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EFFECTS OF PD149163 ON MEDIAL PREFRONTAL CORTICAL DOPAMINE RELEASE IN RATS

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EFFECTS OF PD149163 ON MEDIAL PREFRONTAL CORTICAL DOPAMINE
RELEASE IN RATS

By

Amber L. LaCrosse

THESIS

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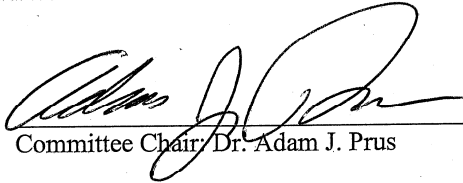
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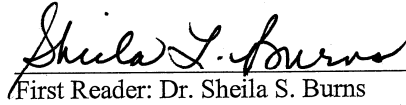
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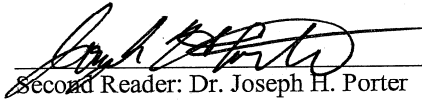
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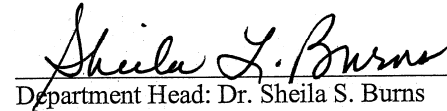
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ABSTRACT

EFFECTS OF PD149163 ON MEDIAL PREFRONTAL CORTICAL DOPAMINE RELEASE IN RATS

By

Amber L. LaCrosse

Schizophrenia is a neuropsychiatric disorder that affects about 1% of the entire population. Schizophrenia is accompanied by positive and negative symptoms as well as cognitive deficits. It has been suggested that the cognitive deficits occur because of a decreased availability of dopamine within the prefrontal cortex. Neurotensin analogs act as neurotensin, an endogenous tridecapeptide found within the CNS, which has shown evidence of increasing dopamine within the prefrontal cortex. The purpose of this study is to further elucidate the mechanisms with which neurotensin acts. PD149163, a neurotensin analog, was administered systemically and paired with several drugs that acted as either antagonists or agonists upon the 5-HT system. Procedures included microdialysis in awake and freely moving male rats. Doses of PD149163 included 0.1 mg/kg and 1.0 mg/kg. The 1.0 dose was effective alone for the increase of dopamine within the prefrontal cortex and was reversed when paired with WAY100638 0.2 mg/kg was administered as a pre-treatment. The 0.1 dose was ineffective alone and when paired with 8-hydroxy-dpat 0.5mg/kg, a 5-HT_{1A} agonist, dopamine levels remained comparable to vehicle. These findings along with previous research add significant support to the suggestion that the 5-HT_{1A} receptor is an important mediator between neurotensin and dopamine activity although further testing is necessary.

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LIST OF ABBREVIATIONS

5-HIAA: 5-hydroxyindoleacetic acid

5-HT: Serotonin

5-HTP: 5-hydroxytryptophan

5-HTT: 5-HT transporter

8-OH-DPAT: 7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol hydrobromide

AC: Adenylate cyclase

CNS: Central nervous system

DA: Dopamine

DOPAC: Dihydroxyphenylacetic acid

ECT: Electroconvulsive shock therapy

EPS: Extrapyramidal side effects

HVA: Homovanillic acid

MAOs: Monoamine oxidases

MK-801: Dizocilpine

mPFC: medial prefrontal cortex

NT69L: (2S)-2-[[[(2S)-2-[[[(2S)-2-[[[(2S)-1-[(2S)-6-amino-2-[[[(2S)-5-

(diaminomethylideneamino)-2-(methylamino)pentanoyl]amino]hexanoyl]pyrrolidine-2-carbonyl]amino]-3-(1H-indol-7-yl)propanoyl]amino]-3,3-dimethylbutanoyl]amino]-4-methylpentanoic acid

PET: Positron emission tomography

PFC: Prefrontal cortex

PI: Phosphoinositol

PPI: Prepulse inhibition

SSRI: Selective serotonin reuptake inhibitor

VMAT2: Vesicular monoamine transporter type two

VTA: Ventral tegmental area

WAY100635: N-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-N-pyridin-2-ylcyclohexanecarboxami

INTRODUCTION

The DSM-IV- TR (Diagnostic and Statistics Manual IV- Text Revision, 2000) characterizes schizophrenia as having several symptoms that are grouped into two categories, positive and negative. Positive symptoms are the types of behaviors that are added to normal human functioning and they include behaviors such as paranoid thoughts of persecution and auditory hallucinations. The negative symptoms are those types of behaviors that are taken away from normal human functioning and this includes behaviors such as anhedonia, which is a word that describes lack of pleasure. Anhedonia leads most patients with schizophrenia to feel depressed, so it is not a surprising statistic that up to 15% of schizophrenics commits suicide (Rowley et al. 2001). Along with experiencing a lack of joy, negative symptoms include social withdrawal, decreased movement, and a reduced emotional response, also known as having a flat affect.

Schizophrenia is a lifelong illness that affects about 1% of the entire population, which is equal to approximately 2-3 million affected people in the United States alone (Regier et al. 1993). Dr. Eugene Bleuler gave schizophrenia its name in 1911. He coined the term for its representation of 'the splitting of psychic function' as seen with this disorder. Schizophrenia is broken down into 5 subtypes. The first is the paranoid type and is characterized by delusions of persecution. The second subtype is described as the catatonic type, characterized by immobility and periods of excessive movement. The third is known as the disorganized type, named appropriately for the display of disorganized behavior and immaturity and silliness in emotional expression. The last two subtypes are the undifferentiated and the residual. The latter is a term used for individuals

with schizophrenia who are not actively showing any symptoms of the disease. The former is used to describe individuals with schizophrenia whose symptoms are not specifically formed enough to fall into one of the other categories. Since symptoms throughout schizophrenia can fluctuate, classification can be difficult to define (Diagnostic and Statistics Manual IV- Text Revision, 2000).

There is, however, another type of adverse symptom associated with schizophrenia. The cognitive deficits for this disease are extremely debilitating and are experienced in every case of schizophrenia ranging in degree from moderate to severe. The deficits are seen within working memory, reference memory, attention, and executive functioning (Green et al. 2004). Schizophrenia was originally named ‘dementia praecox,’ by Emil Kraepelin in 1893 because of the cognitive deficits he observed. Dementia Praecox means “premature mental deterioration”. The cognitive deficits are so severely damaging that those individuals who suffer from schizophrenia are unable to maintain relationships, employment, or maintain appropriate daily hygiene practices (Silver et al. 2003.)

The quality of life for a person suffering from schizophrenia is poor. Schizophrenia is typically diagnosed for males in their early 20s and for females in their later 20s. This means that adequate treatment is necessary for the remainder of the person’s life, which carries an enormous financial burden. These costs include drug treatment, residential accommodation, physician and other healthcare services, and loss of productivity in the work place (Rowley, 2007). Schizophrenia affects males and females equally. Ethnicities, geographic region, season of birth, and socioeconomic environment have not been useful predictors of schizophrenia. Schizophrenia, does

however, have a strong genetic link. In a study of monozygotic twins 48 percent showed a concordance with schizophrenia. These findings suggest that there is a genetic risk for schizophrenia, but that non-genetic factors, such as the environment, must play a role as well (Rowley et al. 2001).

Early History of Treatments for Schizophrenia

Effectively treating all symptoms of schizophrenia is extremely difficult; each person suffering from schizophrenia has different symptoms and therefore each treatment plan needs to be tailored to the individual. The treatments that are currently available such as psychotherapy and pharmacotherapy have limited efficacy. The symptoms that are easiest to effectively treat are delusions and paranoia. The cognitive deficits are currently poorly treated, and thus, few improvements are seen as functional outcomes in treated individuals with schizophrenia (Meltzer, 1999).

Treatment for schizophrenia has gone through several changes over the years, but before pharmacotherapy was an option for this disorder, schizophrenia was only managed through isolation of the afflicted. In the early 1800s, mental illness was thought to be a family problem. Some families actually dealt with their family member's illness by digging a hole in the ground for them inside their homes and forcing them to live and eat in their own waste. Individuals with a mental illness were hidden away from the world and were forced to live like animals (Shorter, 1997).

There was however, a more sinister way of dealing with the mental disorder. Schizophrenia has strong genetic links; therefore, it was thought that the best way to rid

society of the disorder would be to practice eugenics, essentially killing off of the diseased so they no longer impacted society. Along with eugenics, forced sterilization of affected family members was also employed to keep the disease from developing. This occurred mainly throughout Nazi Germany, the U.S. and Scandinavia (Shorter, 1997).

There were many treatments used before the discovery of antipsychotics in the 1950s. Through an accident, it was realized that after a high fever occurred, the adverse symptoms of schizophrenia were subdued. This is when medicine was first practiced to treat the disorder; the medicine was an injection of a mix of sulfur and oil that was used to cause a high fever. Along with inducing high fevers, diabetic comas were also induced to cause seizures. When a seizure occurred because of a diabetic coma, a decrease in symptoms associated with schizophrenia was also observed. These therapies ultimately were shown to be unsuccessful and were abandoned, as two newer procedures were developed: electroshock therapy and the frontal lobotomy (Shorter, 1997).

Electroconvulsive shock therapy (ECT) is the first form of treatment that was effective for treating the positive symptoms of schizophrenia. However, these improvements proved temporary and were made at a great risk to the patient. ECT was developed in 1938 by Italian Ugo Cerletti with help from Lucio Bini. His rationale for electrically inducing seizures began when he theorized that people who suffered from epilepsy had a reduced incidence of schizophrenia. Even though he was wrong in his theory, the treatment turned out to be somewhat effective. This was huge step forward, as schizophrenia had been previously unmanageable. ECT had the most use during the mid 1940s to 1950s. Despite this treatment being the first one with some limited success, there were still some major concerns. First, ECT is dangerous, particularly during its first

years of use in the 1940s when proper sedative drugs were not used and patients fractured bones through the process of the seizure. Second, the treatment was only partially effective. ECT was able to treat the positive symptoms related to schizophrenia but had no affect on the negative symptoms. The subtype of schizophrenia that most responded to ECT is catatonia. ECT is still considered a valid form of treatment for schizophrenia today but is considered a treatment of last resort (Shorter, 1997).

The next line of treatment for schizophrenia came in a most destructive form. Frontal lobotomies were developed by the Portuguese Physician Egas Moniz during the 1930s. The procedure for a lobotomy involved destroying brain tissue in the frontal lobe. A successful lobotomy left the patient emotionless, indifferent, and basically well behaved. The fact that the lobotomy left a person emotionally blunted had no effect on its popularity. The procedures for lobotomies varied, but the most famous was developed by the American neurologist Walter Freeman. He labeled his unique procedure as the frontal transorbital lobotomy, which became less technically known as the “ice pick” lobotomy. Freeman traveled the world performing lobotomies for an audience of reporters and journalists. His procedure was extremely simple involving only an ice pick-like instrument and a hammer. He placed the ice pick into an area just above the eyeball and hammered the ice pick about an inch into the frontal lobe of the brain. Although effective at inhibiting the difficult behavior produced by schizophrenia, ice pick lobotomies fell out of favor because of the inertia, lack of responsiveness, and decreased attention span seen in patients after treatment. Also, lobotomies were being performed in areas that were not sterilized by people who were not qualified practitioners and this led to fatalities caused by infection and epileptic seizures from the extensive brain damage. Between the

years of 1940 and 1950 over 40,000 lobotomies were performed and there are still individuals alive today who have had the procedure performed (Mashour et al. 2005).

The Development of Antipsychotic Medications

The development of the first line of antipsychotic drugs came accidentally in 1952. The first clear antipsychotic drug was chlorpromazine, also known as Thorazine. Phenothiazine, an agent that is currently used in various antipsychotics and antihistamines, was originally developed to be used as a pre-anesthetic drug, but it was observed that when given to a person with schizophrenia they were calmed by the drug (Meyer & Simpson 1997). Numerous drugs like chlorpromazine followed and all are collectively known as typical antipsychotics or first generation antipsychotic drugs. Although chlorpromazine was the first developed, the most widely used typical antipsychotic drug is haloperidol. Haloperidol (Haldol) is a butyrophenone that was discovered a few years after chlorpromazine in 1958 and is still widely used as a sedative and for the treatment of schizophrenia today (Adams et al. 2009).

The dopamine hypothesis of schizophrenia posits that there is a hyper-dopaminergic neurotransmission in people who have schizophrenia. In the early 1960s this hypothesis gained support when Arvid Carlsson made the important discovery that antipsychotic drugs increase dopamine turnover. The increase in dopamine turnover suggested that antipsychotics were treating people with schizophrenia by blocking dopamine receptors and prevented them from being activated (Carlsson et al. 1999). The dopamine hypothesis gained further support in 1975 when Philip Seeman discovered that typical antipsychotics were acting on dopamine D₂ receptors. Seeman wanted to find the

target area in the brain that antipsychotics were effecting. He did this by analyzing the mechanisms of the earlier antipsychotics, particularly, chlorpromazine. Seeman found that antipsychotics were acting as receptor antagonists, blocking nerve impulses that would have stimulated dopamine D2 receptors (Seeman, 2004).

Dopamine (DA) is a monoamine that is part of the catecholamine neurotransmitter system and is tightly regulated by several feedback mechanisms. DA synthesis begins through tyrosine hydroxylase and is stored in synaptic vesicles. DA is released from the presynaptic terminal following calcium influx, and is terminated by reuptake of DA into the terminal by DA transporters or by degradation by monoamine oxidases (MAOs). MAOs convert DA into dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Therefore, an increase in metabolites reflects an increase in DA release or turnover. DA receptors are all G-protein coupled receptors. D₂ and D₅ type DA receptors increase adenylate cyclase (AC) activity through group 5 type G proteins, while D₂, D₃ & D₄ type DA receptors decrease AC activity via G₁ type G proteins. D₂ receptors come in short and long isoforms and not only affect G₅ and G₁ proteins, but affect many second messenger systems as well. D₂ receptors play a role in the increase of phosphoinositol (PI) hydrolysis, regulation of phospholipase A₂, intracellular Ca²⁺ levels and K⁺ currents (Cooper, 1991). DA receptors are located presynaptically on DA neurons as well as postsynaptically. Postsynaptic DA receptors consist of all 5 DA receptor subtypes, whereas D₁, D₂, and D₃ receptor subtypes serve as autoreceptors and are found in the perikarya, dendrites, and axon terminals of DA neurons. Autoreceptor activation normalizes DA transmission by either decreasing or increasing DA release, firing rates and DA synthesis in DA neurons. DA has a higher affinity for the D₂ autoreceptor than

for other DA receptor subtypes. At low concentrations, DA receptor agonists produce decreases in DA function; while at higher concentrations, postsynaptic DA receptors are also activated and lead to an increase in DA neurotransmission (Wolf, 1987). DA synapses appear to allow for diffusion of DA into extracellular fluid and activation of DA receptors distant from the release site, known as volume neurotransmission. DA is transported from the cytoplasm of the cell to the interior of the synaptic vesicles primarily by the type 2 vesicular monoamine transporter (VMAT2). Manipulating DA can occur from the synthesis of DA to the activity at the postsynaptic DA receptors (Cooper, 1991). Approximately 80 – 90% of the cell bodies of DA neurons in the brain are found in the midbrain. The main DA pathways are DA-producing cell bodies the substantia nigra that project to regions of the basal ganglia such as the dorsal striatum and in the ventral tegmental area which project to limbic and forebrain cortical structures (Seeman, 2004).

Typical antipsychotics have the ability to treat the positive symptoms associated with schizophrenia but have limited effects on the negative symptoms (Leucht et al. 2008). Typical antipsychotics are D₂ receptor antagonists which have the ability to stop auditory hallucinations by reducing DA transmission within the basal ganglia (Owens, 1999). There is an unwanted side effect caused by typical antipsychotics as a result of reduced DA transmission within the limbic system. These adverse symptoms are known as extrapyramidal side effects (EPS) and produce a Parkinsonian-like state characterized by tremors, muscle rigidity, dystonia, and dyskinesia (Owens, 1999). The cause of EPS is due to the mechanism of action for antipsychotics. Antipsychotics are D₂ receptor antagonists and they work by lowering the activity of dopamine in the brain, particularly in the limbic system, the area of the brain believed to be responsible for the positive

symptoms associated with schizophrenia. These D₂ receptor antagonists also block dopamine in the nigrostriatal dopamine pathway, which is an important pathway for the movement system. Lowering dopamine levels in the basal ganglia causes motor disruption, resulting in EPS. EPS is so common with typical antipsychotics that it was once looked at as a property for the drug when testing animals in behavioral models. These drugs, although still in clinical use, are less often prescribed than newer ‘atypical’ antipsychotics in western countries. The low cost of typical antipsychotics makes them widely distributed among more impoverished countries (Owens, 1999).

Typical antipsychotics have several negative attributes. If prescribed a typical antipsychotic for a significant length of time, permanent motor damage may occur, resulting in a disorder called Tardive Dyskinesia. Tardive Dyskinesia is a motor disorder; features of this disorder include grimacing, tongue protrusion, lip-smacking, puckering and pursing of the lips and rapid blinking (Owens, 1999). Typical antipsychotic drugs are also known for elevating levels of prolactin within the blood stream. Symptoms of hyperprolactinemia include reduced lactation, decreased libido, and disruption of menstrual cycles, erectile dysfunction, and hypogonadism. Prolactin is a peptide that is released by the anterior pituitary gland, and its release is governed by D₂ receptor activity. Hyperprolactinemia is seen much more frequently with typical antipsychotics than ‘atypical’, or second generation, antipsychotics. Studies conducted using rats have shown increased levels of prolactin in the blood lasting up to 24 hours following treatment with a typical antipsychotic, whereas this increase is only 1-2 hours following treatment with an atypical antipsychotic drug (Gudelsky and Porter 1980; Gudelsky, 1981.)

The Role of Serotonin in the Actions of Antipsychotics

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized from the amino acid tryptophan, and the cell bodies of serotonin-producing neurons are primarily found within the medulla, pons, and midbrain, particularly within a network of brainstem nuclei called the raphe nuclei. Synthesis of serotonin involves the enzyme tryptophan hydroxylase that converts tryptophan to 5-hydroxytryptophan (5-HTP). 5-HTP is then converted to 5-HT by aromatic amino acid decarboxylase. Serotonin is transported into synaptic vesicles by the same vesicular transporter as DA, VMAT2. As with DA, serotonergic autoreceptors control the release of serotonin. Terminal autoreceptors directly inhibit 5-HT release, but other autoreceptors that lie on the cells body and dendrites indirectly inhibit the production of 5-HT by slowing down the rate of firing of serotonergic neurons. After the release of 5-HT, it is rapidly removed from the synaptic cleft by proteins on the nerve terminal called the 5-HT transporter (5-HTT). The activity of 5-HTT can be pharmacologically inhibited by (selective serotonin reuptake inhibitors, SSRIs), which elevates synaptic levels of 5-HT and is the primary mechanism of action of antidepressant drugs such as Prozac (fluoxetine) and Zoloft (sertraline hydrochloride). Serotonin is broken down by MAO to its primary metabolite 5-hydroxyindoleacetic acid (5-HIAA; Gingrich and Hen, 2001).

Atypical antipsychotics include several antagonist and inverse agonist properties that involve the serotonin, epinephrine, and histamine receptor systems. The functional roles for each receptor system are unclear, but it is plausible that serotonin may be partly

responsible for the effects of the second generation atypical antipsychotics (Seeman, 2002; Meltzer, 1999). Clozapine, the first atypical antipsychotic, has a high affinity for the 5-HT_{2A} receptor. This may account for the ability clozapine to treat patients who have been labeled treatment-resistant and may also contribute to the drugs inability to produce EPS (Ichikawa & Meltzer, 1999; Meltzer et al. 1989).

Antipsychotics were discovered in the 1950s. Clozapine, the first atypical antipsychotic drug, was developed by the Swiss pharmaceutical company Wander in 1958 (Crilly, 2007). Although the first atypical class of antipsychotics was discovered only one year after haloperidol in 1958, these antipsychotic drugs were not used to treat schizophrenia until the 1970s and 1980s. The delay was due to the drugs' inability to cause EPS-like effects in animals, which at that time was believed to be necessary for a drug to have antipsychotic properties. Within the first year of testing clozapine, scientists dismissed the drug as an antipsychotic when the drug failed to cause EPS in animal models for schizophrenia (Hippius, 1989). Years later, scientists discovered that clozapine was in fact an antipsychotic that simply did not cause EPS. This is why antipsychotics like clozapine were classified as being 'atypical' since they did not meet previously established criteria that were standard for typical antipsychotics. Clozapine was found to be very efficacious in the treatment of schizophrenia, and many subsequent atypical antipsychotics were developed based on the original findings and chemical structure of clozapine. These include olanzapine (Zyprexa) and risperidone (Risperidal), both of which are widely used today. These novel antipsychotics have become the first line of treatment for schizophrenia (Leucht et al. 1998).

Atypical antipsychotics are not only popular because of their failure to cause EPS, but also because they ameliorate some of the negative symptoms of schizophrenia, which typical antipsychotics were unable to do. The last remaining obstacle to developing an antipsychotic that reduces all major symptoms of schizophrenia is the reversal of cognitive deficits. Although atypical antipsychotics do have some cognition-enhancing properties, these effects are modest at best (Meltzer & McGurk, 1999; Woodward et al. 2005). Other important beneficial effects of atypical antipsychotics such as clozapine, olanzapine, and risperidone versus those of typical antipsychotics were that they decreased the risk of suicide and were more efficacious in treating “treatment resistant” patients’ (Barak et al. 2004; Meltzer, 2001; Meltzer et al. 2003).

Despite their improved efficacy and side effect profile, atypical antipsychotics still carry risks associated with their use. Although they reduce the occurrence of EPS, these side effects can still occur at high doses of atypical antipsychotics. Another adverse side effect is a significant increase in body weight (Kroeze et al. 2003; Wirshing et al. 1999), which can lead to an increase risk of type II diabetes (Tschoner et al. 2009). Other problems associated with the use of atypical antipsychotics are sexual dysfunction, obsessive compulsive symptoms, convulsions, and a cardiovascular effect known as the ‘QT interval prolongation,’ which is essentially a prolonging of the duration of a heart beat. This can lead to more serious problems such as torsades de pointes, the rapid polymorphic ventricular tachycardia and in some cases cardiac arrest (Stollberger et al. 2005).

Clozapine is pharmacologically different than the other atypical antipsychotics and comes with its own major adverse effects. Clozapine was responsible for several

deaths that occurred at a hospital in Finland. The Finnish Epidemic took place in the mid 1970s, where several patients died as a result of clozapine use. This epidemic led to the discovery that clozapine causes agranulocytosis which is a depletion of white blood cells within the body. Agranulocytosis occurs in only about 1 percent of patients taking clozapine and if detected early through blood monitoring is not fatal. However, after the Finnish Epidemic there was a sharp decline in the use of clozapine for obvious reasons. Patients who are currently prescribed clozapine must have weekly blood samples taken to monitor for the development of agranulocytosis (Crilly, 2007).

Since the atypical antipsychotics do not cause EPS or elevate prolactin levels, this suggests that atypical antipsychotics have a different neuropharmacological mechanism of action compared to typical antipsychotic drugs. One of the main differences between typical and atypical antipsychotics is that atypical antipsychotics have a greater affinity for the 5HT_{2A} receptor over the D₂ receptor (Meltzer et al. 1989; Meltzer, 1999). Atypical antipsychotics such as quetiapine, remoxipride, clozapine, olanzapine, sertindole, siprasidone and amisulpride all bind with lower affinity to D₂ receptors than DA and dissociate from the receptor at a faster rate. Typical antipsychotics dissociate from D₂ receptors within approximately 30 minutes of initial binding, but atypical antipsychotics dissociate from the receptor over a much shorter period of time (approximately 60 seconds; Meltzer, 1999). This faster dissociation means that atypical antipsychotics occupy D₂ receptors more transiently and allow normal DA function to resume sooner; this may be the reason why atypical antipsychotics do not elicit the motor symptoms seen with the use of typical antipsychotics. There are two main theories for how atypical antipsychotics exert their antipsychotic effects in the brain. The first is that they

dissociate from D₂ receptors at a faster rate (“fast-off theory”), which is sufficient for alleviation of the positive symptoms of schizophrenia but insufficient to cause EPS. The second theory is that the atypical antipsychotics act as antagonists at 5-HT_{2A} receptors (Seeman, 2004). In support of the 5-HT_{2A} antagonist mechanism of atypical antipsychotics, postmortem studies of people who were long-term users of atypical antipsychotics showed a loss of 5-HT_{2A} receptor density, suggesting that long-term atypical antipsychotic use reduces neurotransmission at 5-HT_{2A} receptors. Additionally, M100907, a selective 5-HT_{2A} antagonist, has been found to have potential antipsychotic actions in various animal models including blockade of amphetamine-induced locomotor activity, blockade of PCP and dizocilpine (MK-801) induced locomotor activity, blockade of MK-801 induced deficits in prepulse inhibition, and antipsychotic properties in the Paw Test (Meltzer, 1999).

It has been suggested that 5-HT_{1A} agonism, along with 5-HT_{2A} antagonism, is of importance to the atypical antipsychotic drug profile. Clozapine and other atypical antipsychotics are indeed 5-HT_{1A} agonists. Atypical antipsychotics increase DA in prefrontal cortex (PFC) and typical antipsychotics do not. The combination of D₂ and 5-HT receptor blockade is crucial for explaining how atypical antipsychotics are able to treat the negative symptoms and cognitive deficits of schizophrenia. For example, the combined administration of the D₂/D₃ antagonist raclopride and the 5-HT_{2A} receptor antagonist M100907 was effective in reducing the conditioned avoidance response, demonstrating that both 5-HT_{2A} and D₂ antagonism is necessary for the efficacy of atypical antipsychotics (Wadenberg et al. 2000).

The theory that the 5-HT_{1A} receptor is an essential mediator for an increase in dopamine release is supported in various studies (Ahlenius, 1989). Co-administration of the 5-HT_{2A} receptor agonist 8-OH-DPAT with the D₂/D₃ receptor antagonist raclopride was observed to have a potentiating effect in the suppression of the conditioned avoidance response, a behavioral task for the testing of antipsychotic properties (Wadenberg & Ahlenius, 1991). Buspirone, an anxiolytic and 5-HT_{1A} partial receptor agonist was shown to decrease amphetamine-induced stereotypies in rats and hyperlocomotion in mice (Gustafsson & Christensson, 1990). Drugs that are 5-HT_{1A} agonists were shown to inhibit the appearance of catalepsy in rodents following administration of the typical antipsychotic haloperidol (Wadenberg, 1996). Haloperidol-induced catalepsy is a common animal model for the testing of EPS. The 5-HT_{1A} agonist 8-OH-DPAT (0.05 mg/kg s.c; 8-hydroxy-N,N-dipropyl-2-aminotetralin) was shown to inhibit the increases in DA release in the nucleus accumbens and the striatum induced by amperozide (10 mg/kg), clozapine (20 mg/kg) and risperidone (0.01 and 0.03 mg/kg). This inhibition was reversed by the 5-HT_{1A} antagonist WAY100635 (0.02 mg/kg; N-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-N-pyridin-2-ylcyclohexanecarboxamide; Ichikawa et al. 1995). There are also several microdialysis studies that support the importance of the 5-HT_{1A} receptor in effective antipsychotic treatments. Clozapine, a moderately potent 5-HT_{1A} receptor agonist, was found to significantly increase DA release in the PFC. Pretreatment with WAY100635 0.1mg/kg attenuated clozapine's effects and WAY100635 alone had no effects on DA release in the PFC. WAY100635 was also found to antagonize the effects of 8-OH-DPAT. These data add significant support that clozapine's effects are related to 5-HT_{1A} mechanisms (Rollema et al. 1997).

Aripiprazole, a novel atypical antipsychotic, increased DA in the PFC in a microdialysis study. The aripiprazole induced DA increases in the PFC were inhibited by WAY100635, lending more support to the importance of the 5-HT_{1A} receptor (Li et al. 2004). Also found through microdialysis was that atypical antipsychotics, quetiapine, iloperidone, and melperone, which are 5-HT_{2A}/D₂ antagonists, also increase DA release in the PFC and these effects are also attenuated by WAY100635. This study showed that quetiapine, iloperidone and melperone are also dependent upon the 5-HT_{1A} receptor in order to produce an increase in DA release in the PFC (Ichikawa et al. 2002).

It has been suggested that the cognitive deficits associated with schizophrenia are due to a decrease of frontal dopamine neurotransmission (Meltzer & McGurk, 1999). The decrease in frontal cortical DA release can be further induced by typical antipsychotics because of their blockade of cortical D₂ receptors. Atypical antipsychotics are able to increase cortical dopamine activity, and it has been suggested that this occurs because of the drug's tendency to block 5-HT_{2A} receptors (Meltzer et al. 2001). Support for this hypothesis has been shown through the use of positron emission tomography (PET) imaging during a verbal fluency task. Participants with schizophrenia had abnormal patterns of left temporal lobe and anterior cingulate activation which was normalized with the use of the drug apomorphine, a drug that acts as a nonspecific dopamine agonist. Apomorphine was given at a dose that had the possibility to actually decrease dopamine because of its ability to stimulate dopamine autoreceptors that regulate dopamine synthesis and release. Significant improvement in performance on the verbal fluency task was observed following administration of clozapine, which was accompanied by an increase in dopamine release in the PFC (Honey et al. 1999). Meltzer has shown that all

atypical antipsychotics increase PFC dopamine neurotransmission within the rat and monkey brain. The ability of atypical antipsychotic drugs to increase dopamine transmission within the PFC is most likely due to the various agonist, partial agonist, and antagonist actions of the drugs on DA and 5-HT receptors (Meltzer, 1999).

Neurotensin as a Novel Target for Antipsychotic Medications

Research has recently focused on other drug targets that could be used in addition to atypical antipsychotics to increase the patient's cognitive ability. Neurotensin is an endogenous neuropeptide that interacts with cholinergic pathways in the central nervous system (Azmi et al. 2006). Neurotensin analogs appear to have antipsychotic properties and possible cognitive enhancing effects. Neurotensin was first isolated in 1973 from bovine hypothalamus by Caraway and Leeman. In 1988, the rat neurotensin gene was isolated and sequenced. Three receptors for neurotensin are contained in the central nervous system (CNS). Neurotensin has a low affinity for neurotensin receptor 1 and also binds to the histamine H1 receptor antagonist levocabastine. Neurotensin has a high affinity for neurotensin receptor 2 but is insensitive to levocabastine. It is currently unclear if neurotensin is an agonist or antagonist at the neurotensin 2 receptor. There is also a third receptor for neurotensin, but this protein has not been adequately characterized (Cusack et al. 1995).

It has been observed that patients with schizophrenia have decreased concentrations of neurotensin in their cerebrospinal fluid (Widerlove et al. 1982; Lindstrom et al. 1988; Nemeroff et al. 1989). Human postmortem studies also support an

alteration of neurotensin neurotransmission in people who have schizophrenia (Kilts, 2001). Neurotensin analogs have the ability to increase neurotensin signaling in the brain which in turn increases dopamine release in the PFC, a region that is hypodopaminergic in people with schizophrenia. Theoretically, deficits in DA transmission in the PFC may be a primary neuroanatomical locus that results in cognitive impairments in schizophrenia. Several animal studies give support to the 'neurotensin model' of schizophrenia; this model hypothesizes that schizophrenic patients have reduced neurotensin, and that increasing neurotensin signaling may be a mechanism of action of antipsychotic drugs. Neurotensin may affect dopamine release in the prefrontal cortex by interacting with mesocortical dopamine neurons. Neurotensin 1 is found all over in the ventral tegmental area (VTA) on dopamine cell bodies and terminals (Dana et al. 1989; Fassio et al. 2000). Activation of neurotensin may cause cell depolarization and in turn cause an increase in spontaneous activity of mesocortical dopamine neurons, which then increases dopamine and neurotensin in the prefrontal cortex (Mercuri et al. 1983; Nalivaiko et al. 1998; Wu & Wang, 1995). Stimulation of the post synaptic dopamine one and neurotensin one and two receptors may cause a positive feedback loop to the VTA. This causes an increase in glutamate which results in a longer activation of dopamine (Sotty et al. 2000). Support for the neurotensin model is demonstrated in several studies. For example, administration of neurotensin in rodents produces behavioral, psychological, and biochemical effects similar to those produced by the administration of antipsychotics (Nemeroff, 1980). Luttinger et al. (1982) found that administration of neurotensin decreases active avoidance behavior in the conditioned avoidance response paradigm. An added study implementing the prepulse inhibition (PPI) model with drug

induced deficits (i.e., PCP or amphetamine) showed a reversal effect using a neurotensin antagonist. Rodents were pretreated with the neurotensin antagonist SR 1492948A, which antagonized or reversed the effects of haloperidol-induced restoration of PPI in isolation-reared rats (Binder et al, 2001). These findings bolster the role for neurotensin neurotransmission in sensory inhibition functions caused by schizophrenia and in the effects of antipsychotic drugs on deficits in sensory inhibition. An additional PPI study using neurotensin infusions into the nucleus accumbens demonstrated a blockade of amphetamine-induced disruption in a dose-dependent manner (Feifel, 1997). A locomotor activity experiment yielded positive results by reversal of amphetamine-induced hyperlocomotion by a 5.0 ug i.c.v. dose of neurotensin. Also, neurotensin had no effect on baseline locomotor activity (Feifel, 1997). Effects of electrical stimulation of the medial forebrain on the in vivo release of dopamine and neurotensin in the PFC showed that both can be increased through increasing the stimulation frequency while holding the number of impulses constant. The blockade of PFC dopamine autoreceptors produces an increase in dopamine release and a decrease in neurotensin release. These effects could be related to the therapeutic effects of antipsychotic drugs. This study suggests that dopamine and neurotensin are differentially released based on stimulation frequency and dopamine autoreceptor activation within a frequency range in which cells normally operate (Bean, 1991).

A microdialysis study has shown evidence that administration of a neurotensin analog, NT69L ((2S)-2-[[[(2S)-2-[[[(2S)-2-[[[(2S)-1-[(2S)-6-amino-2-[[[(2S)-5-(diaminomethylideneamino)-2-(methylamino)pentanoyl]amino]hexanoyl]pyrrolidine-2-carbonyl]amino]-3-(1H-indol-7-yl)propanoyl]amino]-3,3-dimethylbutanoyl]amino]-4-

methylpentanoic acid) significantly increased dopamine neurotransmission in the PFC. Pretreatment with the 5-HT_{1A} antagonist WAY100635 attenuated NT69L-induced increases in PFC dopamine release. These results suggest that the 5-HT_{1A} receptor is an important mediator of the regulation of dopamine within the PFC by neurotensin. When the effective dose of NT69L was given in combination with the typical antipsychotic haloperidol, there was no increase in PFC dopamine release. The lack of effect of haloperidol and positive effect of NT69L gives further support to the notion that the 5-HT_{1A} receptor is crucial for the increase of dopamine within the PFC induced by atypical antipsychotics. This new evidence for the role of neurotensin signaling in regulation of PFC dopamine release suggests that the neurotensin system could be targeted for improving the efficacy of atypical antipsychotics (Prus et al. 2007).

Another neurotensin analog that has had effective results in the increase of dopamine within the PFC is PD149163. PD149163 selectively binds to the neurotensin 1 receptor (Petrie, 2004). After several pre-clinical animal studies, it has been suggested that this drug could have antipsychotic-like efficacy (Feifel et al. 2008). Acute systemic administration of PD149163 has been shown to activate both interneurons and pyramidal cells in the PFC adding more support for the drug's ability to increase dopamine within the PFC (Petrie, 2004). Also of importance to the potential for PD149163 to be a novel atypical antipsychotic is the fact that it does not produce evidence of tolerance after repeated administration. Repeated systemic administration of PD149163 failed to show evidence of tolerance to the ability of this compound to suppress amphetamine-induced hyperlocomotion, supporting the notion that PD149163 or similarly acting neurotensin ligands may be useful as novel antipsychotics (Feifel, 2008).

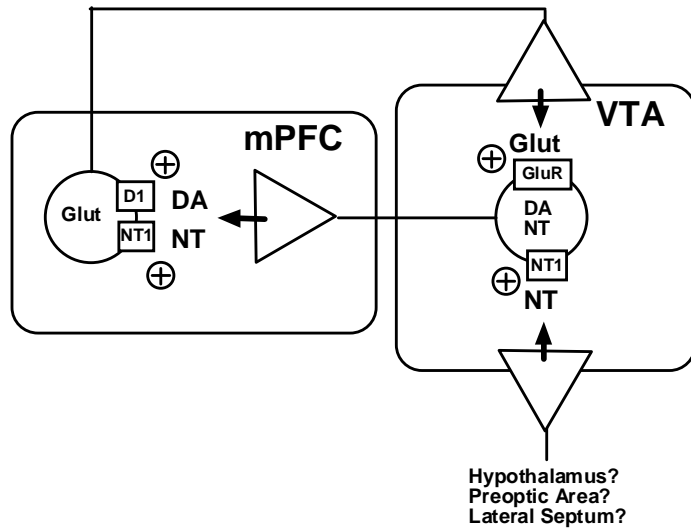


Figure 1. Interconnectivity between ventral tegmental area (VTA) and the medial prefrontal cortex (mPFC).

Figure used with permission from Dr. Adam Prus

RATIONALE

This study sought to further evaluate the role of 5-HT_{1A} receptors in the ability of neurotensin analogs to increase medial prefrontal cortical dopamine release using *in vivo* microdialysis in rats. First, the effects of the neurotensin analog PD149163 on medial prefrontal cortical dopamine release was evaluated. After PD149163 was found to have statistically significant increases in medial prefrontal cortical dopamine levels, the 5-HT_{1A} receptor antagonist WAY100635 was administered prior to PD149163 to show that PD149163-induced increases in medial prefrontal cortical dopamine release are attenuated by 5-HT_{1A} receptor blockade. Lastly, the 5-HT_{1A} receptor agonist 8-OH-DPAT was administered prior to PD149163 to determine that PD149163-induced increases in medial prefrontal cortical dopamine release can be potentiated by 5-HT_{1A} receptor activation.

METHODS

Subjects

Subjects were male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) weighing 250-300 grams. They were housed three per cage prior to surgery and housed singly after surgery at Northern Michigan University. The colony where animals were housed was held at a consistent temperature of 22 degrees Celsius and was also held on a consistent 12 hour light/dark cycle (7am/7pm). Rats had free access to food and water at all times. Animals were maintained in accordance with the guidelines described in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and all protocols and facilities were approved by the Institutional Animal Care and Use Committee at Northern Michigan University.

Drugs

The neurotensin agonist PD149163 was provided by the National Institute of Mental Health Drug Repository, Bethesda, MD, and WAY100635 Maleate Salt (5-HT_{1A} antagonist) was purchased from Sigma Aldrich, St. Louis, MO. (±)-8-Hydroxy-2-(di-n-propylamino) tetralin hydro-bromide was also purchased from Sigma Aldrich. PD149163, WAY100635 and 8-OH-DPAT were dissolved in distilled water. Doses for WAY100635 and 8-OH-DPAT were chosen based on previous literature (Prus et. al,

2007; Ichikawa, 2001). Doses for PD149163 were determined from previous unpublished studies in this laboratory. All drugs were administered through a subcutaneous catheter.

Biochemical assays

Dialysate samples were of 45 uL volume and were divided in half to detect DA and 5HT using an HPLC system. Dialysate samples were applied onto a high-performance liquid chromatography (HPLC) with electrochemical detection and analyzed with a Millennium chromatogram manager (Waters Corporation, Milford, MA USA). Dopamine was separated using a reverse phase column (Atlantis dC18 3 um, 1.0 x 100 mm; Waters Corporation). The mobile phase consisted of 48 mM anhydrous citric acid and 24mM sodium acetate trihydrate containing 0.5 mM EDTA-NA2, 10 mM NaCl, 2 mM dodecyl sulfate sodium salt, and 17% (v/v) acetonitrile, adjusted to pH 4.8 with concentrated NaOH, and was pumped at 0.05 mL/min (LC-10AD, Shimadzu, Kyoto, Japan).

Surgery

Prior to stereotaxic surgery for guide cannula implantation, rats were anesthetized with an equithesin mixture (810 mg pentobarbital, 4.3 g choral hydrate, 2.12 mg of MgSO₄, 14 mL ethanol, and 29 mL propylene glycol and brought to a final volume of 100 mL with sterilized H₂O). After deep anesthesia was attained, rats were mounted in a stereotaxic frame (Stoelting, Wood dale, IL, USA.) Two stainless guide cannulas (21-gauge) with dummy probes were placed and fixed by cranioplastic cement (Lang Dental

Manufacturing Co. Incorporated, Wheeling, IL) onto the cortex dorsal to the right mPFC . Stereotaxic coordinates of each probe, when implanted, were anterior +3.2, later +1.5, and vertical – 1.6 mm relative to bregma. The incisor bar level was set at 3.0 mm. The coordinates were obtained from a stereotaxic atlas (Paxinos & Watson, 1998).

Microdialysis

The microdialysis probes, (Biomed Precision Apparatus Co., Mianyang, China) were implanted into the mPFC 3-5 days after cannulation of surgery, while rats were anesthetized with isoflurane (Phoenix Pharmaceutical Inc, St. Joseph, MO). After probes were inserted, a subcutaneous catheter was implanted into the intrascapular space of the rats. The subcutaneous catheter consisted microbore Tygon tubing (TGY-010, 0.03 um o.d., 0.5 mm i.d.; Bioanalytical Systems Inc., West Lafayette, Indiana). Rats were then individually housed overnight in a dialysis cage. Following overnight perfusion of 0.5 uL/min of Dulbecco's phosphate-buffered saline solution (Sigma Aldrich, St. Louis, MO), the flow rate was raised to 1.5 uL/min and dialysate samples were collected every 30 minutes. After stable baseline dopamine values in the dialysates were obtained, drug and vehicle were administered through the subcutaneous catheter. Injection one was given at the 0 minute mark and injection two was administered at the 30 minute mark, following a standard pre-treatment treatment design (Ichikawa et al. 2001). Doses of drug and time of injections are shown on table 1. Each dose of drug was tested in 6-8 rats. The location of each microdialysis probe was verified at the end of each experiment by brain dissection.

Table 1: Treatment conditions

Treatment	Doses	
	Injection Time	
	0	30
PD149163	0.1 mg/kg	N/A
	1.0 mg/kg	N/A
WAY100635 0.2 mg/kg + PD149163 1.0mg/kg	WAY100635 VEH	PD149163 VEH
	WAY100635 0.2 mg/kg	PD149163 VEH
	WAY100635 VEH	PD149163 1.0 mg/kg
	WAY100635 0.2 mg/kg	PD149163 1.0 mg/kg
(+) -8-OH-DPAT 0.05 mg/kg + PD149163 0.1 mg/kg	8-OH-DPAT VEH	PD149163 VEH
	8-OH-DPAT VEH	PD149163 0.1 mg/kg
	8-OH-DPAT 0.05 mg/kg	PD149163 VEH
	8-OH-DPAT 0.05 mg/kg	PD149163 0.1 mg/kg

DATA ANALYSES

This study was a between-subjects design with two independent variables, one being the type of drug given (PD149163, WAY100636 or 8-OH-DPAT), the second being time, starting with -60 minutes to 120 minutes at 30 minute intervals. The percentage of baseline dopamine output was the dependent variable. A two-way mixed design analysis of variance was used to analyze data. The Bonferroni test was used to test interaction comparisons and to determine group differences. A probability of $p < 0.05$ was considered significant in this study. All results are given as means \pm S.E.M. All analyses were performed using GraphPad Prism version 5.01 for Windows (Graphpad Software, San Diego, CA).

RESULTS

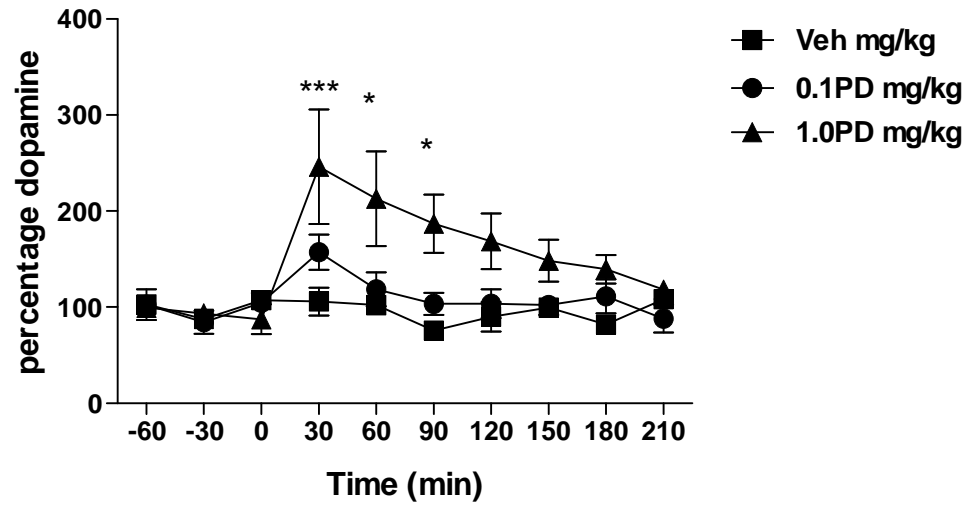
PD149163 significantly increased DA release in the medial prefrontal cortex (mPFC), (treatment, $F(2, 144) = 4.14, P < 0.05$; time, $F(9, 144) = 4.14, P < 0.0001$; interaction, $F(18, 144) = 2.22, P < 0.01$). The 1.0 mg/kg dose of PD149163 was found to have significantly greater effects on mPFC DA release compared to 0.1 mg/kg and vehicle. The 1.0 mg/kg dose of PD149163 produced a significantly greater increase in mPFC DA levels compared to vehicle at 30 minutes after injection until 90 minutes after injection. The 0.1 mg/kg dose of PD149163 produced significantly less DA release than the 1.0 mg/kg of PD149163 60 minutes post injection. PD149163 had increases in mPFC DA release at 0 vs. 30 and 60 minutes.

WAY100635 significantly attenuated the PD149163 induced increase of DA in the mPFC, (treatment, $F(3, 198) = 8.70, P < 0.01$; time, $F(9, 198) = 9.05, P < 0.0001$; interaction, $F(27, 198) = 2.79, P < 0.0001$). WAY100635 0.2 mg/kg was found to significantly attenuate the induced increases of DA in the mPFC by PD149163. WAY100635 0.2 mg/kg in combination with vehicle compared to vehicle in combination with vehicle were greater at 150 minutes post injection. Vehicle in combination with vehicle compared to vehicle in combination with 1.0 mg/kg PD149163 was significantly greater at 60 minutes post injection to 120 minutes post injection, and then again at 180 minutes post injection. WAY100635 0.2 mg/kg in combination with vehicle compared to vehicle in combination with 1.0 mg/kg PD149163 were significantly lower 60 minutes post injection to 210 minutes post injection. WAY100635 0.2 mg/kg in combination with

1.0 mg/kg PD149163 compared to vehicle in combination with 1.0 mg/kg PD149163 was significantly lower at 30 minutes post injection and again at 90 minutes post to 150 minutes post injection. WAY100635 in combination with PD149163 had decreases at 0 vs. 60 minutes to 210 minutes.

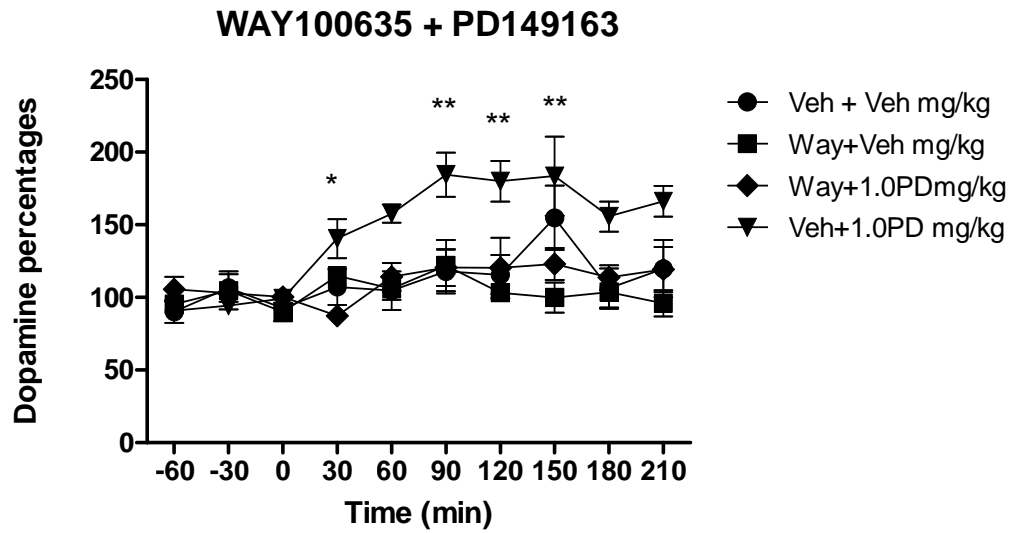
Dose combinations of 0.05 mg/kg of 8-OH-DPAT and 0.1 mg/kg PD149163 had no significant effect on DA release in the mPFC, treatment, $F(3,171) = 2.12, P > 0.05$; time, $F(9,171) = 3.69, P < 0.0001$; interaction, $F(27, 171) = 1.83, P < 0.05$. Vehicle and vehicle compared to 0.1 mg/kg PD149163 and 0.05 mg/kg 8-OH-DPAT increases at 60 and 90 minutes post injection. 8-OH-DPAT in combination with PD149163 showed increases in mPFC DA release at 0 vs. 60, 90, and 120 minutes.

Effects of PD149163 on dopamine release in the prefrontal cortex



Vehicle = Distilled H₂O
* = Significance at 0.05
** = Significance at 0.01
*** = Significance at 0.001

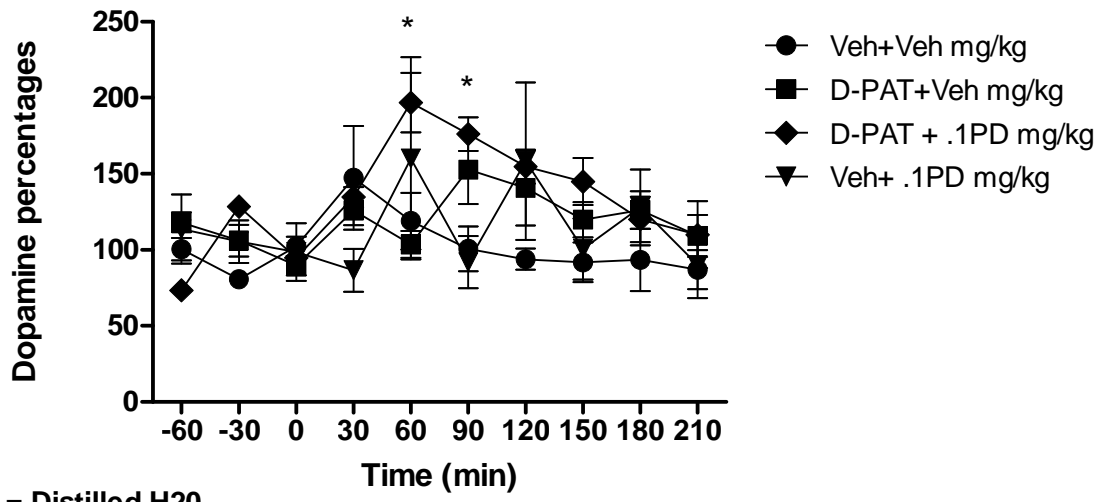
Figure 2. Effects of PD149163 on the release of dopamine in rats



Vehicle = Distilled H₂O
 * = Significance at 0.05
 ** = Significance at 0.01
 *** = Significance at 0.001

Figure 3. Effects of WAY100635 in combination with PD149163 on the release of dopamine in rats

8-OH-DPAT in combination with PD149163



Vehicle = Distilled H₂O

* = Significance at 0.05

** = Significance at 0.01

*** = Significance at 0.001

Figure 4. Effects of 8-OH-DPAT in combination with PD149163 on the release of dopamine in rats

DISCUSSION

This microdialysis study demonstrated that the neurotensin analog PD149163 can increase DA release in the mPFC in rats. The 1.0 mg/kg dose of PD149163 effectively increased the level of DA within the PFC. These effects were attenuated by the addition of the 5-HT_{1A} antagonist WAY100635, adding substantial support that the 5-HT_{1A} receptor is important for mediating the effects of PD149163's ability to cause a DA efflux in the mPFC. The 0.1 mg/kg dose of PD149163 had no significant effect upon DA neurotransmission in the mPFC and when paired with 8-OH-DPAT at a 0.05 mg/kg dose, there was a significant increased difference compared to vehicle at 60 and 90 minutes post injection; however, this difference was not significant as compared to 1.0 mg/kg PD149163 or 8-OH-DPAT alone.

PD149163 increased dopamine release in the mPFC significantly at the 1.0 mg/kg dose. Dopamine percentages were increased to 250% as compared to vehicle alone (at 100%), and remained above 200% until 90 minutes post PD149163 injection. This showed that PD149163 was able to significantly increase DA in the mPFC. PD149163 compared to NT69L, a neurotensin analog, showed a larger increase in dopamine percentage at the 1.0 mg/kg dose ; NT69L dopamine percentages were below 150% at 1.0 mg/kg dose, but at the 3.0 mg/kg produced an increase in dopamine percentage of above or at 150% at the 60 minute post injection mark till the 90 minutes post injection mark when dopamine percentages increased to over 200% at the 120 minute mark and remained above 150% until 210 minutes post injection (Prus et al. 2007). The fact that

both of the neurotensin analogs, PD149163 and NT69L, have the ability to increase efflux in the mPFC, suggests that they produce affects similar to those of atypical antipsychotic drugs. As described in the introduction, neurotensin is a peptide within the CNS that is able to increase DA in the mPFC (Cusak et al. 1995). PD149163 compared to atypical antipsychotic olanzapine given at a 1.0 mg/kg dose showed a similar result of a DA increase of 150% at 60 minutes post injection, moving up to 200% at the 90 minute mark and staying there until the 150 minute mark. DA percentages for olanzapine remained above 150% until the end of the study at 210 minutes post injection (Ichikawa et al, 2001). Clozapine, given at 2.0 mg/kg dose produced a dopamine increase 250% at the 60 minute mark post injection and increased to 400% at the 90 minute mark and continued to increase to 450% at 120 to 150 minutes post injection. The DA levels remained above 350% until the end of the study at 210 minutes post injection (Ichikawa et al. 2001). This comparison adds support to the idea that PD149163 may function as a novel atypical antipsychotic. Typical antipsychotics compared to PD149263 in the present study showed an increase in DA, produced by 0.1 mg/kg of haloperidol, of just below 200% at the 60 minute post injection mark and decreased to just above 150% at the 90 minute mark and continued to decrease to below 150% but above 100% until the end of the study at 210 minutes post injection (Liegeois et al. 2002). Typical antipsychotics do not produce the DA efflux that is seen with PD149163, adding further support for the use of PD149163 as a novel atypical antipsychotic.

WAY100635 0.2 mg/kg in combination with PD149163 1.0 mg/kg produced DA percentages of 100% at the 30 minute mark post injection which remained below 150% until the end of the study at 210 minutes post injection, which was comparable to vehicle

that produced similar changes in DA percentages. This demonstrated that the 5-HT_{1A} receptor plays an important role for DA increase in the mPFC. WAY100635 0.2 mg/kg in combination with NT69L produced DA percentage values that stayed below 150% from 30 minutes post injection to the end of the study at 210 minutes post injection (Prus et al. 2007), suggesting that neurotensin analogs are dependent upon the 5-HT_{1A} receptor to produce an increase in DA in the mPFC. WAY100635 at 0.2 mg/kg in combination with 1.0 mg/kg olanzapine produced DA percentages of just above 150% 60 minutes post injection and decreased to 150% until the end of the study at 210 minutes post injection. WAY100635 0.2 mg/kg in combination with 20 mg/kg of clozapine produced DA percentages of 200% at 60 minutes post injection and 250% DA until 150 minutes post injection, thereafter decreasing below 200% until the end of the study at 210 minutes post injection (Ichikawa et al. 2001). This showed that the 5-HT_{1A} receptor is an important mechanism for both PD149163 and other atypical antipsychotics to produce an increase in DA in the mPFC, and further supports the suggestion that PD149163 may be a novel atypical antipsychotic.

8-OH-DPAT, a 5-HT_{1A} agonist, given at a dose of 0.05 mg/kg in combination with PD149163 yielded negative findings. DA percentages of this combination produced DA increases of less than 200% from 60 minutes post injection until the end of the study at 210 minutes post injection. Previous studies have shown that it is possible to combine 8-OH-DPAT with an atypical antipsychotic and gain increases in PFC DA release. Ichickawa et al. (1999) found that when 8-OH-DPAT 0.05mg/kg was paired with sulpiride 10 mg/kg, an atypical antipsychotic, there were increases of DA release 30 minutes post injection that reached a percentage of 175% and peaked at 200% at 60

minutes post injection and when sulpiride had been given alone DA percentages remained comparable to vehicle levels never reaching above 150%. Also, adding even more support to the plausibility of this concept, WAY100635 completely attenuated the combination's increase in DA release. 8-OH-DPAT has been shown to reverse haloperidol induced EPS in cebus monkeys, and was comparable to apomorphine. This showed also that the 5-HT_{1A} receptor has an important role in the mechanisms of atypical antipsychotics (Christoffersen et al. 1997). Behaviorally 8-OH-DPAT has shown success in combination with raclopride in the conditioned avoidance response task. Raclopride by itself showed no suppression in the task but when given in combination with 8-OH-DPAT did suppress avoidance behavior (Wadenberg et al. 1990). Our negative findings could be due to a number of variables including a lack of thorough dose investigation. The only dose tested in the present study was 0.05 mg/kg, and although this is a standard dose, our negative findings could be due to too low of a dose. Also, 8-OH-DPAT is only a 5-HT_{1A} partial agonist; PD149163 could cause possible positive findings if paired with a full 5-HT_{1A} agonist. Finally, studies testing combinations of 8-OH-DPAT and atypical antipsychotics' are not so prevalent. Further investigation of 8-OH-DPAT at different doses and in different drug combinations is necessary to fully explain these results.

PD149163 has potential as a possible addition to the current treatments of schizophrenia. Atypical antipsychotics can only modestly treat the cognitive deficits associated with schizophrenia at this time and PD149163 could possibly have the effects of a cognitive enhancer. A sub-chronic PCP-induced deficit in the novel object recognition task was found to be reversible by clozapine but not by haloperidol (Grayson et al. 2007). The affects of PD14963 on the DA neurotransmission system is similar to

that of atypical antipsychotics. PD149163 showed positive effects as a cognitive enhancer in a novel object recognition task that involved an induced memory deficit by scopolamine. This deficit was reversed by PD149163 at a 3 µg dose injected through the intracerebroventricular route (Azmi et al. 2006).

This evidence, along with previous studies involving NT69L strongly suggests that PD149163 could serve as a possible novel atypical antipsychotic. However, it is obvious that the mechanisms need to be clearly defined in order to effectively utilize the drugs influence on DA neurotransmission. Additional testing of combinations involving PD149163 and series of 5-HT antagonists and agonists at different doses is necessary. In the future it would be wise to test PD149163 paired with drugs that act on the 5-HT_{2A} receptor as antagonists and agonists to further elucidate how PD149163 works.

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APPENDIX A

This is the approval page from the Northern Michigan University Institutional Animal Care and Use Committee granting permission to conduct this study.




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Continuing Education & Sponsored Programs
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Marquette, MI 49855-5325

MEMORANDUM

December 10, 2007

TO: Dr. Adam Prus
Department of Psychology

FROM: Cynthia A. Prosen, Ph.D. 
Dean of Graduate Studies & Research

RE: **Application to use Vertebrate Animals**
Application # IACUC 091
Approval Period: 01/1/2008-12/31/2011

The Institutional Animal Care and Use Committee have approved your application to use vertebrate animals in research, "Neutotensin analogs for neurocognitive deficits in schizophrenia".

If you have any questions, please contact me.

kjm