixative overnight, cryoprotected by 20% sucrose/ PBS, frozen, and sectioned (20-μm thick).

**ISH**

ISH was performed as previously described (Ma et al. 2007). In brief, cryosections were incubated with digoxin (DIG)-labelled cRNA followed by incubation of alkaline phosphatase-conjugated anti-DIG antibody. The colour development was performed by incubation with NBT/BCIP followed by Nuclear Fast Red stain. Mouse 5-HT₁A cDNA (nt 2114–4089 corresponding to NM_008308) and TrkB-R isoforms, a specific cDNA (nt 2256–4320 corresponding to NM_001025074) were cloned into pBluescript and pCI vector, respectively. EST clone (BC052014) was obtained from Open Biosystems (USA): this clone contains mouse TrkB cDNA (nt 277–2424 corresponding to NM_008745) and shares a common region of both isoforms. These plasmids were used for generating cRNA probes.

**Double fluorescent ISH**

Cryosections were incubated with DIG-labelled 5-HT₁A receptor (5-HT₁AR) cRNA probe and FITC-labelled TrkB-specific cRNA. After stringent wash, sections were incubated with horseradish peroxidase (HRP)-conjugated anti-DIG antibody (1:1000) and labelled with Cy3 by using tyramide signal amplification (TSA) system (NEL744, PerkinElmer, USA). Followed by quenching with 1% H₂O₂, sections were incubated with HRP-conjugated anti-FITC antibody (1:1500) and labelled with FITC by using TSA system (NEL741). 5-HT₁AR mRNA was labelled with Cy3 and TrkB-R isoform was labelled with FITC.

**Data analysis and statistics**

**Microdialysis data analysis**

The microdialysis data were expressed as mean (± S.E.M.) values from baseline, which is the average of the four basal values before vehicle or drug injection. We also calculated area under the curve (AUC; mean ± S.E.M.) values, which are expressed as percentage of basal [5-HT]ₑₓ. These AUC values were calculated as the amount of 5-HT outflow collected either during the 60–120 min period (Fig. 1a) post-injection of BDNF or appropriate vehicle. Within each group of mice, comparisons between basal and post-treatment
5-HT levels at individual time-points were analysed using ANOVA for repeated measures.

**Behavioural analysis**

Two-way ANOVA with pre-treatment (vehicle, paroxetine) and drug treatment (BDNF, vehicle) factors were applied to three different measures in the mouse FST: immobility, climbing and swimming duration and also for the following parameters in the centre in the OF paradigm: session time, number of entries, and distance travelled.

All statistical analyses were conducted using the computer software Statview 5.0 for IBM compatible (Abacus Concepts Inc., USA). Significant main effects were resolved using Fisher’s protected least significant difference (PLSD) post-hoc test. Statistical significance was set at p ≤ 0.05. In the Results section, p values are in comparison to the control group, unless otherwise stated.

**Results**

**BDNF-potentiated SSRI-induced increase in dialysate 5-HT levels in the vHi**

Systemic administration of a SSRI: BDNF-potentiated paroxetine-induced increase in dialysate 5-HT levels in the vHi

Paroxetine was administered 1 h prior to intrahippocampal BDNF infusion in order to reach the plateau of [5-HT]ext levels observed following the blockade of SERT as previously described by Guilloux et al. (2006), with co-administration of paroxetine + pindolol.

ANOVA for repeated measures revealed that a systemic administration of paroxetine significantly increased [5-HT]ext during the t60–t90 period [p < 0.001 compared to the respective basal values; F(8,104) = 13.6, p < 0.001]. At t90, i.e. 15 min after intrahippocampal BDNF injection, [5-HT]ext in the paroxetine/BDNF-treated group, was significantly higher than those measured in the paroxetine/vehicle group (p < 0.05, Fig. 1a). A statistical analysis of AUC values over the t60–t120 period confirmed that intrahippocampal perfusion of BDNF (100 ng) significantly potentiated paroxetine-induced increase in [5-HT]ext (p < 0.05, Fig. 1a, inset).

In order to confirm these latter effects obtained after a systemic administration of a SSRI we induced a local, brain region-specific blockade of SERT. The effects of an intrahippocampal BDNF perfusion were studied when SERT was blocked in the hippocampus by the local infusion of the most selective SSRI citalopram (1 µM) using reverse dialysis. This citalopram dose was chosen according to Romero & Artigas (1997).

**Intrahippocampal injection of a SSRI: BDNF potentiated the effect of a local perfusion of citalopram (1 µM)**

The presence of citalopram (1 µM) in aCSF induced a significant increase in [5-HT]ext from t0 to t60 in both citalopram/vehicle and citalopram/BDNF groups (p < 0.001). ANOVA for repeated measures revealed that BDNF injection potentiated citalopram-induced increase in [5-HT]ext (p < 0.05 compared to the citalopram/vehicle-treated group; Fig. 1b). This effect was revealed 15 min after intrahippocampal BDNF injection at t15 (p < 0.05 compared to the citalopram/vehicle-treated group).

Taken together, intrahippocampal microdialysis data obtained with paroxetine and citalopram point to the regional specificity of the effects of BDNF in the vHi. Since an increase in [5-HT]ext following blockade of SERT induced an antidepressant-like effect in the mouse FST (Page et al. 1999), we assessed whether the potentiation of SSRI-induced increase in [5-HT]ext with BDNF could modulate immobility duration in this behavioural model.

**Antidepressant-like activity of BDNF on paroxetine-induced antidepressant-like activity in the FST**

As previously reported with other SSRIs (Holick et al. 2008), paroxetine as well as BDNF reduced the total immobility time (p < 0.01 and p < 0.05, respectively). Co-administration of BDNF with paroxetine elicited similar effects (p > 0.05 compared to the effects of paroxetine alone). A two-way ANOVA on immobility duration during the last 4 min of the 6-min testing period revealed a significant effect of pre-treatment factor [F(1,30) = 43.08, p < 0.001; Fig. 2a].

We then assessed whether the antidepressant-like activity of BDNF alone or in combination with paroxetine resulted in an activation of serotonergic and/or noradrenergic systems. In a marked contrast to paroxetine (p < 0.001), BDNF alone had no effects on climbing behaviour (p > 0.05). Surprisingly, when combined with paroxetine, intrahippocampal BDNF injection blocked paroxetine-induced increase in climbing behaviour (p < 0.01). Since BDNF increased swimming behaviour, there must be a concomitant decrease in climbing behaviour. A two-way ANOVA on climbing behaviour during the last 4 min of the 6-min testing period revealed a significant effect of pre-treatment [F(1,30) = 15.95, p < 0.001] and treatment factors [F(1,30) = 5.32, p < 0.05; Fig. 2b].
Both BDNF and paroxetine induced an increase in swimming behaviour ($p<0.05$). Consequently the antidepressant-like activity observed in the BDNF group (Fig. 2a) is probably due to an increase in swimming activity. Interestingly, intrahippocampal injection of BDNF potentiated the effect of paroxetine on swimming behaviour ($p<0.05$ between the paroxetine/vehicle group versus paroxetine/BDNF-treated group, or $p<0.01$ between the paroxetine/BDNF-treated group versus BDNF/vehicle group). A two-way ANOVA on swimming behaviour during the last 4 min of the 6-min testing period revealed a significant effect of pre-treatment [$F(1,30) = 16.3, p<0.001$] and treatment factor [$F(1,30) = 9.65, p<0.01$; Fig. 2c).

**BDN-elicited anxiogenic-like behaviour in the OF and EPM paradigms**

Mice treated acutely with paroxetine (4 mg/kg) or vehicle followed by a BDNF (100 ng) or vehicle perfusion, were tested in the OF paradigm to assess anxiety and locomotion measures. BDNF induced anxiogenic-like profile since a significant decrease was observed in centre time ($p<0.001$), number of centre entries ($p<0.01$) and centre distance travelled ($p<0.001$). The anxiogenic-like effect of intrahippocampal BDNF perfusion did not affect the locomotor activity as we observed significant increase in the distance travelled in periphery (see Supplementary data, available online) for these BDNF-treated animals. A two-way ANOVA revealed a significant effect of treatment for all three above-cited parameters: time [$F(1,45) = 6.35, p<0.05$], number of entries [$F(1,45) = 3.5, p<0.05$], and distance travelled [$F(1,45) = 6.45, p<0.05$; Fig. 3a–c].

An acute administration of paroxetine (4 mg/kg) did not modify basal anxiety levels as reported by another group (Hascoet et al. 2000) or locomotor activity, since all three parameters were unchanged in comparison to the vehicle-treated group (Fig. 3a–c). In contrast to the SSRI, the benzodiazepine diazepam (1 mg/kg i.p.) used here as a control displayed an anxiolytic-like activity as it induced an increase in centre time ($p<0.05$) and number of centre entries ($p<0.01$) without affecting the locomotor activity (Fig. 3b, c).

The anxiogenic-like effects of intrahippocampal BDNF injection were prevented by a pre-treatment of paroxetine since this SSRI in combination with BDNF did not affect either the time spent in the centre ($p>0.05$), or the number of entries in the centre ($p>0.05$).

To confirm the anxiogenic-like effects of intrahippocampal BDNF injection, the same protocol used in FST and OF paradigm was applied to the EPM. BDNF also induced an anxiogenic-like profile in this paradigm since a significant decrease was observed in the time spent in open arms ($p<0.001$, Fig. 3d). Paroxetine blocked BDNF-induced anxiogenic-like effect ($p<0.05$), while paroxetine alone had no effects ($p>0.05$). Two-way ANOVA revealed a significant effect of treatment factor [$F(1,20) = 5.61, p<0.05$] and pretreatment factor [$F(1,20) = 4.87, p<0.05$]. This effect was not due to changes in locomotor activity as the number of entries in closed arms was not modified by BDNF treatment or pretreatment with paroxetine (Fig. 3e): a two-way ANOVA did not reveal significant

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**Fig. 2.** Effects of intrahippocampal perfusion of BDNF on paroxetine-induced antidepressant-like activity in the forced swim test (FST) in mice. For the FST experiment data (expressed in seconds), are mean ± S.E.M. of time spent in (a) immobility, (b) climbing and (c) swimming (8–12 mice per group). * $p<0.05$, *** $p<0.001$ compared to the vehicle-treated group; §$p<0.05$, §§$p<0.01$ compared to the BDNF/vehicle-treated group and paroxetine/vehicle-treated group (two-way ANOVA, Fisher’s PLSD post-hoc test).
effect of treatment factor \( F(1, 20) = 1.3, \ p > 0.05 \) or pretreatment factor \( F(1, 20) = 0.06, \ p > 0.05 \). The anxiogenic-like effect of BDNF is probably specific to BDNF since an intrahippocampal injection of NGF induced an anxiolytic like effect (see Supplementary Fig. S2, online).

In addition, measurements of the distance travelled in the periphery in the OF paradigm demonstrated that the effects of BDNF were unlikely to be due to a general decrease in the locomotor activity (see Supplementary Fig. S1, online).

**ISH**

To assess the biological relevance of the serotonergic effects of BDNF, we performed ISH experiments for TrkB (the high-affinity receptor of BDNF) mRNA in the hippocampus and both TrkB-R and 5-HT1A-R in the DRN. Both forms of the TrkB-R, i.e. full-length (Fig. 4a, panel 1) and truncated (Fig. 4a, panel 2) are expressed throughout the vHi in different layers of the dentate gyrus and also in the hilus (Fig. 4c).

Interestingly, TrkB-R mRNA is expressed in the DRN in which the cell bodies of the serotonergic neurons are expressed. Thus we identified that TrkB-R mRNA is co-expressed with 5-HT1AR mRNA. Consequently, we can predict that TrkB-R is expressed in serotonergic neurons (Fig. 4b).

**Discussion**

The present study aimed at investigating the therapeutic potentiality of the co-administration of an antidepressant with BDNF by using neurochemical (i.e. intracerebral *in-vivo* microdialysis) and behavioural approaches with animal models predictive of anxiolytic- and/or antidepressant-like activity. Microdialysis experiments provided evidence that hippocampal 5-HT outflow caused by a systemic injection of a SSRI can be potentiated by a single intrahippocampal injection of BDNF in adult mice. We then assessed whether BDNF-induced potentiation of 5-HT neurotransmission had behavioural consequences in the mouse FST. Indeed, BDNF potentiated the effect of paroxetine on swimming behaviour. Thus, there is a correlation between microdialysis results and those obtained in the FST suggesting that changes
in the antidepressant-like activity induced by BDNF + paroxetine were mediated by increases in 5-HT neurotransmission in the vHi. Meanwhile, BDNF alone induced an anxiogenic-like activity in the OF and EPM tests that was prevented by paroxetine. Using ISH, we also demonstrated that TrkB-R was present at both pre- and post-synaptic levels in the DRN and hippocampus, respectively. At presynaptic levels, TrkB-R mRNA was co-localized with 5-HT$_{1A}$R suggesting their expression on serotonergic neurons. Taken together, our results confirm the occurrence of tight interactions between BDNF and 5-HT systems within the brain that could lead to the development of new therapeutic approaches.

Microdialysis experiments performed in the vHi revealed that BDNF potentiated the effect of a SSRI given either systemically or locally on [5-HT]$_{\text{ext}}$. The effect observed with BDNF in the presence of a SSRI was robust as it has been observed with two different SSRIs. Indeed, in the presence of citalopram (1 $\mu$M) locally perfused by reverse microdialysis in the hippocampus, BDNF potentiated dialysate 5-HT levels. This technique is known to reduce the variability of the measures without affecting the physiological control of nerve terminal release through the activation of somatodendritic 5-HT$_{1A}$R by 5-HT (Romero & Artigas, 1997).

As [5-HT]$_{\text{ext}}$ measured with microdialysis reflects a balance between the release of 5-HT and its uptake, one can assume that, when SERT was blocked by the SSRI, BDNF-induced potentiation of [5-HT]$_{\text{ext}}$ specifically resulted from an increase in 5-HT release. This finding is consistent with a previous in-vitro study showing the capacity of BDNF to stimulate 5-HT release from rat striatal synaptosomes (Goggi et al. 2002).

Together with our in-vivo data obtained in mice, these findings suggest that BDNF may activate TrkB-R by modulating synaptic 5-HT neurotransmission in the brain.

In addition, the present data are in line with experiments carried out in BDNF-deficient mice. Indeed, in-vivo microdialysis (Guiard et al. 2008) and chronamperometry (Daws et al. 2007) techniques revealed that a 50% decrease in BDNF brain production blunted the function of SERT in the hippocampus.

Recently, we demonstrated that an acute intra-hippocampal BDNF injection induced an increase in 5-HT uptake, thus leading to a decrease in [5-HT]$_{\text{ext}}$ (Bennamsour et al. 2008). These results thus suggest that in the absence of the transporter blockade, the effect of BDNF on 5-HT uptake in the adult hippocampus prevail over its effect on 5-HT release. Conversely, when SERT is inhibited by a SSRI, the 5-HT neurotransmission is stimulated and the effect of BDNF on 5-HT release is unveiled.

The FST is one of the most useful tools for antidepressants screening. Swimming, climbing and immobility behaviours were distinguished from each other according to the procedure previously described (Dulawa et al. 2004; Holick et al. 2008). Swimming behaviour relies on the serotonergic system, and...
climbing behaviour on the noradrenergic system in rats (Page et al. 1999). This was evidenced by the observation that the inhibition of 5-HT synthesis by PCPA prevented fluoxetine effects on swimming behaviour, but not those of desipramine, a noradrenaline reuptake inhibitor, on climbing behaviour (Page et al. 1999). Surprisingly, here, the SSRI paroxetine increased both climbing and swimming behaviours suggesting its capacity to affect both 5-HT and noradrenaline neurotransmissions. These results are in agreement with initial data demonstrating that acute paroxetine at a similar dose and route of administration as in the present study increased both [5-HT]_ext and noradrenaline levels in the frontal cortex in mice (David et al. 2003). However, in the FST, an increase in climbing behaviour following SSRI administration was not observed in others in rats (Detke et al. 1995). Differences between these results could be due to a species-dependent effect of SSRIs (rats vs. mice). Taken together, our data indicate that BDNF alone induced an antidepressant-like activity, thus confirming previous results obtained in rats after a single intracerebroventricular administration of BDNF (Hoshaw et al. 2005). Interestingly, the potentiation of antidepressant-like activity of a SSRI by BDNF may offer new alternatives for antidepressant therapy.

We also observed that BDNF decreased the total immobility time associated with an increase in swimming behaviour. The latter finding is consistent with results previously obtained by other groups. For instance, a single bilateral infusion of BDNF into the dentate gyrus or CA3 pyramidal cell layers, at a comparable dose used in the present study, elicited antidepressant-like effects in the rat FST, mainly by increasing the swimming behaviour (Shirayama et al. 2002). Additionally, Govindarajan et al. (2006) found that transgenic overexpression of BDNF caused antidepressant-like effects because these mice exhibit improved performance on the FST with a reduced latency time compared to their controls. However, an intrahippocampal BDNF injection did not increase basal [5-HT]_ext in the hippocampus (Benmansour et al. 2008). Thus, behavioural responses to BDNF in the FST may involve TrkB-R expressed at pre-synaptic level, while the effects of BDNF on dialysate 5-HT levels in adult hippocampus probably reflect pre-synaptic events.

An intrahippocampal injection of BDNF potentiated the effects of a systemic administration of paroxetine on swimming behaviour. Thus, this latter result was correlated with the microdialysis data suggesting that the potentiation of paroxetine effects on hippocampal [5-HT]_ext by BDNF could account for the potentiation observed in the FST. This additive effect may also involve endogenous BDNF release in the hippocampus in response to paroxetine administration (Martinowich & Lu, 2008). Indeed it has recently been shown that an acute antidepressant administration can induce a rapid TrkB-R phosphorylation within 30 min, thereby giving evidence that antidepressants promote BDNF release (Rantamaki et al. 2007). Supporting this concept, the antidepressant-like effect of citalopram in the FST was abolished in mouse overexpressing the truncated form of TrkB-R (Rantamaki et al. 2007). From this latter observation, one could infer that normal TrkB-R signalling is required for antidepressant-like effects in the mouse FST. Thus, the potentiation of paroxetine effects by BDNF on swimming behaviour could be the consequence of higher extracellular BDNF levels resulting from paroxetine effects on both endogenous BDNF release and exogenously applied BDNF into the vHi.

BDNF alone induced an anxiogenic-like effect in the OF and EPM paradigms. This result is consistent with previous data showing that transgenic over-expression of BDNF had an unexpected facilitatory effect on anxiety-like behaviour (Govindarajan et al. 2006). It suggests that BDNF plays a distinct role in animal models of anxiety and depression. Indeed, BDNF inhibits depressive-like behaviour, while it facilitates anxiety-like behaviour in mice. In addition, the weak anxiogenic-like activity of an acute dose of paroxetine in the OF paradigm agree with the literature (Hascoet et al. 2000). However, the observation that paroxetine blocked the effects of BDNF on anxiety-like behaviour is a new finding that held our attention. Together, our data suggest that high levels of 5-HT in adult hippocampus prevented the effects of BDNF. It can be hypothesized that during chronic SSRI treatment, endogenous BDNF could be released (Martinowich & Lu, 2008), which in turn produces anxiogenic-like activity. Such an undesirable effect of BDNF could participate, at least partially, in limiting the efficacy of antidepressant drugs. The effect observed with BDNF seems to be specific to BDNF and does not generalize across growth factors. Indeed, an intrahippocampal injection of NGF, an other neurotrophic factors induced an anxiolytic-like effect in the EPM, which is the opposite to the effect observed with BDNF in this behavioural test (see Supplementary Fig. S2A, S2B, online).

Our neurochemical and behavioural data confirm previous studies showing that BDNF interacts with the serotonergic system in the adult hippocampus.
(Guiard et al. 2008). The possibility that BDNF directly interacts with this system, was assessed by using the ISH technique: its high-affinity TrkB-R is known to be present on 5-HT neurons (Papatoupin & Reichardt, 2001). The expression of TrkB-R mRNA by serotonergic neurons in the DRN was already studied in rats using the ISH technique (Madhav et al. 2001). These authors showed that intracerebroventricular injection of the 5-HT-specific neurotoxin, 5,7-dihydroxytryptamine, which lesions serotonergic cell bodies in the DRN as well as their ascending projections into the hippocampus, caused a dramatic loss of TrkB mRNA from serotonergic cell bodies of the DRN. In addition, the effect of chronic antidepressant treatment on TrkB-R expression was first studied by Wynken et al. (2006) (in rat brain cortex and hippocampus using immunoblotting), then by Saarelainen et al. (2003) and Rantamaki et al. (2007). However, the co-localization of TrkB-R mRNA with 5-HT1AR mRNA is described here in DRN in mice for the first time. Such a co-localization at presynaptic levels brings new information as it suggests that the effects of BDNF on the brain’s serotonergic system observed either at the neurochemical level (5-HT release) or at the behavioural level, were due to its direct action on 5-HT neurons, which may involve a specific raphe-hippocampus pathway.

In conclusion, our results demonstrate that BDNF and serotonergic systems do interact in the mouse hippocampus. Although the impact of antidepressant drugs on BDNF levels is well documented, the reciprocal interaction is not (i.e. the effect of BDNF on 5-HT neuronal activity). As a consequence, the combination of BDNF and SSRI might be a new area of investigation to fasten the delay of onset of antidepressant drugs. Moreover, as revealed in our microdialysis experiment, the injection of BDNF has a limited effect in time because TrkB-R is quickly down-regulated following ligand binding (Sommerfeld et al. 2000). This should also be taken into account should the BDNF–TrkB system be considered as a target for future antidepressant therapy.

Note

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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Statement of Interest

None.

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