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ORIGINAL INVESTIGATION

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Chronic administration of the delta opioid receptor agonist (+)BW373U86 and antidepressants on behavior in the forced swim test and BDNF mRNA expression in rats

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Abstract *Rationale:* Selective delta opioid receptor agonists have been shown to produce antidepressant-like behavioral effects and increase brain-derived neurotrophic factor (BDNF) mRNA expression when given acutely, but the chronic effects of delta agonists have been less well characterized. *Objective:* The present study examined the effects of chronic exposure to the delta agonist (+)BW 373U86 (BW) on antidepressant-like behavior in the forced swim test and on BDNF mRNA expression in comparison to chronic treatment with the antidepressants fluoxetine, desipramine, bupropion, and tranylcypromine. *Methods:* Sprague–Dawley rats were treated chronic ally with one of the above treatments and were tested for antidepressant effects in the forced swim test, and assayed for BDNF mRNA expression by in situ hybridization. *Results:* Acute administration of 10 mg/kg BW produced a significant antidepressant-like effect in the forced swim test, while chronic (8- or 21-day) BW administration did not produce a significant antidepressant-like effect. When 10 mg/kg BW was administered for 8 days, it produced a significant increase in BDNF mRNA expression in the frontal cortex, while having no effect on BDNF expression when given for 21 days. Chronic bupropion and desipramine significantly decreased BDNF expression in the dentate gyrus of the hippocampus, while fluoxetine had no effect in any brain region. Chronic tranylcypromine produced a significant increase in BDNF expression in the CA1 region of the hippocampus. *Conclusions:* Chronic exposure to BW produces tolerance to most effects, although at differential rates. In addition, increased BDNF mRNA expression does not appear to be a common effect of chronic administration of various antidepressants.

Keywords Delta opioid receptor · (+)BW373U86 · BDNF · Antidepressant · Forced swim test · In situ hybridization

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Introduction

Currently prescribed antidepressants increase the synaptic concentrations of one or more of the monoamine neurotransmitters. The increase in monoamines occurs shortly after the drug is given; however, humans do not report a reduction in depressive symptoms for several weeks. In addition, many antidepressants produce undesirable side effects, and are ineffective in certain patients. There is a need, therefore, to find additional targets for the treatment of depression.

The delta opioid receptor (DOR) is one potential target for the development of novel antidepressants. The DOR agonists (+)BW373U86 and SNC80 have been shown to produce antidepressant-like effects in the rat forced swim assay (Broom et al. 2002a), a test that reliably predicts the ability of a compound to produce antidepressant effects in humans. SNC80 has also been shown to have antidepressant-like and anxiolytic-like properties in rats and mice (Saitoh et al. 2004). Likewise, DOR knockout mice show depressive-like and anxiety-like behaviors in several behavioral tests, indicating that the DOR system is important for normal emotional behavior in mice (Filliol et al. 2000).

In addition, the DOR agonist (+)BW373U86 has been shown to dose-dependently increase brain-derived neurotrophic factor (BDNF) mRNA expression in the frontal cortex, hippocampus, and basolateral amygdala after acute administration (Torregrossa et al. 2004). The ability of DOR agonists to increase BDNF mRNA expression may be important for their antidepressant potential because many commonly prescribed antidepressants and electroconvulsive shock (ECS) therapy have been reported to increase BDNF mRNA expression in one or more of these regions (Nibuya et al. 1995; Russo-Neustadt et al. 1999; DeFoubert et al. 2004). Increases in neurotrophic factor expression and increased activity at neurotrophin receptors has been hypothesized to be important for the clinical effectiveness of antidepressant drugs (Duman 2004).

A large amount of research has been done on the connection between BDNF and depression based on the observation that chronic antidepressant treatment increases BDNF mRNA expression; however, this finding has not often been repeated. In fact, several studies have suggested that the antidepressants fluoxetine and desipramine have no effect on BDNF mRNA expression depending on the time of sacrifice and the exons examined (Coppell et al. 2003; Dias et al. 2003). Therefore, one aim of the present study was to determine whether chronic administration of several antidepressants would increase BDNF or TrkB mRNA expression.

In addition, the effect of chronic exposure to selective DOR agonists has not been studied extensively in vivo. Tolerance develops to the convulsant and analgesic effects in mice (Comer et al. 1993; Hong et al. 1998) and to the convulsant effects in rats (Broom et al. 2002b). In addition, Brandt et al. (2001) found that chronic exposure to the DOR agonist SNC80 resulted in tolerance to SNC 80's effect on reductions in food-maintained responding in rhesus monkeys. The effect of chronic DOR agonist treatment on behavior in the forced swim test and on regulation of BDNF and TrkB mRNA expression has not been characterized.

Therefore, there were two primary aims of the present study. The first aim was to characterize the ability of a DOR agonist to produce antidepressant-like effects and increase BDNF mRNA expression after chronic administration to better understand the potential of these compounds as antidepressants in humans, and secondly, to determine if chronic treatment with various clinically used antidepressants can increase BDNF mRNA expression and produce antidepressant-like effects, in part to directly compare DOR agonists to known antidepressants using the methodology of our laboratory, and to clarify the effects of chronic antidepressants on BDNF expression.

Materials and methods

Animals

Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were used for all experiments. They were delivered weighing 250–300 g and were housed three per cage with ad libitum access to food and water. Animal rooms were kept on a 12-h light/dark cycle, with lights on at 0630 hours and a temperature of 21°C. Experiments were carried out in accordance with the Declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. The study protocols were approved by the University of Michigan University Committee on the Use and Care of Animals.

Drugs

(+)BW373U86.2HCl ((+)-4-[(αR)-[(2S,5R)-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl](3-hydroxyphenyl)methyl]-*N*,*N*-diethylbenzamide dihydrochloride) was synthesized by modifications of published protocols (Bishop and McNutt, 1995) and was dissolved in sterile water. Desipramine, bupropion, tranylcypromine (Sigma, St. Louis, MO, USA), and fluoxetine (Eli Lilly, Indianapolis, IN) were dissolved in sterile water.

Experimental treatment

Animals were allowed to acclimate to the research facility for at least 2 days after delivery for all experiments. In experiment 1, three groups of rats were injected with 10 mg/kg (+)BW373U86 (BW) or sterile water vehicle subcutaneously (s.c.) once a day for 1, 8, or 21 days. In all groups, animals were monitored for convulsions in observation cages for 20 min after injection on every day of treatment. The rat was then returned to its home cage. On the last day of treatment (1st, 8th, or 21st day), groups of vehicle-treated (n=6) and BW-treated (n=6) rats were tested in a 15-min forced swim test (described below) 1 h after the last injection. Another set of animals that were injected with vehicle or BW for 8 or 21 days were sacrificed 3 h after the last injection (n=5 for 8-day group, n=6 for 21-day group), and the brains were dissected and frozen in isopentane on dry ice. The brains were then sectioned at a thickness of 20 μm, and slides were stored at −80°C for later analysis by in situ hybridization (described below). Animals were sacrificed 3 h after the last injection of BW because previous work in our laboratory has shown that the maximum effect of BW on BDNF mRNA expression is seen at this time point (Torregrossa et al. unpublished observations).

Experiment 2 aimed to repeat the findings of previous studies. Therefore, rats were treated with doses of desipramine and tranylcypromine that were previously reported to increase BDNF mRNA expression (Nibuya et al. 1995). Fluoxetine and bupropion were given at doses that were previously shown to be active in the forced swim test in rats (Broom et al. 2002b; Torregrossa et al. 2004, respectively). Rats were divided into five groups that received vehicle (n=5), 15 mg/kg desipramine (n=4), 15 mg/kg fluoxetine (n=6), or 30 mg/kg bupropion (n=6) intraperitoneally (i.p.)

for 21 days, or 7.5 mg/kg tranylcypromine i.p. for 7 days followed by 10 mg/kg tranylcypromine for the next 14 days (n=4). Three hours after the last injection on the 21st day, brains were dissected as described in Experiment 1. Another set of rats was given vehicle (n=6), desipramine (n=5), or tranylcypromine (n=4) for 21 days as just described. One hour after the last injection on the 21st day, the rats were tested in the 15-min forced swim test as described below.

Forced swim test

The forced swim test was conducted as described by Torregrossa et al. (2004). Briefly, rats were videotaped from above during a 15-min swim period in a cylindrical container (46×20 cm height×diameter) filled to 30 cm with 25°C (±1) water. An observer blind to treatment scored the videotapes, classifying behaviors every 5 s for the entire 15-min period. The behaviors observed were immobility, swimming, and climbing, which were defined as described by Broom et al. (2002a,b) and Detke et al. (1997).

In situ hybridization histochemistry

Brain-derived neurotrophic factor and TrkB mRNA levels were determined by a double-label in situ hybridization with a [^{35}S]-labeled antisense BDNF or TrkB RNA probe as described by Torregrossa et al. (2004). The rat TrkB cDNA (described by Middlemas et al. 1991) was donated by Dr. Hunter (The Salk Institute). The rat BDNF cDNA (described by Isackson et al. 1991) was donated by Drs. Gall and Lauterborn (University of California, Irvine). To verify that the rat BDNF probe produces little to no nonspecific binding, control slides were hybridized with a [^{35}S]-labeled sense strand BDNF cRNA probe, which was made using the T7 RNA polymerase from linearized BDNF cDNA. These slides were processed for in situ hybridization in the same manner as slides labeled with the antisense probe.

The slides were exposed on Kodak XAR film (Eastman Kodak, Rochester, NY), and films were developed after 14 days for BDNF and 3 days for TrkB.

Quantification of radioactive signal

Brain-derived neurotrophic factor and TrkB mRNA levels were quantified using NIH Image (Scion Image Corp., Frederick, MD) software. BDNF mRNA expression after BW administration was examined in the frontal cortex; the CA1, the CA3, and the dentate gyrus regions of the hippocampus; the basolateral amygdaloid complex; the endopiriform nucleus; primary olfactory cortex; and the postcingulate cortex. All of these regions, with the exception of the dentate gyrus and postcingulate cortex, have increased BDNF mRNA expression after acute BW administration. BDNF mRNA expression for the antidepressant-treated groups and TrkB mRNA expression for all groups was examined in the frontal cortex and the CA1, CA3, and dentate gyrus regions of the hippocampus. Each brain region was analyzed by creating an outline around the region and measuring both the left and right sides of the brain from rostral–caudal sections 100–200 μm apart. At least six sections per region per rat were quantified. The signal measurements were corrected for background and were determined as the mean radioactive intensity per pixel for that region. These signal values for each section were then averaged to obtain the mean signal for each region in each rat. These data points were then averaged per group and compared statistically.

Statistical analysis

All comparisons between multiple groups were conducted by one-way ANOVA. Tukey's post hoc test was used to determine differences between groups where $p<0.05$ was considered significant.

Results

Effects of chronic exposure to a delta agonist

Rats received injections of 10 mg/kg BW or vehicle every day for 21 days. These animals were weighed every day and monitored for convulsions and any overt signs of physical dependence (i.e., withdrawal), but withdrawal signs were not quantified. Figure 1a illustrates the average weight of each group of animals over the course of the 21 days of treatment. There was no difference in weight between the vehicle- and BW-treated groups on any day of treatment. In contrast, animals receiving desipramine, fluoxetine, and tranylcypromine for 21 days all lost weight after the initial days of treatment, and the average weights of all three groups were significantly different from vehicle-treated rats beginning on day 6 and on every subsequent day of treatment (Fig. 1b). Rats receiving bupropion initially lost a very small amount of weight but did not significantly differ from vehicle on any day but day 2.

In addition, the rats receiving BW showed no outward signs of physical dependence or withdrawal on a day-to-day basis. However, rats receiving desipramine, fluoxetine, and tranylcypromine often had diarrhea and were aggressive toward the experimenter and other rats in its home cage.

Finally, on the first day of BW administration, five of six rats had a clonic convulsion within 20 min of injection (data not shown). On every subsequent day of BW administration, none of the rats displayed any sort of convulsive activity.

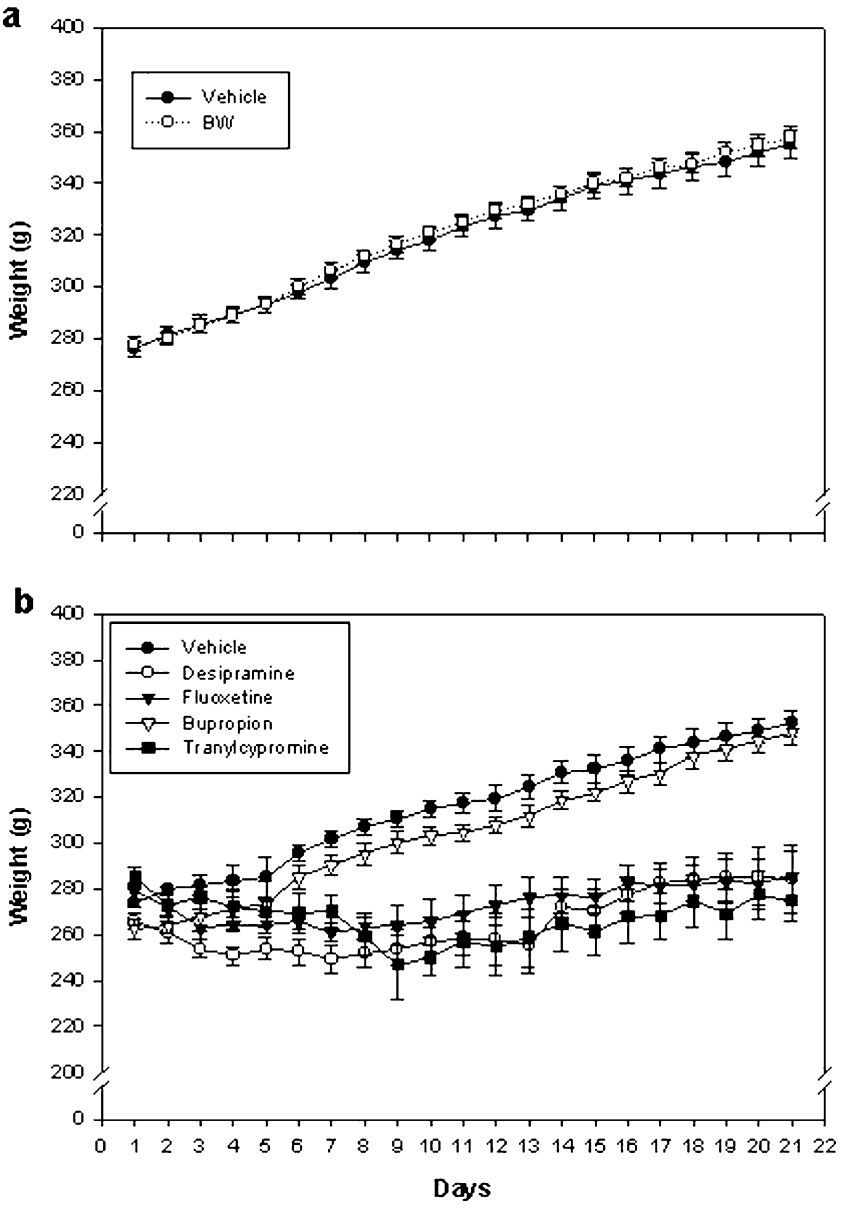

Fig. 1 The body weight of rats receiving **a** chronic 10 mg/kg (+)BW373U86 (BW) or **b** chronic antidepressant treatment. The weight in grams of each rat was recorded every day prior to injection of test compound and is expressed as the group mean on each day± SEM. The weight of BW-treated rats did not significantly differ from vehicle-treated rats. DMI, FLX, and TCP all significantly reduced body weight compared to vehicle controls on days 6–21

Forced swim test

A single injection of 10 mg/kg BW given 1 h prior to a 15-min forced swim test produced a significant reduction in immobility, with concomitant significant increases in both swimming and climbing (Fig. 2a). The reduction in immobility suggests an antidepressant-like effect of BW, which has been previously reported (Broom et al. 2002a,b). Conversely, when 10 mg/kg BW is administered for 8 or 21 days before the forced swim test; there is no longer any significant reduction in immobility when compared to controls (Fig. 2b,c).

In contrast, the antidepressants desipramine and tranylcypromine both produced significant reductions in immobility in the forced swim test after 21 days of administration (Fig. 3). Desipramine also produced a significant increase in climbing, while tranylcypromine produced significant increases in both swimming and climbing.

Regulation of BDNF and TrkB mRNA expression

Brain-derived neurotrophic factor mRNA expression was measured in animals that had been treated with 10 mg/kg BW for 8 and 21 days. After 8 days of BW administration, BDNF mRNA expression was significantly increased in the frontal cortex (Fig. 4). There were no significant changes in BDNF mRNA expression in any other brain region after 8 days of BW. In addition, 21 days of BW administration had no effect on BDNF mRNA expression in any brain region (data not shown). Finally, neither 8- nor 21-day exposure to BW had any effect on TrkB mRNA expression in any brain region examined (data not shown).

Several antidepressants have been reported to regulate BDNF and TrkB mRNA expression particularly in the hippocampus (Nibuya et al. 1995). Therefore, we examined the effect of chronic (21-day) exposure to the anti-

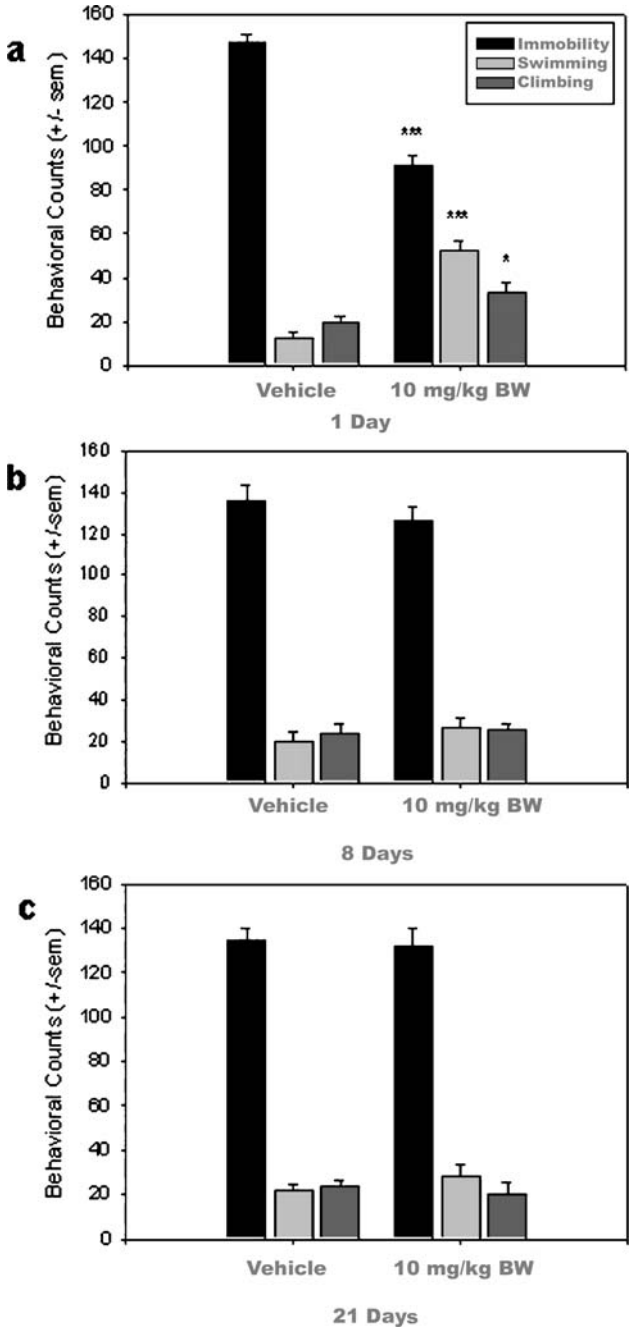

Fig. 2 Effect of acute and chronic 10 mg/kg BW on behavior in the forced swim test. **a** An acute injection of 10 mg/kg BW significantly reduces immobility ($p<0.001$) and significantly increases swimming ($p<0.001$) and climbing ($p<0.05$) in the forced swim test, indicating an antidepressant like effect. **b** 10 mg/kg BW given for 8 days has no significant effect on behavior in the forced swim test. **c** 10 mg/kg BW given for 21 days also has no significant effect on behavior in the forced swim test. * indicates $p<0.05$ and ***, $p<0.001$

depressants desipramine, bupropion, fluoxetine, and tranylcypromine on BDNF and TrkB mRNA expression in the frontal cortex, and the CA1, CA3, and dentate gyrus regions of the hippocampus. None of the compounds produced a significant change in BDNF expression in the frontal cortex. Both desipramine and bupropion significantly decreased BDNF mRNA expression in the dentate gyrus, while having no effect in the other regions of the hippocampus. Fluoxetine had no effect on BDNF mRNA expression in any brain region examined. On the other hand, tranylcypromine significantly increased BDNF mRNA expression in the CA1 region of the hippocampus and tended to increase expression in the CA3 and dentate gyrus regions, although not significantly (Fig. 5). Finally, none of the antidepressants tested had any effect on TrkB mRNA expression in the frontal cortex or hippocampus (data not shown).

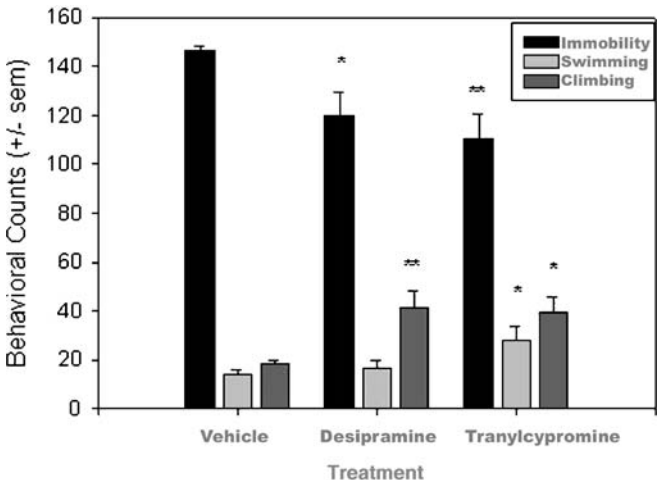

Fig. 3 Effects of chronic DMI and chronic TCP on behavior in the forced swim test. Chronic (21-day) treatment with 15 mg/kg DMI significantly reduces immobility ($p<0.05$) and significantly increases climbing ($p<0.01$) in the forced swim test. TCP, given at a dose of 7.5 mg/kg for 7 days and 10 mg/kg for the following 14 days, significantly reduces immobility ($p<0.01$), and significantly increases swimming ($p<0.05$) and climbing ($p<0.05$) in the forced swim test. * indicates $p<0.05$ and **, $p<0.01$

Discussion

The delta opioid receptor agonist BW has been shown to produce convulsions, antidepressant-like effects, and regulates BDNF mRNA expression upon acute administration (Comer et al. 1993; Broom et al. 2002a,b; Torregrossa et al. 2004). However, few studies have examined the behavioral or neurobiological effects of chronic exposure to selective delta agonists. The present study examined the effects of chronic delta agonist administration on weight, convulsions, antidepressant-like effects, and BDNF mRNA expression. In addition, this study compared the effects of BW to several antidepressant drugs.

Chronic exposure to 10 mg/kg BW did not affect weight or amount of weight gain in rats when compared to controls unlike mu opioid receptor agonists, which may cause weight loss in rats under chronic exposure conditions (Azar et al. 2004). In addition, chronic BW treatment did not result in any other obvious behavioral signs of physical dependence (withdrawal occurrence between doses). Chronic treatment with fluoxetine, desipramine, and tranylcypromine, but not bupropion, resulted in initial weight loss and either continued weight loss or minimal weight gain

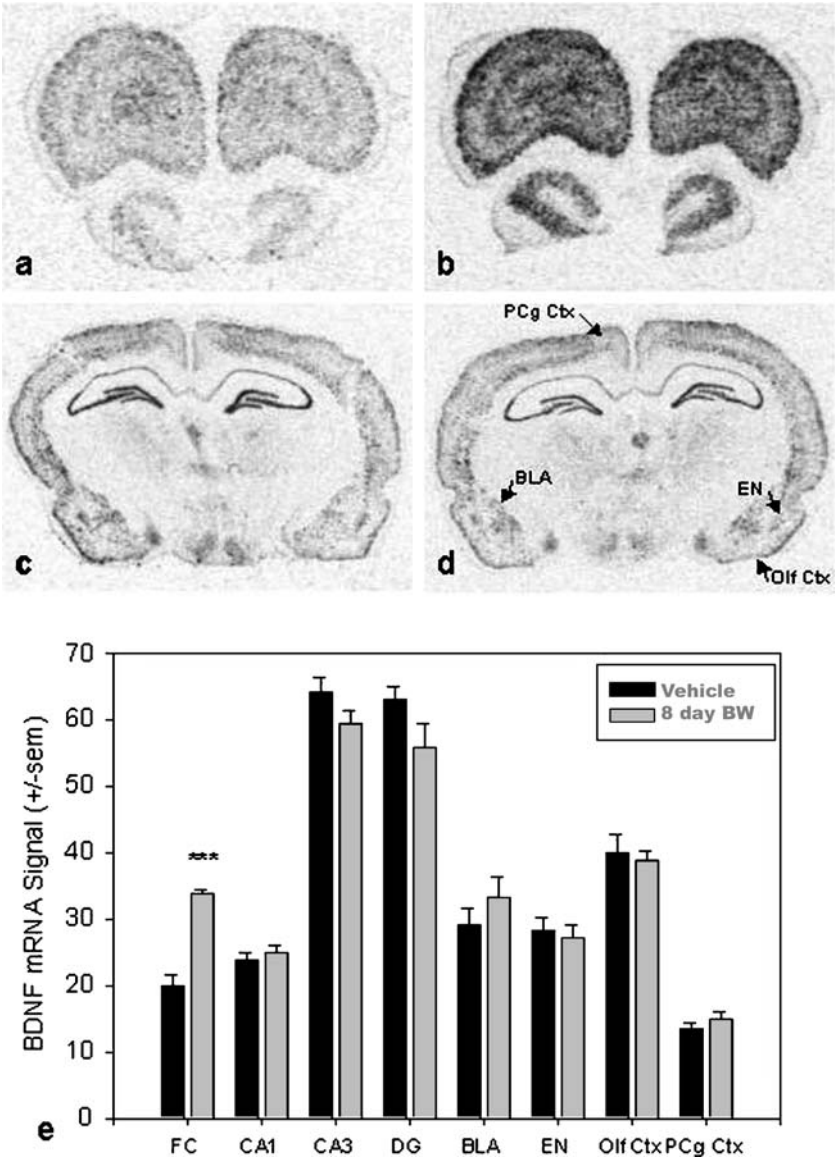

Fig. 4 Brain-derived neurotrophic factor mRNA expression after 8 days of treatment with vehicle or 10 mg/kg BW. **a**–**d** Photomicrographs from x-ray films exposed for 14 days after in situ hybridization with the antisense cRNA probe to rat BDNF mRNA. Photos show sections through the frontal cortex and at the level of the hippocampus. **a**, **c** Sections from a vehicle-treated animal. **b**, **d** Sections from an animal treated with 10 mg/kg BW for 8 days. **e** Quantification of BDNF mRNA signal in frontal cortex, CA1, CA3, and dentate gyrus of hippocampus, basolateral amygdala (*BLA*), endopiriform nucleus (*EN*), olfactory cortex (*Olf Ctx*), and postcingulate cortex (*PCg Ctx*), expressed as mean±SEM. *** indicates $p<0.001$

when compared to controls. In addition, these animals were often very irritable and aggressive.

A single administration of 10 mg/kg BW produces a clonic convulsion in most animals and produces a significant antidepressant-like effect in the forced swim test. When 10 mg/kg BW is given on two consecutive days, none of the animals will have a convulsion on the second day; however, the antidepressant-like effect is maintained (Broom et al. 2002b). In the present study, 10 mg/kg BW was given for 8 and 21 days. None of the animals had a convulsion on any day save for the first day of treatment. BW administered for 8 and 21 days did not produce a significant antidepressant-like effect in the forced swim test, indicating that tolerance does eventually develop to the antidepressant-like effects of BW. In contrast, the antidepressants desipramine and tranylcypromine produced antidepressant effects in the forced swim test after a 21-day administration, indicating that tolerance does not develop to the behavioral effects of these antidepressants under these conditions.

In addition, we have previously shown that 10 mg/kg BW increases BDNF mRNA expression in several brain regions, including the frontal cortex, when given acutely, and increases BDNF mRNA expression in the frontal cortex when given on two consecutive days (Torregrossa et al. 2004). In this study, we found that 8 days of treatment with 10 mg/kg BW continued to produce a significant increase in BDNF mRNA expression in the frontal cortex, whereas at 21 days of treatment, this effect disappeared. BDNF mRNA expression is increased throughout the frontal cortex, suggesting a possible nonspecific effect; however, delta receptors are expressed throughout the frontal cortex (Mansour et al. 1993), so it is possible that the increase in BDNF mRNA expression is directly regulated by delta opioid receptor activation. Delta opioid receptors can activate the mitogen-activated protein kinase (MAPK)

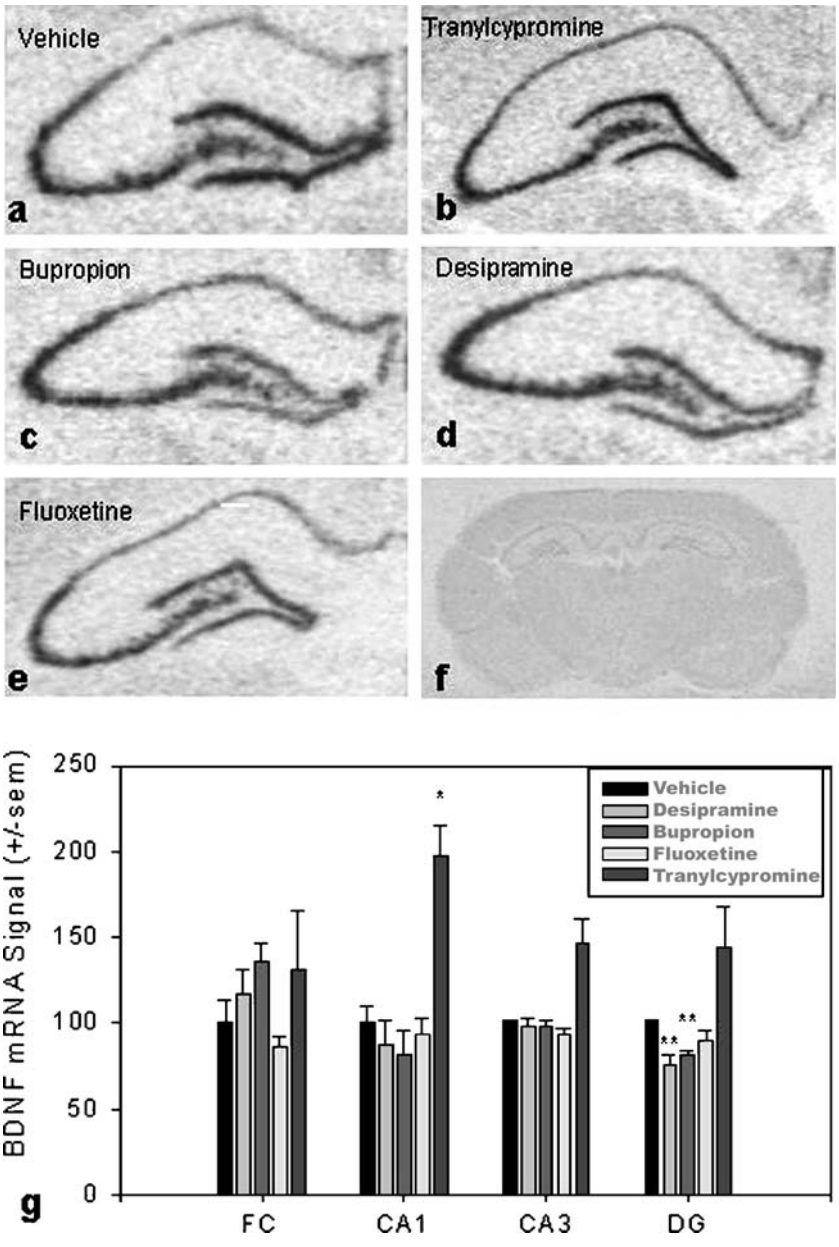

Fig. 5 Brain-derived neurotrophic factor mRNA expression after chronic antidepressant treatment. **a**–**e** Photomicrographs from x-ray films exposed for 14 days after in situ hybridization with the antisense cRNA probe to rat BDNF mRNA. Photos show sections through the hippocampus. **a** Section of 21-day vehicle treatment. **b** Section from chronic TCP treatment **c** Section from chronic bupropion treatment. **d** Section from chronic DMI treatment. **e** Section from chronic fluoxetine treatment. **f** Photomicrograph from a section through the hippocampus after in situ hybridization with the sense cRNA probe to rat BDNF mRNA, illustrating that there is no nonspecific binding. **g** Quantification of BDNF mRNA signal in frontal cortex, CA1, CA3, and dentate gyrus of hippocampus expressed as percent of vehicle control. * indicates $p<0.05$ and **, $p<0.01$

pathway (Kramer et al. 2002; Persson et al. 2003), and activation of the MAPK pathway can lead to up-regulation of BDNF mRNA expression (Wu et al. 2004), providing a potential mechanism of delta-induced increases in BDNF mRNA expression.

It is unclear why tolerance develops to increases in BDNF mRNA expression in all brain regions other than the frontal cortex after a single administration. The largest effect of BW on BDNF expression is seen in the frontal cortex, so while there is some tolerance after a single injection, a significant increase can still be seen with multiple days of injections, whereas in the brain regions where there are acutely smaller increases in BDNF, all effect is lost with the "partial" tolerance that develops from the first injection. However, it may also be the case that delta receptors regulate BDNF expression differentially depending on the brain region.

Alternatively, the apparent tolerance that develops to increases in BDNF mRNA expression over time can be explained by feedback regulation of the system. The presumed constant increase in BDNF expression caused by over 8 days of BW treatment should lead to an increase in BDNF protein, which could, in turn, negatively feedback to reduce BDNF mRNA expression. While this is a possibility that should certainly be tested, a study by Saarelainen et al. (2001) suggests that BDNF actually feeds back to positively alter its expression. In their study, transgenic mice were developed that express the dominant negative form of the TrkB receptor, which results in a reduction in BDNF signaling. It was found that kainic

acid induced up-regulation of BDNF mRNA was greatly reduced in the transgenic mice compared to wild types, implying that BDNF signaling through TrkB is necessary to produce a maximal increase in BDNF mRNA expression.

Therefore, it is most likely that the lack of BDNF expression seen after 21 days of BW is due to tolerance development, but the time course of tolerance development differs depending on the end point studied. This can be explained by differences in the receptor reserve required to produce each effect. There may be a greater receptor reserve available for the production of antidepressant-like behavioral effects than for producing convulsions, and an even greater reserve available for producing increases in BDNF mRNA expression in the frontal cortex. In support of this hypothesis, we previously found that a dose of 1 mg/kg BW significantly increased BDNF mRNA expression in the frontal cortex, while this dose is insufficient to produce convulsions, antinociception, or antidepressant-like effects (Torregrossa et al. 2004; Broom et al. 2002a).

Increases in the expression of growth factors and their receptors, particularly BDNF and its high-affinity tyrosine kinase receptor, TrkB, has been hypothesized to be important for the therapeutic effects of antidepressant drugs. Chronic administration of several antidepressant drugs and ECS was reported to increase the expression of BDNF in the hippocampus (Nibuya et al. 1995). Moreover, BDNF injected directly into the midbrain or hippocampal CA3 or dentate gyrus regions has been shown to produce antidepressant-like effects in the forced swim test (Siuciak et al. 1997; Shirayama et al. 2002). Therefore, a treatment that increases activity of the BDNF/TrkB system acutely may reverse the symptoms of depression more rapidly than current therapies. A fast-acting antidepressant is beneficial for reducing hospitalization times for the severely depressed and to potentially help suicidal patients more rapidly than current antidepressants. The selective non-peptidic delta opioid receptor agonists, like BW, may be fast-acting antidepressants because of their ability to acutely up-regulate BDNF mRNA expression. Long-term treatment with delta agonists is likely to result in tolerance development to some of the effects, but BDNF expression may continue to be increased for at least 1 week of administration, and this may provide sufficient stimulus for rapid recovery from a depressive episode. Therefore, DOR agonists are potentially very important compounds to develop for the acute treatment of a depressive episode. If chronic therapy becomes necessary to maintain proper mental health, then the dose may need to be escalated, or the compound may need to be taken once every few days to allow the tolerance to reverse between administrations.

However, the development of DOR agonists for human use will require finding a compound that can produce antidepressant-like effects at much lower doses than it produces convulsions. Work in our laboratory has shown that slowing the entry of DOR agonists into the brain by either slow intravenous infusion or oral administration can enhance the separation between doses that produce antidepressant-like effects without a convulsion and those that produce convulsions (Jutkiewicz et al. 2005). Therefore, it should be possible to develop DOR agonists that can be administered in a way to produce antidepressant-like effects and increases in BDNF mRNA expression without producing convulsions.

Increased expression of BDNF and/or TrkB expression, however, may not be required for the therapeutic actions of currently used antidepressants. The present study attempted to clarify the confusion in literature by examining whether 21 days of treatment with the antidepressants desipramine, fluoxetine, bupropion, and tranylcypromine can indeed regulate BDNF or TrkB mRNA expression. We found that only tranylcypromine significantly increased BDNF expression in the hippocampus. In fact, both desipramine and bupropion significantly decreased BDNF mRNA expression in the dentate gyrus of the hippocampus, while fluoxetine had no effect. In addition, none of the antidepressants had any effect on TrkB mRNA expression in the frontal cortex or hippocampus. Therefore, increased expression of BDNF and/or TrkB does not appear to be a common effect of chronic treatment with various antidepressants.

Several other laboratories have reported no significant increases in BDNF expression after chronic antidepressant treatment. Miro et al. (2002) found that 14 days of 3 mg/kg intraperitoneal fluoxetine significantly decreased BDNF mRNA expression in several regions, including the CA and dentate gyrus regions of the hippocampus. Coppell et al. (2003) found that fluoxetine significantly reduced BDNF expression in the hippocampus when animals were sacrificed 4 h after the last injection but significantly increased BDNF expression in the hippocampus when animals were sacrificed 24 h after the last injection, suggesting that fluoxetine induced increases in BDNF mRNA expression may only be seen 24 h after the last treatment.

While Nibuya et al. (1995) found that 21 days of desipramine increased BDNF mRNA expression in the hippocampus when animals were sacrificed 3 h after the last treatment, we found the opposite effect. Coppell et al. (2003) found that 2 weeks of desipramine had no effect on BDNF expression when the animals were sacrificed 24 h after injection. In contrast, Jacobsen and Mork (2004) found that 21 days of 10 mg/kg desipramine did moderately, but significantly, increase BDNF expression in the dentate gyrus. Therefore, the literature regarding the effects of desipramine on BDNF expression is quite variable, and the differences may be due to differences in dosing regimens, time of sacrifice, and/or in the methods used to quantify mRNA levels.

The effect of tranylcypromine on BDNF expression appears more consistent, and concurs with our finding in the present study that tranylcypromine significantly increases BDNF expression in the hippocampus (Nibuya et al. 1995; Russo-Neustadt et al. 1999). The present study is the first to examine the effects of bupropion, a preferential dopamine reuptake inhibitor, on BDNF mRNA expression. We found that chronic bupropion significantly

decreased BDNF expression in the dentate gyrus and had no effect in any other brain region. While chronic bupropion has not been studied previously. Nibuya et al. (1995) did examine the effects of chronic 15 mg/kg cocaine i.p. on BDNF expression. Due to the similar mechanism of action of cocaine and bupropion, one might expect to see similar effects on gene expression. In fact, Nibuya et al. (1995) found no significant effect of chronic cocaine on BDNF expression in any brain region.

In conclusion, while acute administration of the selective delta opioid receptor agonist BW consistently produces behavioral antidepressant-like effects and increases BDNF mRNA expression in the frontal cortex, tolerance does eventually develop to these effects after chronic treatment. However, chronic treatment with the antidepressants fluoxetine, desipramine, and bupropion did not produce a significant increase in BDNF mRNA expression in any brain region, indicating that an increase in BDNF gene expression after chronic treatment is not a common effect of all antidepressants, and may not be necessary for the clinical effectiveness of these drugs. Alternatively, treatments like ECS and tranylcypromine that show increases in BDNF expression may be more effective in treating depression. Therefore, the rapid increase in BDNF expression produced by delta opioid receptor agonists may indicate that these drugs can provide an alternative antidepressant treatment for treatment resistant depression or in cases where a more rapid clinical response is desired.

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