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Antidepressant-like effects of novel triple reuptake inhibitors, PRC025 and PRC050

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Abstract

Most currently prescribed antidepressants act by selectively increasing the synaptic availability of serotonin or norepinephrine, or through action on both serotonin and norepinephrine. However, most therapies require several weeks of treatment before improvement of symptoms is observed and not all patients respond to antidepressant treatment. One strategy that has emerged in new antidepressant development is the use of triple reuptake inhibitors, which inhibit reuptake of serotonin, norepinephrine, and dopamine. These compounds have been hypothesized to have a more rapid onset of activity and better efficacy over single or dual reuptake inhibitor antidepressants in part due to the addition of the dopamine component. We have developed novel compounds that are analogs of venlafaxine, of which two, racemic PRC025 ((2*SR*, 3*RS*)-*N*,*N*-dimethyl-3-cyclohexyl-3-hydroxy-2-(2′-naphthyl)propylamine) and PRC050 ((2*RS*,3*RS*)-*N*-methyl-3-hydroxy-2-(2′-naphthyl)-3-phenylpropylamine), are highly potent at human serotonin, norepinephrine, and dopamine transporters and inhibit the reuptake of serotonin, norepinephrine, and dopamine into rat brain synaptosomes. These compounds were tested in animal models used to evaluate potential antidepressants: the forced swim test in rats and the tail suspension test in mice. In the forced swim test, both PRC025 and PRC050 reduced the time spent immobile and increased the time spent swimming, comparable to the effects seen with imipramine, a tricyclic antidepressant. In addition, both PRC025 and PRC050 were effective in reducing the time spent immobile in the tail suspension test, again with effects comparable to imipramine. Therefore it appears that our compounds may possess antidepressant activity and represent a new class of triple reuptake inhibitors.

Keywords: Antidepressant; Reuptake inhibitor; Norepinephrine; Serotonin; Dopamine

1. Introduction

Depression is a serious illness that, according to the World Health Organization, is one of the top causes of disability worldwide. In the United States, depression has a lifetime prevalence of around 10% to 20% in the adult population, yet depression remains under-diagnosed and under-treated. About 65% of patients ultimately respond to antidepressant drug therapy whereas, about 15% of depressed patients are resistant to all known forms of therapy (reviewed in Richelson, 2001). Although many of the underlying causes of depression are still unclear, our basic understanding of the disease has come from observing the effects of antidepressant drugs on neurotransmitter systems. Most currently prescribed antidepressants act by increasing the synaptic availability of serotonin or norepinephrine, both serotonin and norepinephrine (e.g. venlafaxine, duloxetine); or serotonin, norepinephrine, and dopamine (e.g., phenelzine). These effects occur via several mechanisms including blocking the transporters for these neurotransmitters to inhibit reuptake into nerve terminals (e.g. venlafaxine, duloxetine), blocking degradation through the inhibition of monoamine oxidase (e.g., phenelzine), or by blocking presynaptic receptors resulting in an increase in neurotransmitter release (e.g., mirtazapine) (Richelson, 2001, 2003). Therefore, the causes of depression have been, in part,

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attributed to the dysregulation of one or all of these neurotransmitters at the synapse, and the ability of antidepressants to restore these deficits has been implicated in their acute mechanism of action for treating depression (Prange et al., 1974; Schildkraut, 1975). While the effects of antidepressants at the synapse occur almost immediately, these drugs need to be administered for weeks before clinical improvement is seen (~50% reduction of symptoms) (Adell et al., 2005; Nemeroff and Owens, 2002), suggesting that other downstream pathways are involved in their full mechanism of action as well as in the pathophysiology of depression.

Despite the availability of a number of antidepressant drugs which primarily mediate serotonergic and/or noradrenergic signaling, a considerable number of depressed individuals do not achieve remission of depressive symptoms with current therapies. One approach in the development of new antidepressant drugs is the use of triple reuptake inhibitors, which block serotonin, norepinephrine, and dopamine reuptake (Carlier et al., 1998; Skolnick et al., 2003a,b). These antidepressants have been hypothesized to have a more rapid onset of activity and better efficacy over currently prescribed serotonin, norepinephrine, or serotonin/norepinephrine reuptake inhibitor antidepressants in part due to the addition of the dopamine component (Skolnick et al., 2003a). From a historical perspective, a role for dopamine in the action of antidepressants as well as in the pathophysiology of depression has been known for many decades. In addition, there is considerable evidence linking mesocorticolimbic dopamine pathways with depression, especially with the anhedonia and lack of motivation observed in many depressed patients (D'Aquila et al., 2000). In collaboration with Dr. Paul Carlier and colleagues, we have developed novel compounds that are analogs of venlafaxine (Carlier et al., 1998). Venlafaxine can block the transport of serotonin and norepinephrine (at high doses), but has only a minimal effect at blocking dopamine transport (Tatsumi et al., 1997). The present study focuses on two of our analogs of venlafaxine, racemic PRC025 ((2*SR*, 3*RS*)-*N*,*N*-dimethyl-3-cyclohexyl-3-hydroxy-2-(2′-naphthyl)propylamine) and PRC050 ((2*RS*, 3*RS*)-*N*-methyl-3-hydroxy-2-(2′-naphthyl)-3-phenylpropylamine) (Fig. 1), which are potent at human serotonin, norepinephrine, and dopamine transporters. These compounds, which are racemic mixtures, were tested in animal models commonly used to evaluate potential antidepressants: the forced swim test in rats and the tail suspension test in mice.

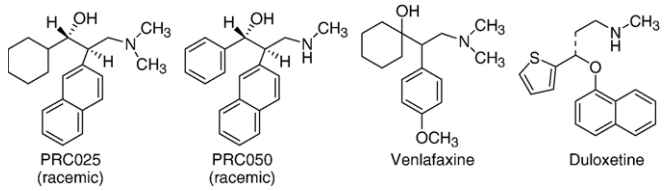

Fig. 1. Structures of PRC025 and PRC050 in comparison to venlafaxine and duloxetine. Only the single 2R,3R enantiomer of PRC025 and PRC050 is drawn, however these compounds are racemic mixtures and thus contain the 2S,3S enantiomers as well.

2. Materials and methods

2.1. Reagents

Imipramine, cocaine, serotonin, norepinephrine, and dopamine were purchased from Sigma (St. Louis, MO). [^{3}H]citalopram, [^{3}H]nisoxetine, [^{3}H]WIN35, 428 ((−)-2-β-Carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate), [^{3}H]serotonin, and [^{3}H]norepinephrine were obtained from Perkin-Elmer (Boston, MA). [^{3}H]dopamine was obtained from GE Healthcare (Buckinghamshire, UK).

2.2. Chemistry

PRC025 was prepared from 2-naphthylacetonitrile and cyclohexanecarboxaldehyde, using the procedures described in Carlier, 1998 (Carlier et al., 1998). The preparation of PRC050 is described in Richelson and Carlier, 2005 (Richelson and Carlier, 2005). Both drugs were administered as the racemic HCl salts.

2.3. Animals

Male Sprague-Dawley rats (200–250 g) and male C57Bl/6 mice (25–35 g) were used for these studies. Animals were housed in a temperature and humidity controlled facility on a 12 h:12 h light/dark cycle. Animals were allowed access to food and water *ad libitum* throughout the study and experiments were conducted during the light phase. All animal procedures were reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee and were consistent with AAALAC guidelines.

2.4. Radioligand binding experiments

The equilibrium dissociation constants (K_d's) for binding to human transporters in membranal preparations from cells expressing these transporters were determined, as previously done in our laboratory (Tatsumi et al., 1997). [^{3}H]citalopram, [^{3}H]nisoxetine, and [^{3}H]WIN35,428 were used as radioligands for human serotonin, norepinephrine, and dopamine transporters, respectively. Each compound was tested in duplicate at 11 different concentrations, spanning 3 orders of magnitude. For the preparation of the homogenates, medium was removed by aspiration and the cells were then washed with 4 ml modified Puck's Dl solution (Richelson and Pfenning, 1984). The cells were then incubated for 5 min at 37 °C in 10 ml solution 1 and 100 mM ethylene glycol-bis(-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). Afterwards cells were removed from the surface by scraping with a rubber spatula, placed in a centrifuge tube, and collected by centrifugation at 1000 ×*g* for 10 min at 4 °C. The pellets were resuspended in the binding assay buffer by use of a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) for 10 s. The mixture was then centrifuged at 35,600 ×*g* for 10 min at 4 °C. The pellets were resuspended in the same volume of the buffer and the centrifugation was repeated. The supernatants were decanted and the final pellets were resuspended in assay buffer and stored at

−80 °C until assayed. The final protein concentration was determined by using the BCA assay (Pierce Biotechnology, Inc., Rockford, IL). Data were analyzed by LIGAND (Munson and Rodbard, 1980) and presented as geometric means, with the standard error of the geometric mean being calculated as described by De Lean et al (De Lean et al., 1982).

2.5. Synaptosomal reuptake

The inhibitor constants (K_i's) of PRC025 and PRC050 for blocking transport of [^{3}H]serotonin, [^{3}H]norepinephrine, and [^{3}H]dopamine into rat brain synaptosomal preparations were determined as previously done in our laboratory (Bolden-Watson and Richelson, 1993; Carlier et al., 1998; Richelson and Pfenning, 1984). Crude rat synaptosomal preparations were prepared from cortical ([^{3}H]serotonin and [^{3}H]norepinephrine transport), and striatal ([^{3}H]dopamine transport) tissues. Each compound was tested in duplicate at 11 different concentrations, spanning 3 orders of magnitude. Male Sprague-Dawley rats (200–250 g) were decapitated and either the cortical (serotonin and norepinephrine) or striatal (dopamine) tissues were rapidly dissected. The tissue was homogenized in a glass Potter–Elvehjem homogenizer with Teflon pestle in 20 volumes (cortex) or 40 volumes (striatum) of oxygenated, ice-cold 5 mM HEPES buffer containing 0.32 M sucrose and 11 mM glucose, pH 7.4. The homogenate was centrifuged at 1000 ×g for 10 min and the resulting supernatant was centrifuged at 20,000 ×g for 20 min. The resultant pellet (P_2) was gently resuspended in oxygenated incubation buffer (10.5 mM HEPES, 128 mM NaCl, 4.95 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.3 mM $CaCl_2$, 10 mM dextrose, 10 μM pargyline, 0.2 mg/ml sodium ascorbate) and added to varying concentrations of drugs. The final concentration of radiolabeled neurotransmitter in the assay was 4 nM [^{3}H]serotonin, 4 nM [^{3}H]norepinephrine, or 2 nM [^{3}H]dopamine. To achieve equilibrium conditions for the antagonists, aliquots of synaptosomes were preincubated for 10 min with drugs at 37 °C in a shaking water bath (80 oscillations/min). The uptake was initiated by the addition of the radiolabeled compound to the synaptosomal protein. The reaction was stopped after 10 min by the addition of 4 ml ice-cold 0.9% (w/v) sodium chloride and rapid filtration through a Whatman GF/B glass fiber filter (presoaked in 0.1% polyethyleneimine) in a 48-place Brandel cell harvester (Brandel, Gaithersburg, MD). The filter was rapidly washed with an additional 8 ml of wash buffer, placed in a scintillation vial containing 5 ml of scintillation cocktail, and counted in a scintillation counter. Specific uptake was calculated as the difference between the total uptake (zero unlabelled neurotransmitter) and nonspecific uptake (excess unlabelled neurotransmitter). Data were analyzed by LIGAND (Munson and Rodbard, 1980) and presented as described above for the radioligand binding assays.

2.6. Forced swim test

When rats are placed in a cylinder of water from which they cannot escape, they will, following initial vigorous activity, remain immobile in the water for the majority of the test. Administration of antidepressants to animals decreases the amount of time spent immobile and increases swimming activity in the chamber (Porsolt et al., 1977). Therefore, this test is widely used to screen novel compounds for potential antidepressant activity (Cryan et al., 2002). Male Sprague-Dawley rats (200–250 g) were individually placed in vertical cylinders (height 40 cm, internal diameter 19 cm) containing water (25 °C) to a level of 15 cm, as originally described by Porsolt et al (Porsolt et al., 1977). Water was changed between trials and the procedure involved a pretest and a 5-min test separated by 24 h. During the pretest, rats (adapted to the experimental room for at least 1 h) were placed in the cylinder for 15 min. Following this initial exposure, the rats were dried with towels and transferred to a "drying cage" situated under a warming lamp. Fifteen minutes later, rats were injected i.p. with imipramine (15 mg/kg) as a positive control, PRC025 (5 or 10 mg/kg), PRC050 (5 or 10 mg/kg), or saline and returned to their home cages. The following day, rats were transferred to the experimental room and acclimated for at least 1 h. Rats were injected intraperitoneally (i.p.) with imipramine, PRC025, PRC050, or saline at 5 h and at 30 min before testing (total of 3 injections) and then placed in the test chambers. A time sampling technique was used to score behavior every 5 s during the 5 min test period as previously described (Detke et al., 1995). At the end of each 5-s interval, the rat's behavior was observed and scored based on the criteria described by Porsolt et al (Porsolt et al., 1977). The rat was considered immobile when floating motionless or making only those movements necessary to keep its head above the surface of the water. Scores for each behavior (swimming or immobility) were expressed as total counts per 5-min session.

2.7. Tail suspension test

The tail suspension test in mice (Steru et al., 1985) appears to be a corroboration of the forced swim test, with possible sensitivity to a broader range of antidepressants. When a mouse is suspended by its tail, there is an initial period of agitation, followed by immobility. This test identifies antidepressant compounds, which decrease the duration of immobility, as in the forced swim test. Male C57Bl/6J mice (25–35 g) were injected i.p. with imipramine (15 mg/kg) as a positive control, PRC025 (10 mg/kg and 5 mg/kg), PRC050 (10 mg/kg and 5 mg/kg), or saline 30 min before testing. Mice were then individually suspended by their tails 35 cm above the tabletop using an adhesive tape placed 1 cm from the tip of the tail. Behavior was scored every 5 s throughout the 6-min test as either mobile or immobile. Mice were considered immobile only when hanging passively and completely motionless. Scores for each behavior were expressed as total counts per 6-min session.

2.8. Locomotor activity

If a compound causes an increase in activity that persists, that activity could invalidate conclusions drawn from the tail suspension test and the forced swim test. Therefore, rats and mice were tested in a plexiglass Opto-Varimex Minor motility chamber (Columbus Instruments, Columbus OH) to determine whether PRC025 or PRC050 affected activity. Animals were

Table 1
Equilibrium dissociation constants (K_d's) for binding to human serotonin (hSERT), norepinephrine (hNET), and dopamine (hDAT) transporters: PRC025, PRC050; reference antidepressants

Compound	K_d (nM)		
	hSERT	hNET	hDAT
PRC025	6.0±0.8	19±2	100±10
PRC050	6.0±0.3	0.40±0.05	120±10
Venlafaxine	9.0±0.3	1060±40	9300±50
Paroxetine	0.13±0.01	40±2	490±20
Imipramine	1.4±0.03	37±2	8500±100
Nomifensine	1010±30	16.0±0.4	56±3
Sertraline	0.29±0.01	420±20	25±2

Values represent the geometric mean of the K_d±SEM of at least three independent experiments for PRC025 and PRC050. Binding data for reference compounds are from taken from Tatsumi et al. (1997).

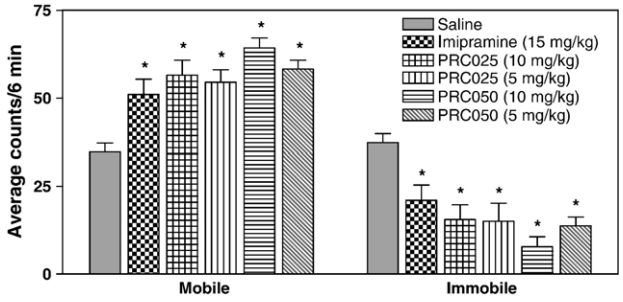

Fig. 2. Effect of PRC025 and PRC050 on the amount of mobility or immobility in mice during the tail suspension test. Mice were injected i.p. with either test compound (doses of 5 or 10 mg/kg) or saline 30 min prior to the test. Behavior was observed every 5 s during the 6-min test period and scored as mobile or immobile. Bars represent the mean number of counts over the 6-min period (±S.E.M.). *$P<0.05$ vs. saline treatment. $n=4$–7 mice per group.

acclimated to the test chamber for 2 h and then were injected with test compounds or saline. Thirty minutes post injection, activity was measured in 10 min intervals for 30 min to correspond to the time of the tail suspension test (mice) or the forced swim test (rats).

2.9. Statistical analysis

For the behavioral tests, statistical analysis was performed using one-way ANOVA followed by the Tukey's test for post-hoc comparisons. $P<0.05$ was considered significant.

3. Results

3.1. Affinities of PRC025 and PRC050 for the human monoamine transporters

Equilibrium dissociation constants (K_d's) for binding of PRC025 and PRC050 to human serotonin, norepinephrine, and dopamine transporters are given in Table 1, along with several reference antidepressants for comparison. Hill coefficients (n_H) for the compounds at each binding site were close to unity (data not shown), suggesting that the binding obeyed the law of mass action. Both PRC025 and PRC050 displayed similar affinities for binding to human serotonin transporter (6 nM) and to human dopamine transporter (~100 nM), while PRC050 was ~50 fold more potent for binding to human norepinephrine transporter than was PRC025 (0.4 nM and 19 nM, respectively).

3.2. Inhibition of synaptosomal serotonin, norepinephrine, and dopamine reuptake

The reuptake data (Table 2) represent the geometric mean of the K_i±SEM of the values for each compound. PRC025 and PRC050 potently inhibited the reuptake of [^{3}H]serotonin and [^{3}H]norepinephrine into rat cortical synaptosomes and [^{3}H]dopamine into rat striatal synaptosomes. The K_i values for inhibition of [^{3}H]serotonin, [^{3}H]norepinephrine, and [^{3}H]dopamine uptake by PRC025 were 6±0.8, 19±2.2, and 53±1 nM, respectively, and by PRC050 were 12±2, 1.2±0.1, 43±7 nM, respectively (Table 2). K_i values for reference antidepressants are included for comparison.

Table 2
Inhibitor constants (K_i's) for reuptake inhibition of serotonin, norepinephrine and dopamine in rat brain synaptosomes: PRC025, PRC050; reference antidepressants

Compound	K_i (nM)		
	[^{3}H]serotonin uptake	[^{3}H]norepinephrine uptake	[^{3}H]dopamine uptake
PRC025	6.0±0.8	10.0±0.5	53±1
PRC050	12±2	1.2±0.1	43±7
Venlafaxine[a]	39±3	210±20	5300±600
Paroxetine[a]	0.73±0.04	33±2	1700±300
Imipramine[a]	41±3	14±1	11000±1000
Nomifensine[b]	1280±80	5.0±0.4	51±8
Sertraline[a]	3.4±0.4	220±40	260±40

Values represent the geometric mean of the K_i±S.E.M. of at least three independent experiments for PRC025 and PRC050. Reuptake data for reference compounds are taken from [a](Bolden-Watson and Richelson, 1993) and [b](Richelson and Pfenning, 1984).

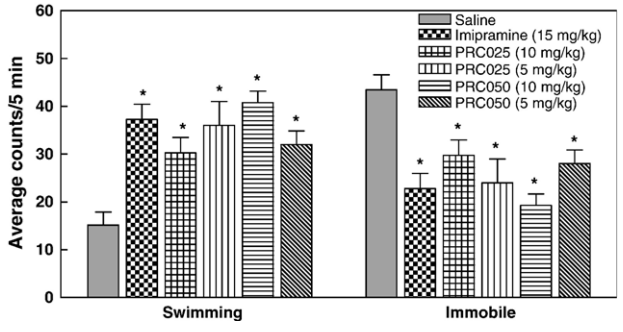

Fig. 3. Effect of PRC025 and PRC050 on the amount of swimming or immobility in rats during the forced swim test. Rats were injected i.p. with test compound (doses of 5 or 10 mg/kg) or saline 24 h, 5 h, and 30 min prior to the test. Behavior was observed every 5 s during the 5-min test period and scored as swimming or immobile. Bars represent the mean number of counts over the 5-min test period (±S.E.M.). *$P<0.05$ vs. saline treatment. $n=4$–7 rats per group.

3.3. Effects of PRC025 and PRC050 in the tail suspension test in mice

Peripheral administration of both PRC025 and PRC050 resulted in a significant decrease in immobility in the tail suspension test in mice at both doses tested (5 mg/kg and 10 mg/kg i.p.) (Fig. 2). These effects were dose-dependent with PRC050, but not with PRC025. The reduction in immobility produced by both PRC025 and PRC050 was similar to those of the reference antidepressant, imipramine, which was tested at a higher dose of 15 mg/kg i.p.

3.4. Effects of PRC025 and PRC050 in the forced swim test in rats

Peripheral administration of PRC050 resulted in a dose-dependent decrease in immobility and an increase in swimming in the forced swim test. PRC025 also produced a significant reduction in immobility and an increase in swimming at both doses tested (5 mg/kg and 10 mg/kg i.p.), although these effects were not dose-dependent (Fig. 3). Similar to the tail suspension test, the reductions in immobility were comparable to imipramine which was tested at a dose of 15 mg/kg i.p.

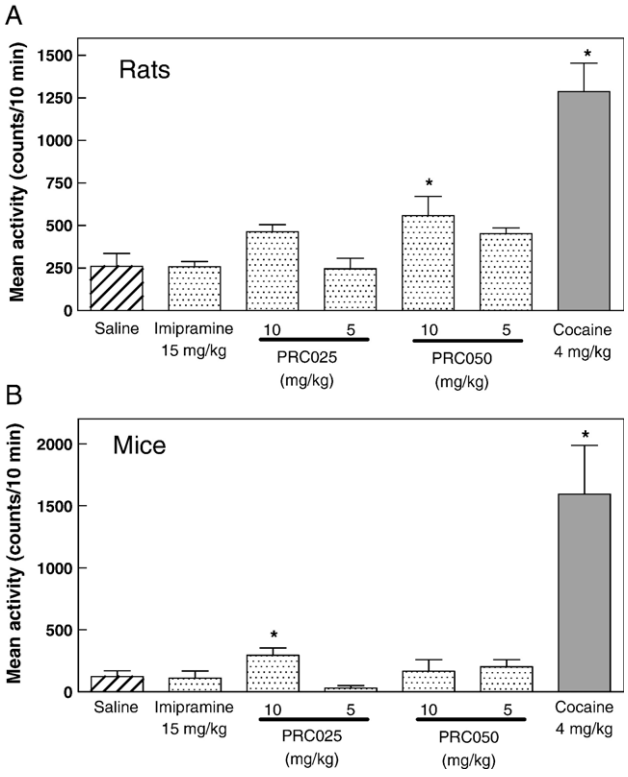

Fig. 4. Effect of PRC025 and PRC050 on locomotor activity. Rats (Fig. 4A) or mice (Fig. 4B) were injected i.p. with either test compound (doses of 5 or 10 mg/kg) or saline and locomotor activity was measured for 30 min beginning 30 min post injection. This time was selected to correspond to the test conditions of the forced swim test (rats) or the tail suspension test (mice). Bars represent the mean number of total activity counts (horizontal + ambulatory) measured every 10 min (±S.E.M). A low dose of cocaine was tested for comparison in rats (4 mg/kg i.p.) and mice (4 mg/kg i.p.). *$P<0.05$ vs. saline treatment. $n=4$–12 animals/group.

3.5. Effects of PRC025 and PRC050 on locomotor activity

Tested at a dose of 10 mg/kg i.p., PRC050 significantly stimulated locomotor activity in rats compared to that for saline controls (Fig. 4A). However, at 5 mg/kg i.p., a dose that was effective in the forced swim test, PRC050 had no significant stimulatory effect on activity. PRC025 did not significantly stimulate locomotor activity in rats at either dose tested (Fig. 4A). In mice (Fig. 4B), PRC025 produced a significant increase in activity at 10 mg/kg i.p. but not at 5 mg/kg i.p., although both doses were effective in the tail suspension test. Interestingly, PRC050 did not significantly stimulate locomotor activity in mice at either dose tested (Fig. 4B). The effects of PRC025 and PRC050 on locomotor activity in rats and mice were small in comparison to the stimulatory effects of a low dose of cocaine.

4. Discussion

The high prevalence of depression and the fact that a significant proportion of individuals do not respond well to any currently marketed antidepressants or treatments support the need for new therapeutics to treat depression. Not only is depression associated with a high risk of suicide, but it is also considered a significant risk factor for the development of coronary artery disease and stroke (Musselman et al., 1998; Pratt et al., 1996). Further, depressed patients have an increased risk of premature death (Harris and Barraclough, 1998), and depression can negatively affect outcomes of other diseases such as cancer, cardiovascular, and endocrine diseases (Peyrot, 2003; Pratt et al., 1996; Reiche et al., 2004; Somerset et al., 2004; Sonino et al., 2004; Spiegel, 1996). About 65% of patients ultimately respond to antidepressant drug therapy (Steffens et al., 1997), while about 15% of depressed patients are resistant to all known forms of therapy (Keller et al., 1992). In addition, most therapies require several weeks of treatment before improvement of signs and symptoms is observed and there are numerous side effects caused by antidepressants (Nemeroff and Owens, 2002). Thus, there is a need for new antidepressant drugs with better efficacy, fewer side effects, and more rapid onset of action.

While most currently prescribed antidepressants act by influencing serotonergic and/or noradrenergic neurotransmission, a growing number of studies suggest that the dopaminergic system may also be an important therapeutic target for the treatment of depression (D'Aquila et al., 2000; Papakostas, 2006). A role for dopamine deficiency in the pathophysiology of depression is supported by studies demonstrating reduced levels of dopamine and its metabolite homovanillic acid in depressed and/or suicidal patients compared to normal individuals (Engstrom et al., 1999; Hamner and Diamond, 1996; Mitani et al., 2006; Papakostas, 2006), as well as findings of increased dopamine D_2/D_3 receptor binding (D'Haenen H and Bossuyt, 1994; Klimek et al., 2002; Shah et al., 1997) and reduced dopamine transporter activity (Klimek et al., 2002; Meyer et al., 2001; Neumeister et al., 2001) in depressed patients. In addition, anhedonia, or the lack of interest in normally pleasurable activities, is a core symptom of depression and has been linked to deficits in the mesolimbic dopamine system, an area important

for mediating reward and incentive motivation (D'Aquila et al., 2000). Chronic treatment with different classes of antidepressants consistently leads to a potentiation of dopamine neurotransmission suggesting that a compound with immediate effects on dopamine signaling may be more effective and faster acting than a drug with no dopaminergic activity, especially for alleviation of symptoms related to reward and motivation (D'Aquila et al., 2000). A recent study examined the effects of treatment with the serotonin/norepinephrine reuptake inhibitor, duloxetine, in combination with the dopamine/norepinephrine reuptake inhibitor, bupropion, in a small sample of patients with major depressive disorder who had not achieved symptom remission with either treatment alone. The combination of these drugs resulted in a significant improvement in depressive symptoms in these patients suggesting that a drug targeting serotonergic, noradrenergic, and dopaminergic neurotransmission simultaneously would likely be effective in treating depression (Papakostas et al., 2006).

Two of our compounds, which are analogs of venlafaxine, racemic PRC025 and racemic PRC050, demonstrated a higher affinity than venlafaxine (Tatsumi et al., 1997) for all 3 human transporters. Additionally, PRC025 and PRC050 potently inhibited serotonin, norepinephrine, and dopamine reuptake in rat synaptosomal preparations, suggesting that these compounds are indeed triple reuptake inhibitors. In tests predictive of antidepressant activity in humans, the forced swim test in rats and the tail suspension test in mice, PRC025 and PRC050 reduced the time spent immobile with effects comparable to imipramine. Although these tests are not models of depression *per se*, they are widely used and demonstrate a high specificity for detecting novel antidepressant drugs (Cryan et al., 2002; Harro, 2004). Therefore, both PRC025 and PRC050 have antidepressant properties based on the results of these tests. Drugs that increase locomotor activity such as psychomotor stimulants can produce false positives in the forced swim test and tail suspension test (Cryan et al., 2005). In these studies, PRC050 at a dose of 10 mg/kg i.p. showed a significant increase in locomotor activity in rats, while PRC025 at a dose of 10 mg/kg i.p. significantly increased locomotor activity in mice. However, these effects were not observed at the lower dose tested (5 mg/kg) in either rats or in mice and this dose was effective in the forced swim and tail suspension tests. In addition, the magnitude of the locomotor stimulation of PRC050 at 10 mg/kg i.p. in rats was similar to that observed with a previously reported triple reuptake inhibitor, DOV 21,947, tested under similar experimental conditions (Skolnick et al., 2003a) and was much smaller than that observed with a low dose of cocaine. Therefore, it is not likely that the increase in locomotor activity observed at the higher dose of PRC050 in rats or of PRC025 in mice would invalidate the results of the tail suspension and forced swim tests.

Based on the results of these studies, PRC025 and PRC050 may possess antidepressant activity and represent a new class of triple reuptake inhibitors. The addition of the dopamine component to serotonin and norepinephrine transporter blockade may result in a more efficacious antidepressant but further studies are needed to investigate this point. This would include testing these compounds in other animal behavioral models which are thought to more closely mimic depression such as chronic stress paradigms which would allow for further investigation into the onset of action and efficacy of PRC025 and PRC050 compared to other classes of antidepressants. In addition, studies with the pure enantiomers of PRC025 and PRC050 are currently in progress.

Acknowledgements

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