PROTEIN KINASE A AND THE FORCED SWIM TEST: A STRAIN COMPARISON

by
Nancy Rogacki

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

2006

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

This manuscript has been read and accepted for the
Graduate Faculty in Psychology in satisfaction of the
dissertation requirement for the degree of Doctor of Philosophy.

_____	_____
Date	Gordon Barr, Ph.D., Chair of Examining Committee
_____	_____
Date	Joseph Glick, Ph.D., Executive Officer

Errol de Souza, Ph.D.

Sathapana Kongsamut, Ph.D.

Victoria Luine, Ph.D.

Vanya Quinones-Jenab, Ph.D.

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK

Abstract

PROTEIN KINASE A AND THE FORCED SWIM TEST:
A STRAIN COMPARISON

by
Nancy Rogacki

Advisor: Professor Gordon A. Barr

The second messenger cyclic AMP (cAMP) has been long documented as affected by antidepressant (AD) treatment. AD drugs typically take days to weeks to produce clinical efficacy. Although cAMP levels may be elevated by short-term AD administration, chronic AD's may be necessary to affect all of the components of the cAMP cascade, including the second messenger's receptor, protein kinase A (PKA). The first report to examine PKA binding in the human depressive brain was not published until 1997 (Lowther, Katona, Crompton, & Horton). PKA density was unaltered as demonstrated via saturation binding in five brain regions in depressive suicides, yet decreased in those currently taking AD drugs. No published account regarding a controlled study of the impact of chronic AD treatment on PKA density exists. Strain differences may exist for AD effects on biochemistry, as well as behavior. Two strains, one "normal" (Sprague-Dawley, SD) and one exhibiting depressive characteristics (Wistar Kyoto, WKY) were tested for changes in both measures as a result of subchronic and chronic AD treatment. These two strains were known previously to differ in their responses to the forced swim test (FST), an animal model of depression that detects AD activity with a high correlation to clinical efficacy. Firstly, the pharmacological profile of a single PKA receptor in two fractions, the cytosolic and particulate, of the frontal cortex was determined. Secondly, strain differences in PKA density and FST response to the clinically utilized AD's desipramine and bupropion were assessed. Alprazolam did not demonstrate an AD-like effect in the FST. The two rat strains differed in their control levels of FST behavior and PKA density, but not affinities. Only the SD strain showed PKA density changes at a low AD dose and only in the particulate fraction.The PKA density changes in both strains in the cytosolic fraction occurred only at the high dose of an AD, perhaps reflecting transcriptional processes. No consistent correlation between biochemistry and behavior was found. Both how the FST measures AD activity with clinical drug effect concordance and if AD effect can be fingerprinted via changes in PKA density remain unsolved.

Key Words: Protein Kinase A, Forced Swim Test, Antidepressant, Depression, Wistar Kyoto, Sprague-Dawley, B_{max}, K_D

Acknowledgements

First and above all, I wish to thank my mother Lucille Rogacki to whom I dedicate this work. I lost her just months before the completion of this degree. I could not have had a better friend, who selflessly did everything for me and went out of her way to be sure I could live out my goals. From my early childhood, she always supported my interest in nature and told me she always knew I would become a scientist. Her generosity toward me on every level will never be forgotten. It is she who helped me see who God really is. She always prayed for me through all of these difficulties. I will not forget her sense of humor and "Luisms". In my life no one will ever or could ever be as crucial. I will wait every day to see you again, LouLou.

To my sister Karen Rusin and my brother Frank Rusin, I thank you both for being dear friends who I can always rely on. And in whose company I always have fun times. Both of you were always willing to listen to my issues regarding this Neverending Story. I thank Karen, as my elder, for having been a role model. You are a great example that the definition of "woman" does not inherently equal "married with children". Thanks to my long-deceased father Anthony Rogacki for instilling in me respect for education and his financial support.

Thanks to Sam Kongsamut for being my advisor of this research at my place of employment. Thanks for getting me to do a great deal more of the pharmacological characterization than I'm sure I would have done. And the "boiling pot" work that clearly sent a message to me at the time as I kept opening *The Bible* to references on boiling pots throughout that difficult time.

Also, my thanks to the committee members at Hunter: Profs. Barr, Luine and Quinones-Jenab. Also, Dr. Errol de Souza who traveled far to attend my defense.

Sincerest thanks to Prof. Phil Zeigler for listening to me and supporting me when I transferred from that Other School. In addition, warm thanks to Prof. Peter Moller for continuing that support. I will be forever indebted to Hunter College for allowing me the tremendous opportunity to complete this degree that will forever change the course of my career.

Thanks to the management of Hoechst Marion Roussel/Aventis Pharmaceuticals for the payment of the supplies for this work that was unrelated to company business and use of the facilities and equipment off hours to conduct this research.

Thanks to the understanding and support of those who finished their Ph.D.'s at HMR/Aventis before me: Drs. Susan Hanak, Fredderike Wirtz-Burger, and my dear friend, Ann Marie Szczepanik. You were all a tremendous help to the maintainence of my personal sanity via the commonality of our experiences. Thanks to my friend Craig P. Smith who these past several years at my job has been a guiding exemplar of the true definition of Scientist.

Thanks to those people in Sam Kongsamut's and Buzzy Morse's groups who looked out for me, offered me their work spaces and supplies, and treated me warmly when I was working in their department to conduct the biochemical assays. Specifically, I would like to thank Dr. Bruce Harris, Margaret Kenney, Henry de Fex, Fernando Camacho and Elaine Powers (my "landlady" who tolerated my obnoxious use of her freezer space). Thanks to Jim Roehr for fielding questions regarding the binding-related equipment and its theory. Special thanks to Hal Hartman who very much went out of his way to get me set up over there when I first got started and also for his numerous discussions with me regarding the history of depression at the predecessor companies.

Absolute love and thanks to a number of tremendous friends, some of which have walked this very long and convoluted road with me. My mother always told me: Put all of your friends in a strainer and you are lucky to find one left. I have had the great privilege to have many more than that left in my strainer. I want to give a very special thanks to a few who were outstanding. Cynthia Morgan, you have stood behind me these almost 20 years since I was at that Other School (biggest mistake of my life). I still have until this day the card that you had sent to me that states: "I Believe in You". I believe in you, too. I appreciate the friendship, support and prayers always. Also my friend Vincent Jorgensen with his repeated advice: Keep Your Eye on the Prize. Thanks to Ruby Sura for standing behind me when I went through everything at that Other School and helping to get me going again at that time.

My friend at sanofi-aventis, Melinda Cilio, is unparalleled. I am eternally grateful for you.

Thanks to the kind statistical help/advice from Dr. S.T. Hardiman. It was a revelation to find a statistician that truly understood preclinical work.

Grateful thanks to Pastor Vance and the people of North Plainfield Baptist Church. Their prayers and friendship sustained me, and also my family, through some very difficult times. Special thanks to Tom Harrity and Paul Smith, who as scientists in the congregation were particularly understanding.

May I never forget the people who have depression and schizophrenia and the real reason why we are doing what we do. I pray for a true and fruitful focus for the rest of my career and that these efforts go someday, somehow towards a way to get people to look at depression differently---and make some real progress for a change rather than continue beating the monoamine hypothesis into the ground.

Finally, thanks to my Lord. Each time a door slammed in my face, You always opened another. The final completion of this degree took so many strange turns. It did not go whatsoever as I originally planned, but this long and winding road had many purposes and lessons along the way. I have no doubt You were always in control. And although not in my time, in Your time my prayers were answered.

Table of Contents

Abbreviations

ACTH	adrenocorticotropin	**hr**	hour
AD	antidepressant	**5-HT**	serotonin
ADP	adenosine diphosphate	**IP**	intraperitoneally
AEBSF	4-(2-Amino-ethyl)benzenesulfonyl fluoride	**MAO**	monoamine oxidase
		mg/kg	milligram/kilogram
		min	minutes
AMP	adenosine monophosphate	***mM***	millimolar
ATP	adenosine triphosphate	**mRNA**	messenger ribonucleic acid
B_{max}	maximum number of binding sites		
BSA	bovine serum albumin	**n**	sample size
cAMP	cyclic AMP	**NaH_2PO_4**	sodium phosphate
cGMP	cyclic GMP	**NE**	norepinephrine
C subunit	catalytic subunit	***nM***	nanomolar
CORT	corticosterone	**PDE**	phosphodiesterase
d	diameter	**PEI**	polyethyleneimine
DA	dopamine	**PKA**	protein kinase A
EDTA	(ethylene dinitrilo)tetra-acetic acid disodium salt	**R subunit**	regulatory subunit
		RI	regulatory subunit I
EEG	electroencephalogram	**RII**	regulatory subunit II
fmol/mg	femtomoles/milligram	**SD**	Sprague-Dawley
FST	forced swim test	**SEM**	standard error of the mean
GABA	gamma amino butyric acid		
GDP	guanosine diphosphate	**SSRI**	selective serotonin re-uptake inhibitor
GMP	guanosine monophosphate		
GTP	guanosine triphosphate	**TCA**	tricyclic antidepressant
h	height	***ul***	microliter
HCl	hydrochloric acid	***um***	micromolar
HPA	hypothalamic-pituitary-adrenal axis	**vs.**	versus
		WKY	Wistar Kyoto

List of Figures

List of Tables

Part One: Protein Kinase A

Chapter 1
An Introduction to cAMP and Its Receptor: Protein Kinase A's Involvement in Depression

Background on cAMP and Depression

"…We pursued this novel biochemical approach, distal to receptor occupancy and involving the signal transduction processes, because it could play an important role in regulating the biological responses of the effector cells to various neurotransmitters…Our results demonstrate that the cAMP-dependent phophorylation system….could represent an intracellular target beyond the receptor level which might be involved in the biochemical mechanisms of action of antidepressant drugs and the pathogenesis of affective disorders…Increasing knowledge of the effects of antidepressant drugs on second messenger systems further supports the hypothesis of an imbalance in neuronal postreceptor mechanisms in depressed patients…"

Racagni, Brunello, Tinelli and Perez (1992), page 54

The second messenger cyclic AMP (cAMP) has been long documented as affected by antidepressant (AD) treatment (see, for example: Pandey & Davis, 1979). Although cAMP levels may be elevated by short-term AD administration, chronic AD's may be necessary to affect all of the components of the cAMP cascade (Duman, Heninger, & Nestler, 1997). Abdullah and Hamadah (1970) had proposed that depression is correlated with a decrement of intracellular cAMP activity, while mania results from the striking elevation of that second messenger. In his 1989 hypothesis, Wachtel described depression as resulting from cAMP deficiency accompanied by phospholipase C dominance and

mania as the converse. Avissar and Schreiber (1992) have furthered this thinking by proposing that AD's induce manic states, and therefore, this explains why AD's can trigger mania in bipolar disorder.

The depressed suicide literature appears to substantiate lowered cAMP activity in some situations. In suicide victims with a history of depression, it was determined that there was a lowered basal level of adenylate cyclase, yet GTPγS or forskolin stimulation of the enzyme was not altered in the frontal cortex (Cowburn, Marcusson, Eriksson, Wiehager, & O'Neill, 1994). In direct contrast, Lowther, Crompton, Katona, and Horton (1996) found no change in basal activity, but trends toward lowered GTPγS or forskolin stimulated activity in frontal and parietal cortex. The researchers were aware of, although could not explain, the prior group's findings.

What impact do AD's have on cAMP? Although cAMP levels may be elevated by short-term AD administration, chronic AD's may be necessary to affect all components of the cAMP cascade (Duman, Heninger, & Nestler, 1997). Upregulation of the cAMP system may occur in some brain regions like the hippocampus, but downregulation has been found in the locus coeruleus; therefore, regional differences must be noted.

The vast majority of the clinically employed AD's have their mechanism of action at G-protein coupled receptors and thus have some direct impact on cAMP functioning since this is one of the first effector systems that will be altered by such a drug. Phosphodiesterase inhibitors, which inhibit metabolism of cAMP, have potential AD activity with more rapid onset of effect (Wachtel, 1983). This is reflected clinically, perhaps due to rolipram's potent dual impact on the norepinephrine (NE) system by

increasing NE turnover presynaptically and inhibiting phosphodiesterases (PDE's) postsynaptically (Horowski & Sastre-Y-Hernandez, 1985). However, not all clinical studies are in agreement. One study, which had compared rolipram with the tricyclic antidepressant (TCA) amitriptyline, found the former less efficacious in major depression with a greater side effect profile (Scott, Perini, Shering, & Whalley, 1991). In direct contrast, a second study found rolipram more efficacious than desipramine in major depression, with a lower side effect profile (Bobon et al., 1988). Because the side effects can be numerous due to rolipram's lack of selectivity for the individual PDE's, it indicates the need for more selective PDE inhibitors in the future when the function of individual PDE's and adenylate cyclases are better understood.

The ability to accurately measure cAMP levels is difficult experimentally, even with fresh tissue. Procedurally, it is easier to assess other components of the cAMP cascade, including the regulatory subunit of protein kinase A (PKA), the cAMP receptor. Cyclic AMP binding to the regulatory subunit of PKA is a major mechanism by which intracellular cAMP influences subsequent intracellular events (Lowther *et al*., 1997). Since PKA binding is upregulated by agents that increase intracellular cAMP, it may be taken as an indicator of steady state intracellular cAMP concentration.

Antidepressant drugs typically take days to weeks to produce clinical efficacy. This in part appears due to impact on the second messenger cAMP. While the mechanisms surrounding this delay in action remain unknown, it suggests underlying alterations at the genomic level (Manji, Potter, & Lenox, 1995). One of the most critical findings

necessary for the comprehension of the affective disorders is the determination of the long-term drug mechanisms of action, and what underlies the delay in effect.

Protein Kinase A's Role in Depression: Postmortem Human Studies

Not only may levels of cAMP itself be impacted by chronic AD treatment, but the second messenger's receptor, cAMP-dependent protein kinase or protein kinase A (PKA) may exhibit changes which are found in those afflicted by depression or in those receiving AD treatment. Protein kinase A is described as a holoenzyme made up of two catalytic (C subunits) and two regulatory subunits (R subunits) (Brandon, Idzerda, & McKnight, 1997). When cAMP binds to an R subunit, the C subunits dissociate and become enzymatically activated. They can then phosphorylate intracellular targets. At least two types of PKA exist, type I and type II, which are differentiated in terms of R subunit, RI or RII. The radioligand [^{3}H]-cAMP can be used to determine binding to R subunits, and is thought to label both RI and RII (Nishino *et al*., 1993).

Information remains scant in regard to PKA regulatory's role in depression. The first report to examine PKA binding in the human depressive brain was not published until 1997 (Lowther, Katona, Crompton, & Horton). Protein kinase A density was unaltered as demonstrated via saturation binding in five brain regions in depressive suicides who were AD free for at least three months, yet decreased in those currently taking AD drugs. In contrast, more recent reports had found decreased PKA density in the prefrontal cortex of depressed suicides regardless of whether they had been treated with AD's or not (Dwivedi, Conley, Roberts, Tamminga, & Pandey, 2002; Dwivedi *et al*., 2004a).

These studies, which utilize brain tissue from suicides, are confounded by numerous variables such as cause of death, harvest of tissue up to 72 hours after death, accuracy of diagnosis, and use of differing psychotropic and non-psychotropic medications. Postmortem clinical studies thus contain numerous methodological limitations. One such study on bipolar patients (Rahman *et al*., 1997) had pooled suicide victims along with those dying of natural causes (although in that study no differences were found between the groups), and the studies often include patients which have overdosed on AD's and other medications. In addition, all patients have been medicated at some point, even if not for several months at time of death, which may alter PKA levels. Perhaps 15.9 % of all depressives and 29.2 % of all bipolar patients commit suicide (Chen & Dilsaver, 1996), so results based on these subsets of patients that have committed suicide may not be applicable to all afflicted with these disorders.

As stated earlier, the findings are contradictory in terms of whether PKA is altered or not in depressives and whether or not this is a result of AD treatment. In a study of PKA's role in bipolar disorder (Rahman *et al*., 1997), [^{3}H]-cAMP binding was found to be reduced across all brain regions assayed in the cytosolic component, but no differences were found in the membrane component. It was suggested that this may result from increased cAMP signalling in this illness, as in *Aplysia* where it was determined that reduced levels of R subunits and the consequent alteration of a decreased R to C subunit ratio may result in increased kinase activity and protein phosphorylation (Greenberg, Castelluci, Bayley, & Schwartz, 1987).

Animal Studies on Protein Kinase A and Depression

No published account regarding a controlled study of the impact of chronic AD treatment on PKA density exists. Controlled laboratory studies may be performed in rodents that have been chronically injected with AD's and then measured for impact on PKA receptor density as measured via [^{3}H]-cAMP binding. After their initial work with suicide victims, the Dwivedi group executed some controlled animal studies in regards to aspects of depression. The first study examined the effects of corticosterone (CORT) on PKA measures. One of the hallmarks of major depression is an overactive hypothalamic-pituitary-adrenal (HPA) axis. A measure of this is the failure of the dexamethasone suppression test, indicative of endogenously high levels of circulating cortisol (Holsboer, Lauer, Schrieber, & Krieg, 1995).

Dwivedi and Pandey (2000) hypothesized that because PKA is crucial to intracellular signaling, the number of binding sites for cAMP to regulatory subunits of PKA may be altered by manipulations of HPA function, here altered specifically by CORT activity. Corticosterone treatment for one day was found to have no significant effect. However, after four and fourteen days of treatment, two doses (50 and 100 mg implanted pellets) decreased the maximum number of binding sites (B_{max}) of both the cytosolic and particulate fractions of [^{3}H]-cAMP binding sites in both cortex and hippocampus of SD rats, with more impact with the higher dose and greater length of implant. In addition, adrenalectomies were performed in other groups tested for the same time parameters. After four and fourteen days, increased B_{max} was found in both fractions and both brain regions, more so for the longer duration. In those rats that received adrenalectomy with

the addition of a CORT implant, a dose-dependent reversal of adrenalectomy-induced increase in B_{max} was shown, with a complete reversal shown for the 100 mg dose.

The Dwivedi group (Shukla *et. al,* 2004) has since made some preliminary reports of chronic AD treatment in the context of this CORT-induced paradigm at the 2004 Society for Neuroscience (San Diego, CA) conference. A reduced PKA density was induced via 21 day implantation of CORT pellet at a dose of 50 mg. Simultaneously, desipramine (10 mg/kg), fluoxetine (5 mg/kg), and the monoamine oxidase inhibitor phenelzine (10 mg/kg) were administered ip to SD rats for the 21 day period. The chronic administration of desipramine and fluoxetine was described by this group as significantly reversing the reduction in PKA density resulting from CORT implantation in both cytosolic and particulate fractions of prefrontal cortex. This was not attempted in rats which had not been treated simultaneously with CORT due to the prediction that no changes would occur in untreated animals (Pradeep Shukla, personal communication, Society of Neuroscience, San Diego, CA, 2004). This determination does appear amenable to changes in frontal cortex and hippocampus due to chronic drug treatment as CORT pellets implanted into control and adrenalectomized rats reduced and increased, respectively, receptor density measured as $B_{max's}$ in those groups (Dwivedi & Pandey, 2000).

Part Two: Forced Swim Test and Strain Differences: Application to Depression

Chapter 2
The Forced Swim Test

Animal Model of Antidepressant Efficacy: The Forced Swim Test

The Forced Swim Test (FST), first introduced in 1977, was described as capable of inducing a state of "behavioral despair" analogous to the lowered mood which is found in human depression (Porsolt, Anton, Blavet, & Jalfre, 1978; Porsolt, Bertin, & Jalfre, 1977; Porsolt, Le Pichon, & Jalfre, 1977). This has sometimes been labeled as the "Porsolt's swim test", but more recently the "behavioral despair test" or "forced swim test", with some researchers making alterations to Porsolt's original protocols. Rats or mice are placed in a beaker of water, a highly stressful situation (Kirby, Chou-Green, Davis, & Lucki, 1997), in which they alternate between bouts of attempts at escape ("persistence") and bouts of giving up ("despair"). During the allotted time, an immobility baseline (time in which the animal makes just enough movement to keep its head about the water) is measured.

This model became well-known for its ability to detect clinical efficacy of AD compounds (Willner, 1984). The time spent immobile (or in "despair") could be reduced by AD's from various drug classes (Borsini & Meli, 1988 ; Porsolt, Anton, Blavet, & Jalfre, 1978). This included TCAs, monoamine oxidase inhibitors, and atypical AD's. Psychostimulant activity of false positives like caffeine and amphetamine could be screened out by also testing the compounds in a model of generalized activity, such as the open field test. In addition, the FST was determined to be sensitive to non-

pharmacological treatments. These included electroconvulsive shock, deprivation of REM sleep, and an enriched environment, which all resulted in a significant reduction of the immobility baseline (Porsolt, Anton, Blavet, & Jalfre, 1978).

The FST has been characterized from the beginning as less sensitive to serotonin (5-hydroxy tryptamine, 5-HT) compounds (Porsolt, Bertin, Blavet, Deniel, & Jalfre, 1979). More recent studies continue to substantiate that conclusion (Lopez-Rubalcalva & Lucki, 2000; Pare, Kluczynski, & Tejani-Butt, 1999). In fact, one review (Borsini, 2000) has claimed that rats are insensitive to serotonin selective re-uptake inhibitors (SSRI's) in the test. However, this may have more to do with strain, dose level, and deviation from the original protocol, as the present laboratory has found SSRI's and 8-OH-DPAT detectable in the FST (Rogacki, Corbett, & Wettstein, 2000).

The FST has also been criticized for various reasons; for example, with mice it is more variable and less selective regarding the compounds that are active, thus resulting in more false positives than in rats (Borsini & Meli, 1988). Another argument is that the prolongation of immobility during the test session can be explained by familiarity with the environment, such that rats learn that previous exposure to the test cylinder during the pre-test session was not a dangerous experience (Borsini, Volterra, & Meli, 1986). This argument has been extended such that the TCA imipramine was interpreted as interfering with memory consolidation since it had the same effects in the FST as the antibiotic anisomycin which interferes with learning (De Pablo, Parra, Segovia, & Guillamon, 1989).

A major fault of the behavioral pharmacology of depression is that for nearly 30 years, there has been little done to establish what exactly is measured by the FST. While a wide variety of criticisms have been made regarding the actual measurement of this test, those who have reviewed various animal models of depression have concluded that the test is a valid indicator of clinical efficacy (Porsolt, 2000; Willner, 1984). What is the FST really measuring?

Previously it was mentioned that the FST was determined as a highly stressful event (Kirby, Chou-Green, Davis, & Lucki, 1997). The first study to outline its impact on monoamine systems was conducted by Weiss and colleagues (1981) who, as stress researchers, were interested in the test as a measure of a stress response. However, their assessments of the monoamine levels induced by the stress of the FST were evaluated as *ex vivo* assays, not as *in vivo* measurements. While many depression researchers continued to describe the test as a "stress response", many years passed before the biochemical and neurochemical correlates were better investigated, partly due to difficulties in technique and partly due to historical reasons, such as the study of stress as separated from the study of depression.

In more recent times it has been possible to assess the effects of the FST on biogenic amine levels via *in vivo* microdialysis. The first such report assesses neurotransmitter levels in the prefrontal cortex on two consecutive days of an 8 min swim, after which samples were taken for up to 3 hours after each session (Jordan, Kramer, Zukas, & Petty, 1994). Although NE was increased by 183% over baseline levels, DA and 5-HT remained stable throughout the first test session. In contrast, the second test session

evidenced a significant increase of all three neurotransmitters measured: NE (310%), DA (441%), and 5-HT (496%), with these elevations remaining for at least one hour following the test. The interpretation was that the stress of the swim test on the previous day sensitized the biogenic amines in the prefrontal cortex to a subsequent swim stress exposure.

The rat FST characteristically includes a 15 min pre-test session on the day prior to the test. If this pre-test is not employed, the immobility baseline is much lower and it is difficult to detect activity for some AD's (personal observation). Here the microdialysis results suggest, at least in cortex over two swim sessions, that it is in actuality increased, not decreased, monoamine levels that result in enhanced "behavioral despair".

Appropriate Strain Choice: The Wistar Kyoto Rat as an Analog of Anxiogenic Depression

Enhanced "behavioral despair" as demonstrated by an elevated immobility baseline in the FST is a characteristic of the WKY rat strain. This strain has evidenced numerous behavioral, biochemical, and neuroendocrine problems analogous to human depression. The WKY's history involves utilization as a normotensive control for the Spontaneously Hypertensive Rat, because the WKY was developed from the same original Wistar stock as that strain, but the WKY lacked the gene responsible for the hypertensive feature (Louis & Howes, 1990).

Pare (1989) first began to characterize the strain in tests of stress ulcer; and found that they were easily stressed and highly ulcerogenic. As he continued his work with these rats, it became apparent that they mirrored depressive symptomatology in many ways. In

addition, it seemed that they also demonstrated anxiogenic characteristics (Pare & Redei, 1993; also, Durand et al., 1999; Soderpalm, 1989) such that they are probably best described as analogous to anxiogenic depression. This is highly utilitarian due to the prevalence of anxiety disorders found in patients with major depressive disorders (Zimmerman, McDermut, & Mattia, 2000).

In addition to their high immobility baseline in the FST, the WKY exhibited "freezing" behavior and low ambulation scores in the open field test, and rapidly acquired a learned helplessness task (Pare, 1994b), indicative of behavioral depression. In addition, hyponeophagia (unconditioned suppression of feeding due to novelty) was demonstrated by fasted WKY rats in response to the presesentation of a single pellet in an open field environment (Pare, 1994a). The interpretation given was that in this model, which resembles reduced feeding seen in the behaviorally depressed (anorexia is often correlated with human depression), this rat is very responsive to stress and exhibits depressive behavior. The WKY are slow to gain weight, fearful of the food hoppers on arrival to the animal facility, and remain consistently a few weeks behind SD rats in body weight gain (personal observation).

Also, one of the hallmarks of depression is a disturbed sleep-wake cycle, which has been found in the WKY rat (Dugovic, Solberg, Redei, Van Reeth, & Turek, 2000). In comparison with the Wistar control strain, the WKY evidenced a 50% increase in REM sleep during the 12 hour light phase and also increased sleep fragmentation during both the light and dark phases. Lower electroencephalogram (EEG) power densities were

observed over the entire frequency ranges. The WKY was therefore described as a genetic analog of depression that is also useful for the study of sleep abnormalities.

Concerning their response to a variety of chronic novel stressors, including water deprivation, heat stress and increased housing density, the WKY evidences some biochemical differences when compared to the "normal" SD rat (Pare & Tejani-Butt, 1996; Tejani-Butt, Pare, & Yang, 1994). In response to these stressors, SD rats evidenced a decrease of 5-HT_{1A} density in the hippocampus while the WKY demonstrated an increase in 5-HT_{1A} density in the hypothalamus and hippocampus. Sprague-Dawley rats showed an increase in 5-HT transporter sites in the cortex, while the WKY revealed a decrease in these sites in the cortex and hippocampus (Pare & Tejani-Butt, 1996). The overall interpretation was that of a hypoactive 5-HT system in the WKY rat.

In regard to the NE system, SD rats demonstrated no change in binding to α_2-adrenergic or β-adrenergic receptors following 3 weeks of chronic novel stressors (Tejani-Butt, Pare, & Yang, 1994). However, the WKY rats showed reduced α_2-adrenergic binding in the amygdala, and significant reductions in the cortex, hippocampus, amygdala, and hypothalamus for β-adrenergic binding. In contrast, a much higher increase in NE transporter sites was found in the hippocampus and amygdala of the WKY as compared to the SD rats. This last finding was interpreted as less NE may be in the synapse of the WKY due to excessive re-uptake. Overall, it was clear that WKY and SD rats regulate their 5-HT and NE systems differently when exposed to chronic novel stress.

The neuroendocrine regulation of the WKY rat was found to be dysregulated in response to stress when compared to two other rat strains, the Wistar and the Fischer 344 (Redei, Pare, & Kluczynski, 1994). The impact of restraint stress on the HPA axis implied that the WKY rats have impaired ability to regulate glucocorticoid negative feedback, which leads to hyperactivated HPA functioning. The sham-operated WKY had higher adrenocorticotropin hormone (ACTH) messenger RNA (mRNA) levels in response to stress. In addition, the other two strains evidenced profound effects due to adrenalectomy, yet the WKY remained unaffected in these measures of ACTH content, thymus weight, and hypothalamic corticotropin-releasing factor mRNA levels. The addition of CORT reversed the adrenalectomy effects of the other strains, but had no effect or increased the impact on the WKY.

Since circadian patterns of hormonal rhythms are frequently disturbed in depressive disorders, the effect of diurnal cycle was measured in the WKY rat with the Wistar as the control strain (Solberg, Turek, Olson, & Redei, 1998). The levels of plasma ACTH and CORT exhibited similar levels and patterns in both strains throughout a 14:10 light-dark cycle until the daily diurnal peak, which occurs at dark onset at their awakening. Although the Wistars began to decline in their hormone levels shortly thereafter, the WKY hormone levels exhibited a sustained increase for nearly 6 hours.

This correlates with data from human depressives, except at times inverse since humans are diurnal, not nocturnal. Specifically, at 7 AM, the highest values for cortisol (the human equivalent of corticosterone) were found in depressed patients, with elevations sustained above normal controls for 6 hours (Weber, *et al*., 2000).

The WKY rat has therefore demonstrated a wide variety of correlates of human depression in the parameters of behavior, biochemistry, and neuroendocrinology. However, no animal model can approximate every facet of this human disease, nor may it be an appropriate analog of all depressive subtypes. None-the-less, it appears as a more appropriate strain than the most commonly used SD rat, which appears in the literature as "normal" on all of the aforementioned measures.

Strain Differences in Forced Swim Test Response to Antidepressants

The FST is an animal model of depression that detects AD activity with a high correlation to clinical efficacy. Compounds which significantly reduce the amount of time spent immobile when immersed in a water-filled beaker are regarded as AD in effect. Therefore AD's will increase behaviors such as climbing, diving, and swimming in the test as part of attempts to escape the inescapable condition. The two rat strains, SD and WKY, have evidenced significantly different immobility baselines in the test (Lopez-Rubalcava & Lucki, 2000; Pare, 1989; Rogacki, Corbett, & Wettstein, 2000; Tejani-Butt, Kluczynski, & Pare, 2003).

Lahmame and Amario (1996) found the WKY to be subsensitive to desipramine and 8-OH-DPAT in the FST, which led this group to describe the strain as a model of "treatment-resistant" depression. Lopez-Rubalcava & Lucki (2000) similarly found that the WKY demonstrated a blunted response to the 5-HT compounds fluoxetine and 8-OH-DPAT, but were more sensitive than the SD strain to the NE-driven desipramine. In contrast, Tejani-Butt *et al.* (2003) found that the SD strain was not sensitive to any AD's tested, unlike the WKY, which was sensitive to desipramine (NE re-uptake inhibitor) and

nomifensine (NE and DA re-uptake inhibitor). They did determine that the WKY was not sensitive to paroxetine (SSRI), which agreed with the other groups' reports that the WKY are not sensitive to 5-HT compounds.

Unlike the other researchers, one group found the WKY to be sensitive to all mechanisms of action (Rogacki, Corbett, & Wettstein, 2000). This included the 5-HT compounds fluoxetine and 8-OH-DPAT. These findings can be explained by differences in methodology, as each group runs the FST with variations in the protocol. Some use an automated test, a data sampling procedure or differ regarding water depth, drug dose, and animal supplier.

Few reports (Tejani-Butt *et al*., 2003; Lahmame *et al.,* 1997) address chronic administration in the FST, especially with a concurrent side-by-side comparison with a subchronic scheme as done by the Lahmame group. Tejani-Butt and colleagues (2003) found that chronic administration over 12 days did not produce AD effects in the SD strain. However, Lahmame *et al.* (1997) found that chronic administration (15 days) altered sensitivity for the WKY strain, such that they became weakly responsive to imipramine. All three strains tested, including the SD rat, revealed a greater AD response to imipramine after chronic administration.

Part Three: Specific Aims

Introduction

Effects of drug treatment on the cAMP-dependent phosphorylation system has been hypothesized as a major end point of AD treatment (Racagni, Brunello, Tinelli & Perez, 1992), yet more than a decade later, the work to test this hypothesis, or even understand the characteristics of the cAMP receptor, remains in its infancy. No clinically available AD drugs have been developed to directly modulate PKA receptors. It is crucial to establish the PKA receptor(s) as a potential target for AD drug development.

Past work with postmortem brain tissue has implicated that the PKA system is altered by chronic AD treatment. One such study demonstrated that PKA density is lowered by AD's (Lowther *et al.*, 1997). A number of uncontrolled variables make interpretation of the human studies difficult such that PKA's role in depression remains unclear. In addition, there is a paucity of this work.

Because some indication of chronic AD effects on PKA have been indicated in the clinical literature, controlled laboratory studies in animals are required to further elucidate the potential impact of AD's on PKA. A single report from the 2004 Society of

Neuroscience conference (San Diego, CA) describes effects of AD's on PKA function (Shukla *et. al*, 2004), with both increase and decrease in receptor density shown for chronic administration of desipramine and fluoxetine, respectively. That work included the co-administration of CORT to induce a decrease in PKA density.

In the present work a number of critical variables were addressed. Among them were rat strain, AD's of differing mechanism of action, acute vs.chronic administration schedule, PKA binding in two fractions (one soluble and present in supernatant, the other membrane bound and present in the pellet) as the mode of second messenger assessment, frontal cortex as the brain region studied, and whether positive effects by drug treatment were reflected in both behavior and biochemistry (or could one occur in absence of the other).

Would two separate injection schemes alter the outcome of either the behavioral test or biochemical assay? If the behavioral assay became less sensitive following chronic injection, perhaps it is measuring the initial increase in cAMP found by more acute treatment, and loss of effect might be reflective of downregulation of cAMP following chronic AD administration (Pandey & Davis, 1979).

Most importantly, would effect of chronic, but not subchronic, AD treatment on PKA binding reflect an explanation for the lag time to alleviate depression seen in patients?

Therefore, the purpose of the experiments of this dissertation was to understand the relationship between PKA and AD treatment on two strains of rat that reflect two differing populations. One was an analog of depression (WKY), one measure of this

being high immobility times in the FST. This strain variation in behavior is much akin to the population variation seen in clinical subjects.

In the current work, three drugs were tested: desipramine (TCA), bupropion (DA re-uptake inhibitor, AD), and alprazolam (benzodiazepine anxiolytic). They were chosen for their differing mechanisms of action, such that the NE, DA, and gamma-amino butyric acid (GABA) systems were tested. The first two drugs remain widely prescribed AD's.

The overriding hypothesis was that the cAMP system, here measured by changes PKA density following AD treatment, will differ in two strains of rat, one "normal" and one exhibiting depressive characteristics in the FST, an animal model of depression that has been shown as sensitive to cAMP-mediated compounds. To test this hypothesis, experiments were designed to focus on:

1. Protein kinase (PKA)
2. Forced swim test (immobility score)
3. Strain difference (SD and WKY)
4. Drug administration paradigm (acute, subchronic and chronic)

Specific Aims

Specific Aim 1. To determine a pharmacological profile of a PKA receptor in two fractions, one soluble (cytosolic) and one membrane bound (particulate). This was determined by cAMP analog binding tested against [^{3}H]-cAMP.

Hypothesis 1. The PKA receptor measured will demonstrate a unique pharmacological binding profile that will identify a single PKA receptor subtype that is the same in both fractions.

Specific Aim 2. To determine strain differences in PKA density and FST response to subchronic and chronic treatment of the AD desipramine, a NE re-uptake inhibitor. This was determined by changes in immobility baseline in the FST and PKA receptor densities and affinities via [^{3}H]-cAMP binding assays.

Hypothesis 2. The two rat strains will differ in their response to desipramine regarding both FST behavior and PKA density, and subchronic vs. chronic drug administration.

Specific Aim 3. To determine strain differences in PKA density and FST response to subchronic and chronic treatment of the AD bupropion, a DA re-uptake inhibitor. This was determined by changes in immobility baseline in the FST and PKA receptor densities and affinities via [^{3}H]-cAMP binding assays.

Hypothesis 3. The two rat strains will differ in their response to bupropion regarding both FST behavior and PKA density and subchronic vs. chronic drug administration.

Specific Aim 4. To determine strain differences in FST response to subchronic and chronic treatment of alprazolam, a benzodiazepine anxiolytic. This was determined by changes in immobility baseline in the FST.

Hypothesis 4. The two rat strains will differ in their response to alprazolam in the FST with one strain or the other demonstrating an AD-like response.

Specific Aim 5. To determine strain differences in control response levels in the FST and [^{3}H]-cAMP binding assays.

Hypothesis 5. The two rat strains will differ in their control levels of FST behavior and control levels of PKA density, but not affinities, in the [^{3}H]-cAMP binding assays.

While it would have been interesting to assess an SSRI (fluoxetine, paroxetine), these drugs cause ulcerations at high doses (30 or 60 mg/kg) when dosed chronically. A study with paroxetine (highly active in the FST for both strains here in preliminary work) had to be terminated in the WKY due to the caustic nature of the drug that could not be overcome by utilizing a different vehicle.

Part Four:

General Methods

Materials/Methods

All animal procedures were conducted in strict accordance with a protocol approved by the Institutional Animal Care and Use Committee at Aventis Pharmaceuticals. This included measures to reduce pain or discomfort as per guidelines set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Subjects

Male WKY and SD rats (10.5-12 weeks of age at the time of brain harvest; Charles River; Raleigh, NC and Kingston, NY, respectively) were housed 2-3 per cage in a temperature-controlled environment on a 12:12 light/dark cycle. Animals were given food (Lab Diet rat pellets) and water *ad libitum*.

Chronic Forced Swim Test Procedure

The overall procedure for chronic studies was as follows: rats of each strain were divided into three groups, which were vehicle control, subchronic drug, and chronic drug. The subchronic injection scheme was that of the typically used FST paradigm: three injections, which are given 24 hours, 5 hours, and 1 hour prior to the FST. All injections were administered intraperitoneally (ip). The chronic drug group received one daily injection between 07:30-08:30 h for 19 consecutive days. In order to make direct comparison with the chronic group for each drug tested, the vehicle control and subchronic drug treatment group also received a daily vehicle injection throughout that

time period. All rats were therefore injected for three weeks such that the vehicle control group could be applied to either the subchronically or chronically injected drug groups.

The subchronically injected drug group received its first drug injection (after 19 days of vehicle) 24 hours prior to the FST and following a 15 min forced swim pre-test session (in a water-filled beaker 40 cm h x 18 cm d x 15 cm deep) on Day 20. They then received two more drug injections 5 and 1 hour prior to the test session. The chronically injected drug group received a single drug injection following the pre-test on day 20 and a final drug injection on Day 21 1 hour before the test session. The vehicle group was treated in the same fashion on Days 20 and 21. The FST was a 5 min test during which immobility time was measured observationally with a stop watch. The timing of the FST, whether it was run in the morning or afternoon, has not been found to affect the immobility baseline measured, nor has it altered any drug effect that has been measured throughout the day (personal observation).

Rats were sacrificed the following morning. Frontal cortices were removed and frozen at that time.

Tissue Preparation and Binding Assay

Tissue preparation

Several methods were considered and that of Rahman *et al*. (1997) was chosen with slight modifications as noted. Rats were killed by decapitation with rapid removal of their brains and dissections of their frontal cortices over ice. Brain samples from the frontal cortex were homogenized with a teflon homogenizer (Glascol; Terre Haute, Indiana) and

then ultrasonicated with a Kontes Micro Ultrasonic Cell Disrupter (Vineland, New Jersey; 30% setting with 10 sec pulses until broken down) in 10 volumes ice-cold buffer.

The homogenization buffer contained 20 *mM* Tris-HCl (pH 7.4 at 25^{o}C), 2 *mM* EDTA, 25 *mM* 2-mercaptoethanol, 0.5 *mM* 4-(2-Amino-ethyl)benzenesulfonyl fluoride (AEBSF), and 10 *ug/ml* leupeptin (all reagents purchased from Sigma Corporation; St. Louis, MO). The homogenization was followed by ultracentrifugation at 48,000 x *g* for 30 minutes. The supernatant was separated and set aside. The pellet was resuspended in the homogenization buffer and centrifuged for 15 minutes at 48,000 x *g*. This was repeated twice for the pellet, but only the first supernatant was reserved. Cytosolic and membrane fractions were stored at -80 ^{o}C until assay.

Binding Assays

[^{3}H]-cAMP binding assays were performed as described by Rahman *et al.* (1997) with slight modification. The incubation buffer contained 20 *mM* sodium phosphate (NaH_2PO_4) buffer (pH 7.4 at 25 ^{o}C), 2 *mM* EDTA, 15 *mM* 2-mercaptoethanol, bovine serum albumin (BSA final concentration; 0.5 mg/ml), 3-isobutyl-1-methylxanthine (IBMX; final concentration; 1.5 *mM*), approximately 40 *ug* cytosolic or membrane protein, and [^{3}H]-cAMP (0.125-25 *nM* for saturation assays, 5 *nM* for competition binding; 32 *Ci/mmol*, Perkin-Elmer; Boston, MA) in a total volume of 500 *ul*. Non-specific binding was defined by 10 *uM* unlabeled cAMP in the saturation assays. Incubation times varied for the time course assays. Protein concentrations varied for the linearity of protein assays.

Incubation was at 25 ^{o}C for 60 min for the competition and saturation assays at which time rapid filtration occurred with a Brandel cell harvester (Brandel; Gaithersburg, MD) across Whatman GF/B glass filters pre-soaked in 0.3% polyethyleneimine (PEI), followed by 3 washes with cold sodium phosphate buffer. Counting of the filters was performed by liquid scintillation spectrometry (Beckman; Fullerton, CA). This followed an overnight equilibration period during which the filters soaked in Ecoscint fluid (National Diagnostics; Atlanta, GA).

Protein Assay

Protein measurements were conducted according to the method of Bradford (1976). Protein dye reagent and BSA standard were purchased from Bio-Rad Laboratories; Hercules, CA). Optical density readings were taken by a Spectramax Plus spectrophotometer (Molecular Devices Corporation; Sunnyvale, CA) using SoftmaxPro software.

Part Five:

Characterization of the PKA Receptor

Chapter 3
Determining a Pharmacological Profile for the PKA Receptor

Introduction

Characterization of [^{3}H]-cAMP binding

Although two types of regulatory PKA exist (RI and RII), and each of these has at least one subtype (RIα and RIIα; RIβ and RIIβ), the literature has described the [^{3}H]-cAMP binding in both cytosolic and particulate fractions and both human and murine brain tissue as indicative of a one-site fit, with one exception: a two-site fit reported by Rahman *et al.* (1997) that revealed one high affinity site and one low affinity site in human postmortem temporal cortex. This was found in the particulate fraction, but not the cytosolic component. Assessment of both fractions is of interest due to the possible migratory nature of PKA which has been proposed to translocate from one compartment to another in the cell (Nestler, Terwilliger, & Duman, 1989).

One determinant by which receptors can be identified is the K_D, or the affinity constant, for the [^{3}H]-cAMP radioligand. Because the focus here will be on frontal cortex, only those determinations will be mentioned. Lowther *et al.* (1997) worked only with the cytosolic component of human tissue. The K_D's ranged from 2.45 to 2.77 *nM*, with no significant differences between control and suicides, or whether they had been AD free or not. In contrast, another group has reported lower K_D's (0.66 to 0.79 *nM*) in

both control and depressed suicides, as well in both cytosolic and particulate fractions (Dwivedi *et al*., 2002; Dwivedi *et al*. 2004a). Yogesh Dwivedi does not consider this K_D to be indicative of binding to a high affinity site (personal communication, Society for Neuroscience, New Orleans, LA, 2003), although Nishino *et al*. (1993) describes a K_D of 0.9 *nM* as a high affinity site. This was in addition to a K_D of 3.6 *nM* of a low affinity site found in the particulate fraction of human temporal cortex. Dwivedi similarly reports low K_D values (0.66 to 0.83 *nM*) for rat frontal cortex (Dwivedi, *et al*., 2004b).

Another measure by which to characterize a receptor is the B_{max}, or receptor number, quantified by femtomoles per milligram (fmol/mg) of protein. In frontal cortex of human controls and suicides, Lowther *et al.* (1997) reported $B_{max's}$ in cytosolic fraction which ranged from 751 fmol/mg in AD-treated suicides to 912 fmol/mg in AD-free suicides. Rahman *et al.* (1997) found in the cytosolic fraction of frontal cortex a B_{max} of 613 fmol/mg in control and 478 fmol/mg in bipolar disorder tissues. The values were 439 fmol/mg and 326 fmol/mg, respectively, in the particulate fractions. In a single normal human control brain, Nishino *et al*. (1993) determined B_{max} values in prefrontal (areas 9 and 10) and frontal cortices (areas 4 and 6). These ranged from 598 fmol/mg to 752 fmol/mg.

In contrast, Dwivedi *et al*. (2004a) showed relatively lower B_{max} values in pre-frontal cortex of 174 fmol/mg in depressed suicides and 268 fmol/mg in controls for the cytosolic component. The particulate fraction yielded values of 85 fmol/mg in depressed suicides and 143 fmol/mg in controls.

Values in rat pre-frontal cortex shown by Rahman *et al.* (1997) were 910 fmol/mg and 1018 fmol/mg for cytosolic and particulate fractions, respectively. In contrast, Dwivedi *et al.* (2002) reported $B_{max's}$ for cortex of 348 fmol/mg and 129 fmol/mg for cytosolic and particulate fractions, respectively. B_{max} values appear then as characteristic of a particular laboratory rather than the source species, as shown by a sometimes ten-fold difference for the same or similar tissue as reported by different groups.

Before assessments of K_D and B_{max} can be made, initial experimental parameters must be determined to assess accurately these measures. Firstly, a time course of binding must be made using an estimate of the radioligand K_D. Published reports estimated that binding reached equilibrium by 40-60 minutes and remained constant up to 120-150 minutes at 25 ^{o}C. Linearity of specific binding with increased protein concentrations was found up to 200 *ug* of final concentration. Final protein concentrations for saturation experiments ranged from 25 *ug* to 80 *ug*. The non-specific binding values were low (5 to 12 % of total binding), even at highest concentrations (10-20 *nM*) of the radioligand tested (Karge *et al*., 2001; Rahman *et al*., 1997).

In the current work, all of the parameters and others were established in the context of this specific laboratory. Also, some comparisons were made of procedures from different laboratories; for instance, the Dwivedi group uses a very high centrifuge speed for a long duration (100,000 *g* for 60 minutes), while other groups adhere more closely to the original Nishino protocol that employs a 48,000 *g* spin. While some groups have used fresh brain tissue for assay purposes, others have used frozen material.

Since the current work yielded $B_{max's}$ that were much higher than literature values for both cytosolic and particulate fractions, although other parameters were in agreement, some additional work was conducted to determine if this binding could be abolished. Each fraction was boiled for 15 or 30 minutes to ascertain whether the binding was solely to a receptor, and not some non-specific protein or other unknown material. Other parameters, including a test of potential filter binding, indicated that a receptor-ligand relationship was being measured.

Analog Binding

Receptor characterization can also be determined by pharmacological profiling via competition binding by which cAMP, a number of its analogs, and unrelated compounds can be tested at several concentrations to assess an inhibition constant, or IC_{50}, or a K_i where the IC_{50} is corrected for radioligand concentration (Cheng & Prusoff, 1973). The compounds tested can be placed in a rank ordering that typifies a consistent profile by which a given receptor can be identified.

Three groups have described competition binding, while the Dwivedi group has not published any such characterization (Lowther *et al*., 1997; Nishino *et al*.,1997; Rahman *et al.,* 1997). Only one group (Rahman *et al.,* 1997) made these determinations in human frontal cortex and nothing has been published on rat tissue. Cyclic AMP demonstrated a potent displacement at nanomolar concentrations, while its analogs cGMP, 5'-AMP, and ATP showed marginal displacement at even micromolar concentrations.

The most detailed profile to date was that shown by Nishino *et al*. (1997) for the cytosolic component of human temporal cortex. They reported a K_i of 4.95 *nM* for

cAMP, but no displacement was shown for several cAMP analogs (3'-AMP, 5'-AMP, 2', 3'-cAMP, 5'-ADP, ATP, 5'-GMP, 5'-GDP, and GTP), as well as none seen for several neuroleptics. In an unspecified region, the human cytosolic fraction tested by Lowther produced a K_i of 7.7 *nM* for cAMP, but no displacement was shown for the cAMP analogs (5'-AMP, 5'-ADP, ATP, 5'-GMP, 5'-GDP), as well as none seen for several AD's.

The present work tested all of the cAMP analogs above, plus several others: rp-cAMP, sp-cAMP, 8-bromo-cAMP, rp-bromo-cAMP, sp-bromo-cAMP, and dibutyryl cAMP. These determinations were made in both SD and WKY rat strains, as well as in both cytosolic and particulate fractions of frontal cortex. This is the most extensive pharmacological characterization made to date of [^{3}H]-cAMP binding, which should produce a fingerprint identification of the receptor(s) being quantified by the assay in each fractional component.

Methods

Subjects

As described in general methods section. Male WKY and SD rats were 7.5-9 weeks of age at the start of the study.

Tissue preparation

As described in the general methods section.

Binding Assays

As described in the general methods section.

Protein Assay

As described in the general methods section.

Filter Blank Binding

In order to determine whether the relatively high specific binding as compared to the literature was the result of extraneous binding to the filter material, a test of filter binding was performed. No protein was added to these filter blank tubes. Instead, 400 *ul* of buffer was added to each tube. Total binding was measured by adding 50 *ul* of [^{3}H]-cAMP and 50 *ul* buffer. Non-specific binding was measured by adding 50 *ul* of [^{3}H]-cAMP and 50 *ul* of 10 *uM* unlabeled cAMP.

Boiling of Brain Tissue

In order to determine whether the relatively high specific binding as compared to the literature was the result of extraneous binding to something other than a receptor, aliquots of cytosolic and particulate brain tissue were boiled for 15 or 30 minutes each in a small flask. Although re-naturing could occur, some brief ultrasonication and mixing of the material was necessary to create a homogeneous preparation to use for binding assays.

Data analysis

Data were analyzed with GraphPad Prism version 3.02 software (GraphPad Software, Inc., San Diego, CA). Non-linear regression/one site hyperbola was used to determine K_D and B_{max}. Non-linear regression/sigmoidal dose response with variable slope was used to determine IC_{50} values. Variability is reported as standard error of the mean.

Results

A characterization of the [^{3}H]-cAMP binding site was conducted to determine its biochemical and pharmacological characteristics. Filter binding does not account for the high level of specific binding as compared to literature values for a receptor identified as a PKA receptor that binds to [^{3}H]-cAMP. In addition, this high level of specific binding was attributed to a receptor to ligand relationship. A large portion of the specific binding was lost after 15 minutes of boiling, with the majority of the specific binding absent following 30 minutes of boiling.

A typical saturation experiment conducted with frontal cortex from the SD rat demonstrated a one-site fit and yielded a B_{max} of 3211 +/- 167 fmol/mg and 5064 +/- 295 fmol/mg in the cytosolic and particulate fraction, respectively. The K_D values from the same experiment were 6.73 +/- 0.49 *nM* and 5.07 +/- 0.17 *nM* for the cytosolic and particulate fraction, respectively.

Figures 3-1 through 3-4 illustrate the competition curves for those analogs that evidenced displacement of [^{3}H]-cAMP. This is shown for each strain and fraction separately. Note the similar appearance of the curves for the individual analogs across these variables. Table 3-1 shows the IC_{50} values for cAMP and its analogs that were tested in both cytosolic and particulate fractions of both SD and WKY rats. The IC_{50} values were comparable for both strains, as well as for both fractions, for cAMP and all of the analogs that were measured.

Discussion

The present work determined B_{max} values in rat frontal cortex that were several fold higher in both cytosolic and particulate fractions of rat pre-frontal cortex than shown by other groups (Dwivedi & Pandey, 2000; Nishino *et al*., 1993; Rahman *et al*.,1997), as well as those B_{max} values that had been determined in human cortex (Dwivedi *et al*., 2002; Lowther *et al*., 1997; Nishino *et al*., 1993; Rahman *et al*.,1997). Filter binding and boiling of the brain tissue were conducted to ascertain whether a valid receptor to ligand relationship was being assessed with the [^{3}H]-cAMP binding assays. Both types of experiments found this to be true, but while the results of the boiling of tissue indicated that degradation of receptor proteins occurred, that investigation could not rule out the existence of other receptor(s), nor could it determine definitively that the receptor studied in this laboratory is the same one studied by other laboratories.

Although the B_{max} values found in the current work were higher than published values, the K_D and IC_{50} values for cAMP were similar to those published by some other groups for rats (Nishino *et al*., 1997) and humans (Lowther *et al*., 1997; Nishino *et al*., 1997). The IC_{50} values that have been published by these researchers for some of the cAMP analogs were also in agreement here (see Table 3-1).

In contrast, the Dwivedi group has shown a higher affinity (K_D = 0.67- 0.73 *nM*) site for saturation binding than the present work or that of other investigators. That group has published no binding profile for the PKA receptor such that no comparison with the IC_{50} of cAMP or its analogs can be made. Due to the high affinity K_D values published by the Dwivedi group for the [^{3}H]-cAMP saturation binding assay in both cytosolic (0.73 *nM*)

and particulate (0.67 *nM*) fractions, as well as the very low B_{max} values in both fractions, perhaps those investigators have measured a high affinity binding site.

While Yogesh Dwivedi (personal communication, Society for Neuroscience, New Orleans, LA, 2003) stated that his group determined very high B_{max} values in an assay when the binding buffer was of an acidic pH, this could not explain the high levels of binding found in the present case. Three levels of pH were tested for the binding assay buffer (acidic, neutral, and basic) here. While the alterations in pH revealed variations in K_D and B_{max}, low pH did not enhance specific binding (data not shown).

Overall, despite high density values above those that have been published previously, it has been shown that the current binding reveals the following: a true receptor to ligand relationship, similar affinity values for cAMP and its analogs in both fractions that are comparable to some other laboratories that have tested these (in the cytosolic fraction), and that therefore probably a single receptor has been assessed in both fractions. This receptor is most likely identifiable as a known PKA receptor.

In addition, the competition binding conducted here in the cytosolic and particulate fractions demonstrated a similar fingerprint in those fractions regarding affinities measured via IC_{50} for the numerous cAMP analogs for which brain binding had not been reported previously. The present work is the most extensive identification that has been made to date for a receptor subtype that binds to [^{3}H]-cAMP. The lack of published work that has demonstrated extensive pharmacological profiling of the receptor(s) being measured by other groups, including those that have worked with the PKA saturation

binding assay, has made absolute identification and comparison to the receptor measured here impossible.

The fact that the same receptor has been identified in both fractions here has crucial impact on the present work. This is because the effect of subchronic and chronic drug treatment was assessed on PKA density throughout the remainder of the work. It is therefore possible to make assessments about the potential migratory nature of PKA that has been proposed to translocate from one compartment to another in the cell (Nestler, Terwilliger, & Duman, 1989). If only one receptor subtype is being assessed, then it may be determined whether that same receptor not only alters its density in one fraction, but two fractions, as a result of drug treatment. This is interesting whether the receptor translocates or simply changes in density by degradation or novel synthesis because this may underlie a mode of AD activity that can be used as a surrogate marker for novel compounds with proposed AD impact.

It must be noted here that the cAMP analogs tested are limited to those that are commercially available such that a more extensive pharmacological profiling is not possible. A family of receptor subtypes may bind dozens of analogs in a similar fashion with only one key ligand differentiating a given receptor. Based on the available data, the assumption will be made here that a single receptor is measured by the present work in both cytolic and particulate fractions of rat frontal cortex, although it is known that more than one regulatory subunit of PKA exists (Brandon, Idzerda, & McKnight, 1997).

[^{3}H]-cAMP Competition Binding for cAMP Analogs: Cytosolic Fraction of the Frontal Cortex from the Sprague-Dawley Rat

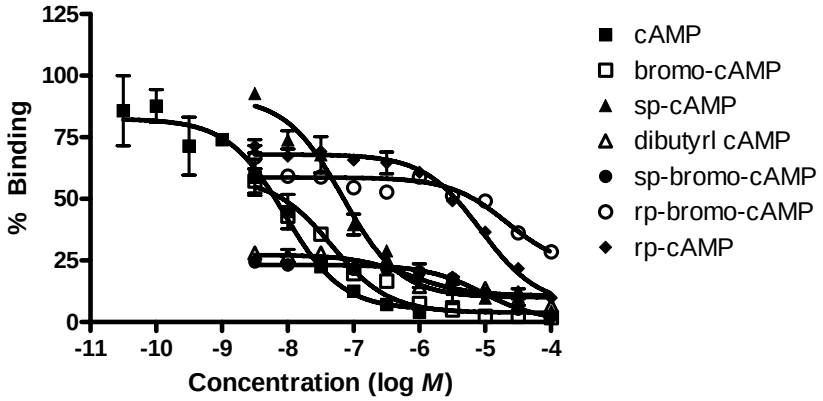

Fig. 3-1. [^{3}H]-cAMP competition binding for cAMP and several of its analogs in the cytosolic fraction of the frontal cortex from the SD rat. Data shown is only for those analogs that evidenced displacement of the radioligand. X axis: Concentrations of each analog in log *M* units.Y axis: Percent binding normalized to 100% of total binding. The line for each analog represents one assay conducted in duplicate with pooled tissue from the frontal cortex of several SD rats.

[^{3}H]-cAMP Competition Binding for cAMP Analogs: Cytosolic Fraction of the Frontal Cortex from the Wistar Kyoto Rat

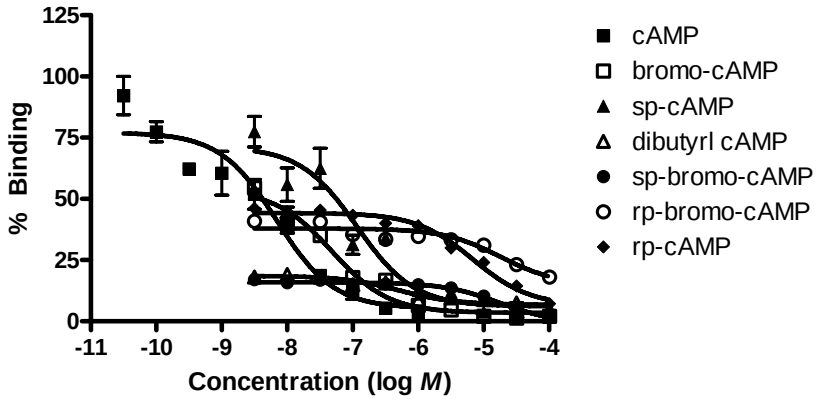

Fig. 3-2. [^{3}H]-cAMP competition binding for cAMP and several of its analogs in the cytosolic fraction of the frontal cortex from the WKY rat. Data shown is only for those analogs that evidenced displacement of the radioligand. X axis: Concentrations of each analog in log *M* units. Y axis: Percent binding normalized to 100% of total binding. The line for each analog represents one assay conducted in duplicate with pooled tissue from the frontal cortex of several WKY rats.

[^{3}H]-cAMP Competition Binding for cAMP Analogs: Particulate Fraction of the Frontal Cortex from the Sprague-Dawley Rat

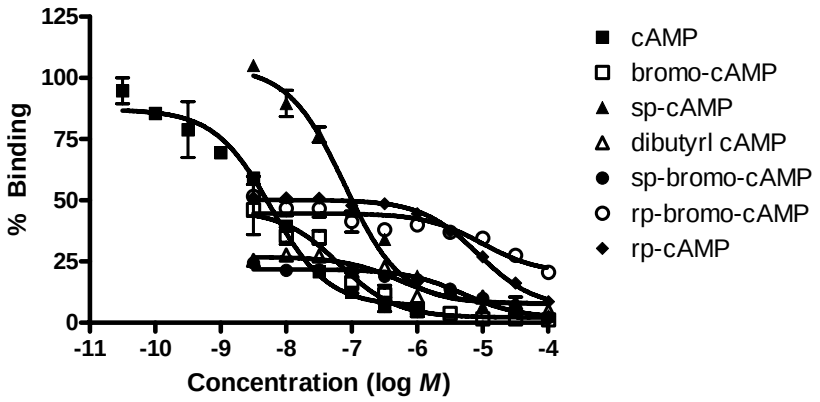

Fig. 3-3. [^{3}H]-cAMP competition binding for cAMP and several of its analogs in the particulate fraction of the frontal cortex from the SD rat. Data shown is only for those analogs that evidenced displacement of the radioligand. X axis: Concentrations of each analog in log *M* units. Y axis: Percent binding normalized to 100% of total binding. The line for each analog represents one assay conducted in duplicate with pooled tissue from the frontal cortex of several SD rats.

[^{3}H]-cAMP Competition Binding for cAMP Analogs: Particulate Fraction of the Frontal Cortex from the Wistar Kyoto Rat

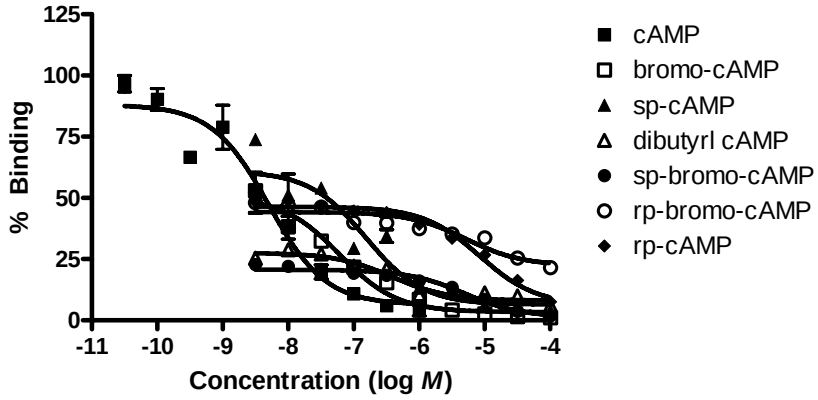

Fig. 3-4. [^{3}H]-cAMP competition binding for cAMP and several of its analogs in the particulate fraction of the frontal cortex from the WKY rat. Data shown is only for those analogs that evidenced displacement of the radioligand. X axis: Concentrations of each analog in log *M* units. Y axis: Percent binding normalized to 100% of total binding. The line for each analog represents one assay conducted in duplicate with pooled tissue from the frontal cortex of several WKY rats.

Binding Constants for cAMP and Analogs (log IC_{50} values)

	Cytosolic				Particulate			
	Sprague-Dawley		Wistar Kyoto		Sprague-Dawley		Wistar Kyoto	
	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)
cAMP	**7.81**	**(0.24)**	**7.70**	**(0.21)**	**7.91**	**(0.19)**	**7.86**	**(0.23)**
rp-cAMP	**4.98**	**(0.08)**	**5.00**	**(0.09)**	**4.95**	**(0.13)**	**4.89**	**(0.10)**
sp-cAMP	**6.37**	**(0.45)**	**6.83**	**(0.13)**	**6.48**	**(0.45)**	**6.77**	**(0.04)**
bromo-cAMP	**7.26**	**(0.14)**	**7.20**	**(0.19)**	**7.37**	**(0.21)**	**7.33**	**(0.10)**
rp-bromo-cAMP	**4.87**	**(0.48)**	**4.85**	**(0.16)**	**5.16**	**(0.48)**	**5.25**	**(0.09)**
sp-bromo-cAMP	**5.04**	**(0.08)**	**4.75**	**(0.11)**	**5.31**	**(0.08)**	**5.31**	**(0.08)**
dibutyryl cAMP	**5.89**	**(0.27)**	**6.29**	**(0.11)**	**6.16**	**(0.08)**	**6.26**	**(0.07)**
3' adenylic acid	**nb**		**nb**		**nb**		**nb**	
5'AMP	**nb**		**nb**		**nb**		**nb**	
GDP	**nb**		**nb**		**nb**		**nb**	
GMP	**nb**		**nb**		**nb**		**nb**	
GTP	**nb**		**nb**		**nb**		**nb**	
ATP	**nb**		**nb**		**nb**		**nb**	

nb= no binding

Table 3-1. Binding constants (log IC_{50}) for cAMP and its analogs. These were assessed (n=2 per assay) in both cytosolic and particulate fractions of frontal cortex in both SD and WKY rats. IC_{50} values appear similar across fractions and strains for every analog, indicating that the same receptor is being measured across these variables.

Part Six: Antidepressants Effects on Forced Swim and Protein Kinase A

Chapter 4
Effects of Desipramine on the Forced Swim Test and Protein Kinase A

Introduction

The TCA's were originally developed for treatment of schizophrenia. While they were found to be ineffective for that indication, a serendipitous finding in the 1950's and 1960's was their utility for depression (Stahl, 2000). Although most of the TCA's block re-uptake pumps for NE, 5-HT, and DA, some are more selective (Stahl, 2000). This ability of the TCA's to relieve depressive symptomatology and their impact on the biogenic amines gave birth to the monoamine hypothesis of depression.

The monoamine theory was based on inference; specifically, that increased levels of monoamines via pharmacological administration alleviated depression (Schildkraut, 1967). The conclusion was therefore drawn that depressives are those who maintain low circulating levels of certain neurotransmitters, such as NE and 5-HT. However, no such direct evidence was ever put forth. In contrast, the hypothesis drew on indirect evidence, such as lowered metabolites of NE and 5-HT in the body fluids of depressives, with the assumption made that lowered neurotransmitter synthesis was the problem (Leonard, 1991). In contrast, the concentration of NE's main metabolite in the cerebrospinal fluid of depressives is not a conclusive indicator of NE function in these patients (Leonard, 2000). Other explanations, such as a metabolic problem like slower degradation of neurotransmitter, were not explored as possibilities for less breakdown products.

Evolution of the monoamine hypothesis has included some reinterpretation and elaboration surrounding the catecholamines, but the general concept has endured regarding the lowered availability of neurotransmitter. This is reflected in the pursuit of research that determines the function of monoamine transport; for example, 5-HT transport is impeded in depressives, but normalized by AD treatment (Leonard, 1991). The NE transporter in human depressives, based mostly on suicide victims, is similarly characterized as decreased, with one study demonstrating this in the midcaudal region of the locus coeruleus (Klimek, *et al*., 1997).

One drug that facilitates primarily NE transport is desipramine, which is more selective for NE over 5-HT (Stahl, 2000). Desipramine remains as a widely prescribed TCA for the treatment of depression (Saddock & Saddock, 2001; Stahl, 2000; Arana & Rosenbaum, 2000).

The impact of desipramine in the FST was discussed earlier in chapter two. Different investigators have found varying results for this drug not only in the test itself, but also for the SD and WKY strains (Lahmame & Amario, 1996; Lopez-Rubalcava & Lucki, 2000; Tejani-Butt *et al.*, 2003). While Lahmame and Amario (1996) found desipramine inactive in the FST for the WKY, other groups found the WKY not only to be sensitive, but more so than the SD strain (Lopez-Rubalcava & Lucki, 2000; Tejani-Butt *et al.*, 2003).

A study by Moyer, Sigg, and Silver (1986) addresses desipramine's effect on PKA. This may have implications for the FST. Since PKA activity was not altered one hour following acute desipramine injection, this may explain why the FST characteristically

cannot detect AD compounds with a single injection in rats. Alterations in PKA activity were found only after a 24 hour delay, when the rats in the FST paradigm are injected again. Although this study describes the catalytic component of PKA, it indicates the ability of desipramine to alter the cAMP-PKA system.

The hypothesis here is that desipramine will demonstrate a significant effect in the FST for one strain (WKY), but not the other (SD). In addition, desipramine will alter PKA density in the frontal cortex, as it has already been demonstrated to impact the PKA catalytic component. Because many of the depressive suicides have been treated chronically with TCA's, this study is also important to interpret the clinical literature regarding AD impact on PKA regulatory.

Methods

Subjects

As in the general methods. Male WKY and SD rats were 7.5-9 weeks of age at the start of the study.

Drug

The TCA desipramine was used at two doses, 1 mg/kg and 10 mg/kg, ip. Desipramine HCl was purchased from Sigma Corporation. The compound was dissolved via vortexing in distilled water with base correction for the salt. The injection volume was 1 ml/kg. Each strain and each dose was run independently over a three week period due to the large number (n=30) which was necessitated by the three separate treatment groups: control, subchronic, and chronic.

Chronic Forced Swim Test Procedure

As in the general methods.

Tissue Preparation and Binding Assay

As in the general methods for frozen tissue with a modified Rahman method (Rahman *et al*., 1977) except frontal cortices from individual rats were homogenized separately with a Caframo (Wiarton, Ontario) teflon stirrer. In order for the binding assay for each fraction to be completed in a single day, n=6 rats were selected randomly from n=10 per treatment group.

Data Analysis

The binding assays were analyzed by non-linear regression/one site hyperbola to determine K_D and B_{max} values with GraphPad Prism version 3.02 software (GraphPad Software, Inc., San Diego, CA). Scatchard analysis was conducted with GraphPad Prism version 4.03 software. The FST results were analyzed by two-way analyses of variance (ANOVA) using SAS version 8 (SAS Institute; Cary, NC). Post hoc comparisons were made by the Bonferroni test. In addition, the K_D and B_{max} values that had been obtained for each animal in each of the three treatment groups were analyzed in the same fashion. Variability is reported as standard error of the mean.

Results

Forced Swim Test

Desipramine at a low dose of 1 mg/kg in the FST (see Figure 4-1) exhibited significant main effects for strain ($F_{(1,59)}= 29.06$, $p < 0.001$), but neither for treatment ($F_{(2,59)}= 1.21$, n.s.), nor for the strain X treatment interaction ($F_{(2,59)}= 1.66$, n.s). The

immobility levels for the vehicle control groups differed significantly ($p<0.01$) between the two strains; however, the subchronic and chronic treatment groups did not differ significantly within strains.

Desipramine at a high dose of 10 mg/kg in the FST (see Figure 4-2) exhibited significant main effects for strain ($F_{(1,59)}= 7.98$, $p< 0.01$), treatment ($F_{(2,59)}= 16.94$, $p<0.001$), and the strain X treatment interaction ($F_{(2,59)}= 7.05$, $p<0.01$). The decreases in immobility shown by SD rats for subchronic (26%) and chronic (11%) dosing were not significant. Both subchronic and chronic dosing produced significant decreases (24%, $p<0.01$ and 41%, $p< 0.001$), respectively, regarding immobility in the WKY rats. The vehicle control groups between the two strains differed significantly ($p<0.05$); however, the subchronic and chronic treatment groups did not differ within strains.

[^{3}H]-cAMP Binding

Desipramine at a low dose of 1 mg/kg in the cytosolic fraction of PKA exhibited significant main effects on B_{max} for strain ($F_{(1,35)}= 104.89$, $p<0.001$), but neither for treatment ($F_{(2,35)}= 0.11$, n.s.) nor the strain X treatment interaction ($F_{(2,35)}= 3.30$, n.s.). See Figure 4-3 for the mean results from individual hyperbolic analyses; Figures 4-4 and 4-5 depict scatchard plots of the same data as a single composite per treatment group. The PKA density of the vehicle control groups differed significantly ($p<0.001$) between the two strains; however, the subchronic and chronic treatment groups did not differ within strains.

Significant main effects occurred for K_D for strain ($F_{(1,35)}= 28.66$, $p<0.0001$) and the

strain X treatment interaction ($F_{(2,35)}$= 8.33, p< 0.01). No effect was shown for treatment ($F_{(2,35)}$= 2.04, n.s.). Although the two strains did not differ in K_D values for their control groups, the control and subchronic values differed significantly (p<0.05), as well as subchronic and chronic values (p<0.05) within the WKY strain. See Table 4-1 for a summary of K_D results for all desipramine experiments.

In the particulate fraction significant main effects occurred for strain ($F_{(1,34)}$= 111.94, p<0.001) and treatment ($F_{(2,34)}$= 6.17, p<0.01), but not the strain X treatment interaction ($F_{(2,34)}$= 1.94, n.s.). See Figure 4-6 for the mean results from individual hyperbolic analyses; Figures 4-7 and 4-8 depict scatchard plots of the same data as a single composite per treatment group. The vehicle control groups between the two strains differed significantly (p<0.001). The SD strain demonstrated an increase (16%) subchronically versus control that was not significant, but the subchronic and chronic treatment groups for that strain had differed significantly (p<0.05) as the B_{max} for the latter group was slightly lower than the control level.

Significant main effects occurred for K_D for strain ($F_{(1,34)}$= 199.40, p<0.0001), but neither for treatment ($F_{(2,34)}$= 0.87, n.s.) nor the strain X treatment interaction ($F_{(2,34)}$= 1.28, n.s.). The two strains did differ in K_D values for their control groups (p<0.0001). Neither the SD nor WKY strain differed across treatment groups within strain.

Desipramine at a high dose of 10 mg/kg in the cytosolic fraction of PKA exhibited main effects on B_{max} for strain ($F_{(1,34)}$= 22.76, p<0.001) and treatment ($F_{(2,34)}$= 7.44, p<0.01), but not the strain X treatment interaction ($F_{(2,34)}$= 0.66, n.s.). See Figure 4-9 for the mean results from individual hyperbolic analyses; Figures 4-10 and 4-11 depict

scatchard plots of the same data as a single composite per treatment group. While the increase (33%) in B_{max} evidenced for the chronic group over the control group for the SD strain was significant ($p< 0.05$), the increase (22%) evidenced by the WKY strain was not significant. Neither of the vehicle control groups between the two strains differed nor the subchronic and chronic treatment groups differed within strains.

Significant main effects occurred for K_D for strain ($F_{(1,34)}= 4.43$, $p<0.05$) and treatment ($F_{(2,34)}= 3.34$, $p<0.05$). No effect was shown for the strain X treatment interaction ($F_{(2,34)}= 0.48$, n.s.). The two strains did not differ in K_D values for their control groups nor were there any differences in K_D within a given strain across treatments.

In the particulate fraction significant main effects occurred for strain ($F_{(1,35)}= 85.51$, $p<0.001$), but neither for treatment ($F_{(2,35)}= 0.65$, n.s.) nor the strain X treatment interaction ($F_{(2,35)}= 1.43$, n.s.). See Figure 4-12 for the mean results from individual hyperbolic analyses; Figures 4-13 and 4-14 depict scatchard plots of the same data as a single composite per treatment group. The vehicle control groups between strains differed significantly ($p<0.001$); however, the subchronic and chronic treatment groups did not differ within strains.

Significant main effects occurred for K_D for strain ($F_{(1,35)}= 531.85$, $p<0.0001$), treatment ($F_{(2,35)}= 6.96$, $p<0.01$) and the strain X treatment interaction ($F_{(2,35)}= 5.02$, $p<0.05$.). The two strains differed in K_D values for their control groups ($p<0.0001$). In addition, the control and chronic groups ($p<0.01$), as well as the subchronic and chronic groups ($p<0.01$) differed within the SD strain. No groups differed across treatments within the WKY strain.

Discussion

As has been previously described here, some groups have found the WKY strain to be more sensitive in the FST than the SD strain to the effects of the NE-reuptake inhibitor desipramine (Lopez-Rubalcava & Lucki, 2000; Tejani-Butt *et al*., 2003). In contrast, another group had found the WKY strain to be subsensitive to desipramine (Lahmame & Amario, 1996).

The current laboratory, using the standard FST paradigm with a scheme of three injections, has found the WKY strain to be characteristically more sensitive to desipramine than the SD strain, much like some former groups (Lopez-Rubalcava & Lucki, 2000; Tejani-Butt *et al*., 2003). The low dose of desipramine was negative in the FST for both rat strains for both injection paradigms as is typically seen in the characteristic subchronic injection paradigm of the FST.

Although not statistically significant, the SD rats did evidence a decrease in immobility (more so subchronically) in response to a high dose of desipramine. This differed from chronic work by Lahmame *et al.* (1997) with the SD rat for another TCA (imipramine) that revealed a greater AD response to the drug after chronic administration. The WKY rat demonstrated significant AD effects to both subchronic and chronic desipramine at the high dose, thereby revealing a greater sensitivity to desipramine overall. Compared to the control level, the AD effect was magnified slightly via chronic treatment for that strain; however, chronic treatment was not different from subchronic when those treatment groups were compared directly.

In part, the apparently greater sensitivity of the WKY strain to the drug may be explained by the significant differences found in both swim tests for the control level of immobility demonstrated between strains. The characteristically higher immobility shown by the WKY strain has been noted in this laboratory previously. This is in agreement with other laboratories (Lopez-Rubalcalva & Lucki, 2000; Pare,1989; Tejani-Butt, Kluczynski, & Pare, 2003). Perhaps the absolute difference in immobility time for the WKY affords that strain an advantageous ability to display significant impact of drug treatments.

PKA density did not differ from control values in either fraction for either strain for the low dose desipramine treatments. The SD strain demonstrated an increase subchronically in the particulate fraction that differed significantly from its own chronic group that evidenced a B_{max} that was slightly below that of the control level. In contrast, both strains evidenced an increase in PKA density in the cytosolic fraction due to chronic treatment with a high dose of desipramine, but this was of a greater magnitude and significant only for the SD strain.

Similarly, an increase in cytosolic PKA density with this same high dose of desipramine was shown in the SD strain by the Dwivedi group, but in the context of reversing a CORT-induced decrease of PKA density (Shukla *et al*., 2004). Perhaps this increase in density may be correlated with the need for chronic treatment to evidence an AD effect with desipramine clinically. Increased PKA density in the cytosol may indicate an AD effect, at least for a NE-driven AD drug. Although those investigators (Shukla *et*

al., 2004) also found a change in the particulate fraction, no such alterations were found in that fraction here.

The PKA density in both the cytosolic and particulate fractions for the control groups was lower, often in a highly significant manner, for the WKY strain as compared to the SD strain. This decrease was pronounced for the latter fraction. Perhaps the lower PKA density underlies some facet of differential regulation of cAMP by the WKY strain, thereby indicative of another one of that strain's depressive characteristics. Although Lowther *et al.* (1997) had found PKA density of the cytosolic fraction unaltered in depressive suicides who were AD free for at least three months, others have reported decreased PKA density in both fractions of the prefrontal cortex of depressed suicides regardless of whether they had been treated with AD's or not (Dwivedi, Conley, Roberts, Tamminga, & Pandey, 2002; Dwivedi *et al*., 2004a). This lower PKA density demonstrated in human depressives was mirrored in the present work by the WKY rat, a strain that exhibits depressive characteristics.

The changes in affinity for [^{3}H]-cAMP as measured by K_D values demonstrated few changes due to drug treatment for either strain. The magnitude of change, which was at most two-fold in one case, is probably not enough to alter PKA receptor function via this parameter.

Forced Swim Test: Effect of Desipramine 1 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

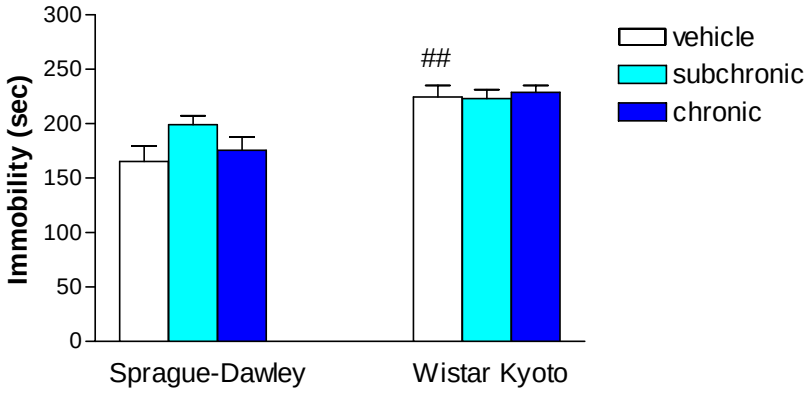

Fig. 4-1. Effect of desipramine at 1 mg/kg in the forced swim test: SD vs. WKY rats. The vehicle and subchronic injection scheme included three ip drug injections: 24 hr, 5 hr, and 1 hr prior to the FST following 19 days of vehicle. The chronic group received one daily ip drug injection for 21 consecutive days. Immobility time in sec (+ SEM) was measured observationally. N=10 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; ##, $p<0.01$ vs. SD control.

Forced Swim Test: Effect of Desipramine 10 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

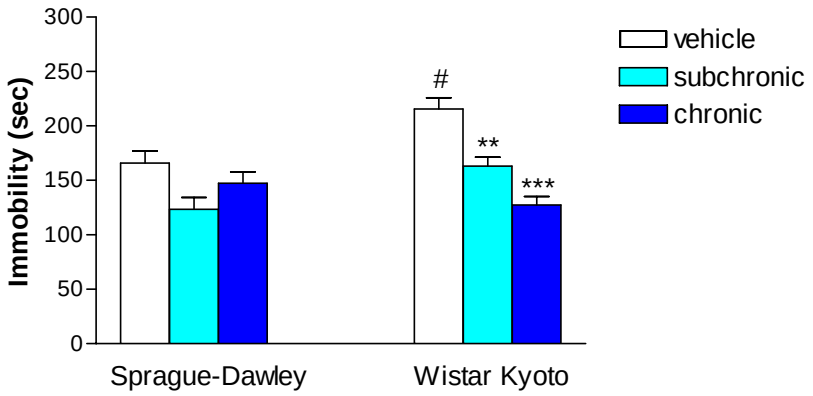

Fig. 4-2. Effect of desipramine at 10 mg/kg in the forced swim test: SD vs. WKY rats. The vehicle and subchronic injection scheme included three ip injections: 24 hr, 5 hr, and 1 hr prior to the FST following 19 days of vehicle. The chronic group received one daily ip drug injection for 21 consecutive days. Immobility time in sec (+ SEM) was measured observationally. N=10 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; #, $p<0.05$ vs. SD control; **, $p<0.01$, ***<0.001 vs. WKY control.

[^{3}H]-cAMP Binding in the Cytosolic Fraction of the Frontal Cortex: Effect of Desipramine 1 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

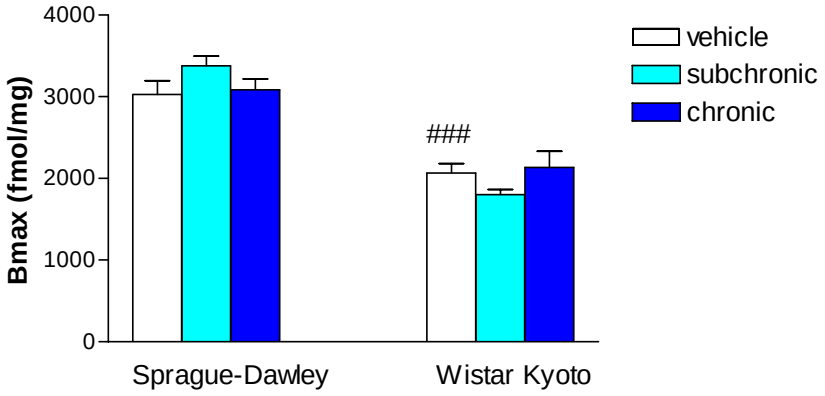

Fig. 4-3. Effect of desipramine at 1 mg/kg on cytosolic PKA binding: SD vs. WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; ###, $p<0.001$ vs. SD control.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Desipramine 1 mg/kg

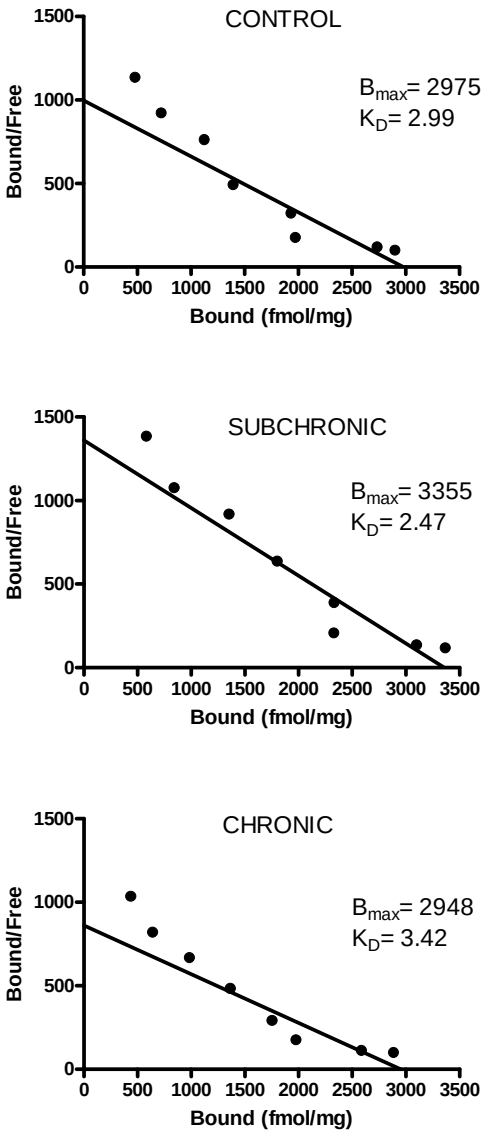

Fig. 4-4. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of SD rats. Control vs. subchronic or chronic desipramine 1 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Desipramine 1 mg/kg

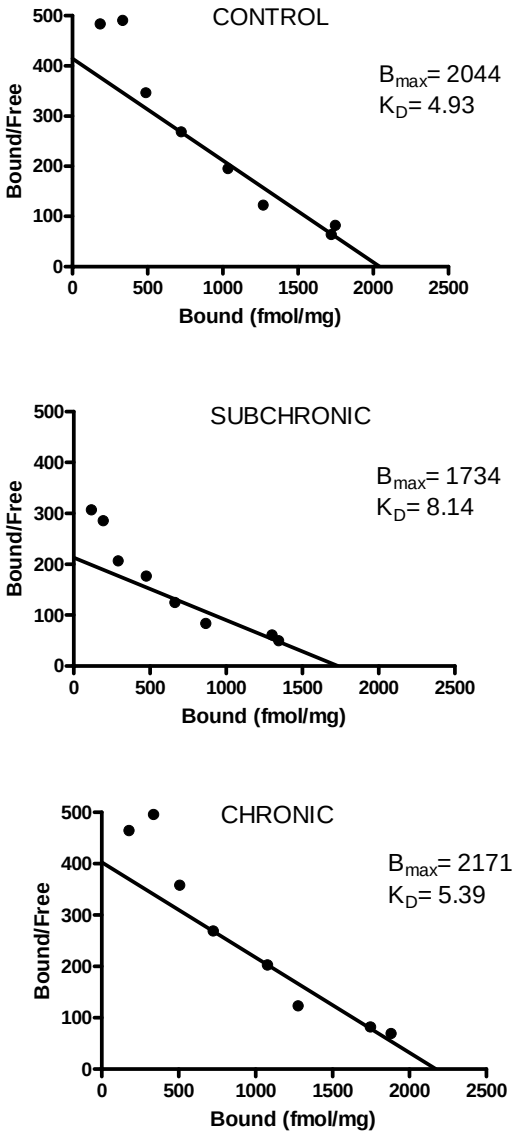

Fig. 4-5. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic desipramine 1 mg/kg injection (ip).). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

[^{3}H]-cAMP Binding in the Particulate Fraction of the Frontal Cortex: Effect of Desipramine 1 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

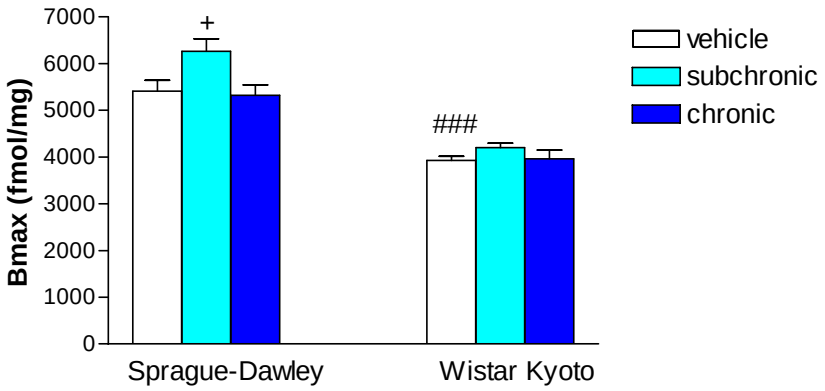

Fig. 4-6. Effect of desipramine at 1 mg/kg on particulate PKA binding: SD vs. WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; ###, $p<0.001$ vs. SD control; +, $p< 0.05$ vs. SD chronic.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Desipramine 1 mg/kg

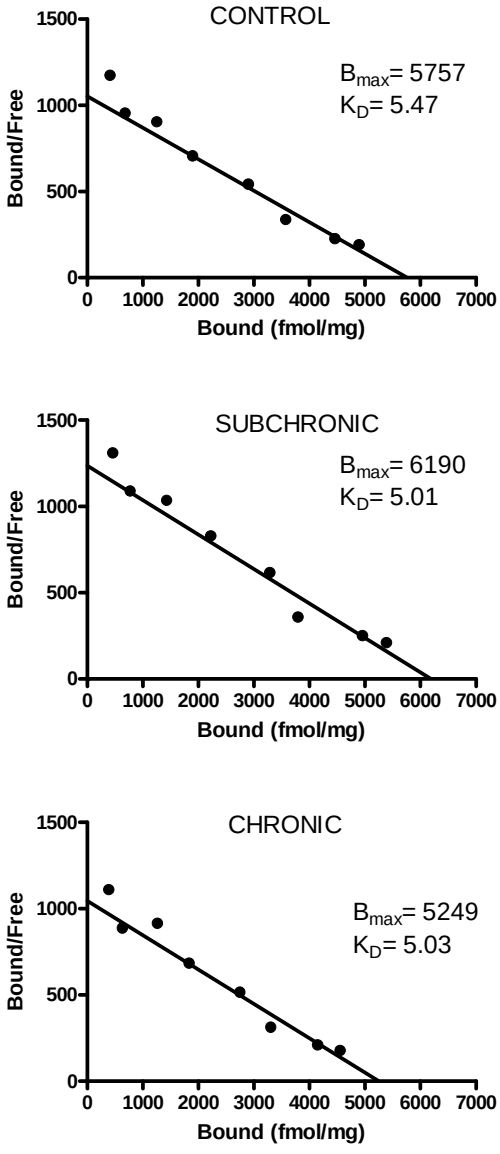

Fig. 4-7. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of SD rats. Control vs. subchronic or chronic desipramine 1 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Desipramine 1 mg/kg

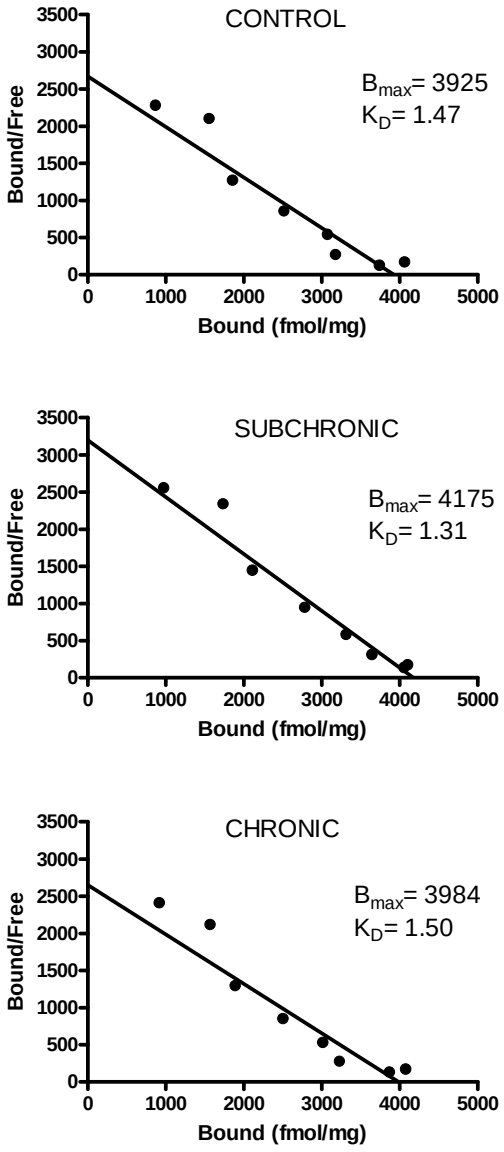

Fig. 4-8. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic desipramine 1 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand.Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

[^{3}H]-cAMP Binding in the Cytosolic Fraction of the Frontal Cortex: Effect of Desipramine 10 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

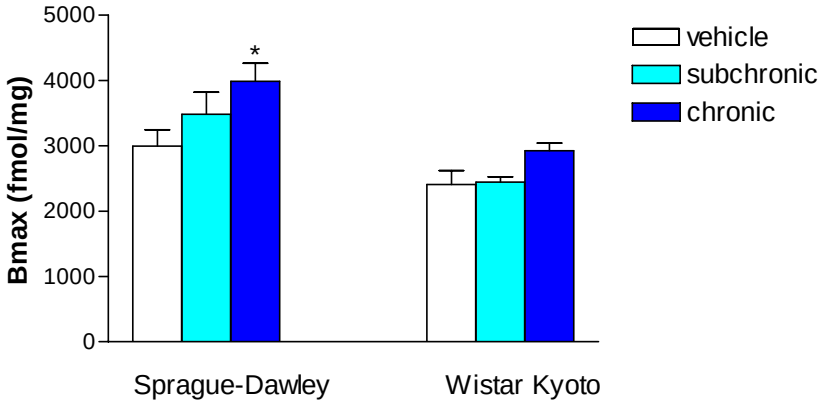

Fig. 4-9. Effect of desipramine at 10 mg/kg on cytosolic PKA binding: SD vs. WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; *, $p<0.05$ vs. SD control.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Desipramine 10 mg/kg

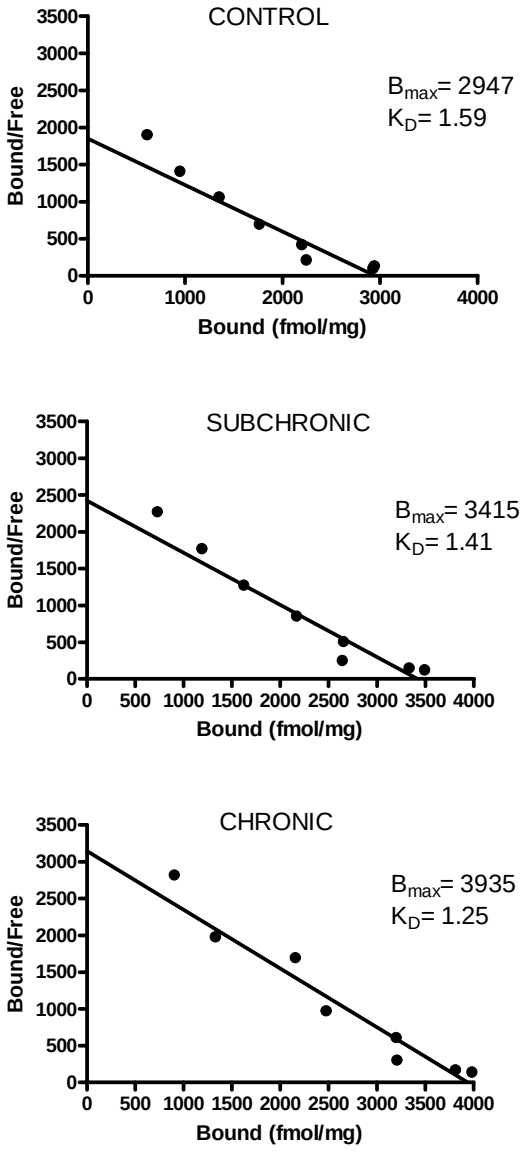

Fig. 4-10. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of SD rats. Control vs. subchronic or chronic desipramine 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Desipramine 10 mg/kg

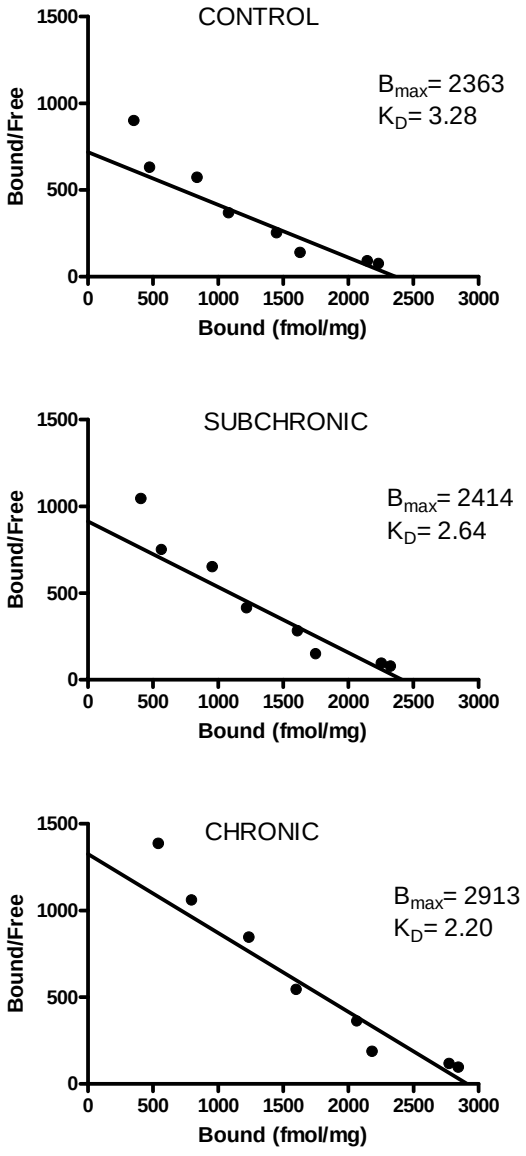

Fig. 4-11. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic desipramine 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

[^{3}H]-cAMP Binding in the Particulate Fraction of the Frontal Cortex: Effect of Desipramine 10 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

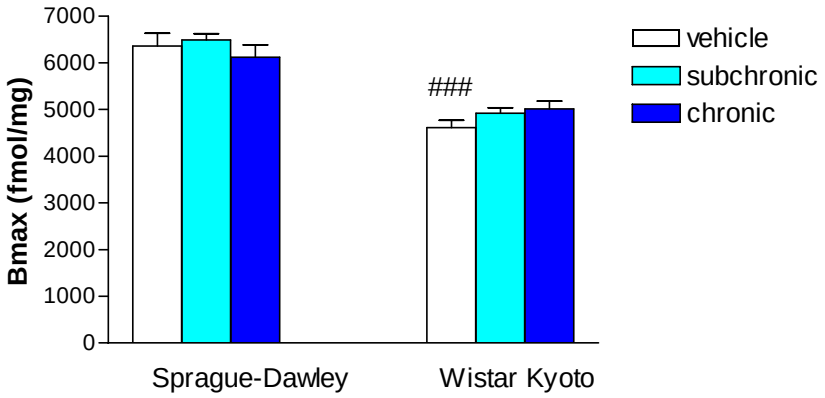

Fig. 4-12. Effect of desipramine at 10 mg/kg on particulate PKA binding: SD vs. WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; ###, $p<0.001$ vs. SD control.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Desipramine 10 mg/kg

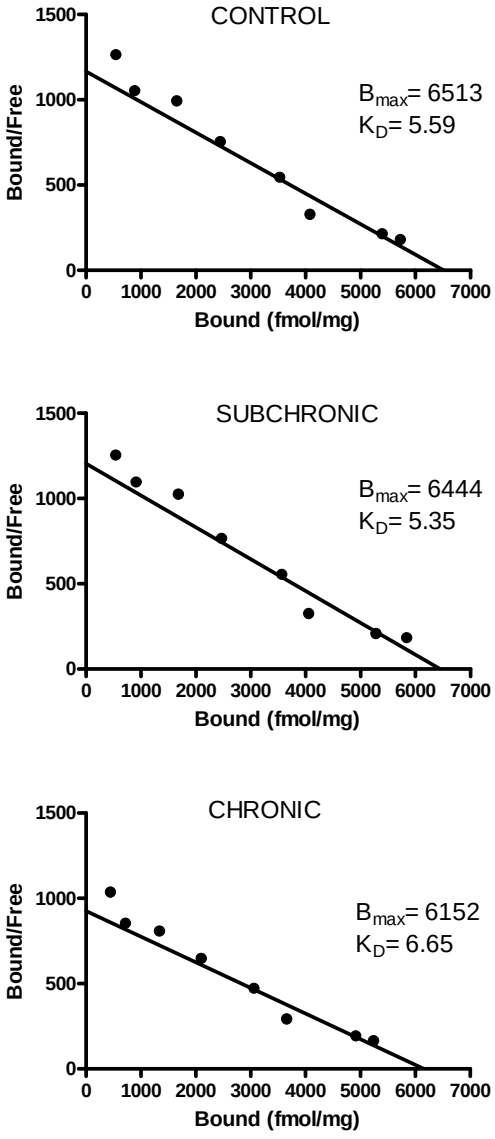

Fig. 4-13. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of SD rats. Control vs. subchronic or chronic desipramine 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Desipramine 10 mg/kg

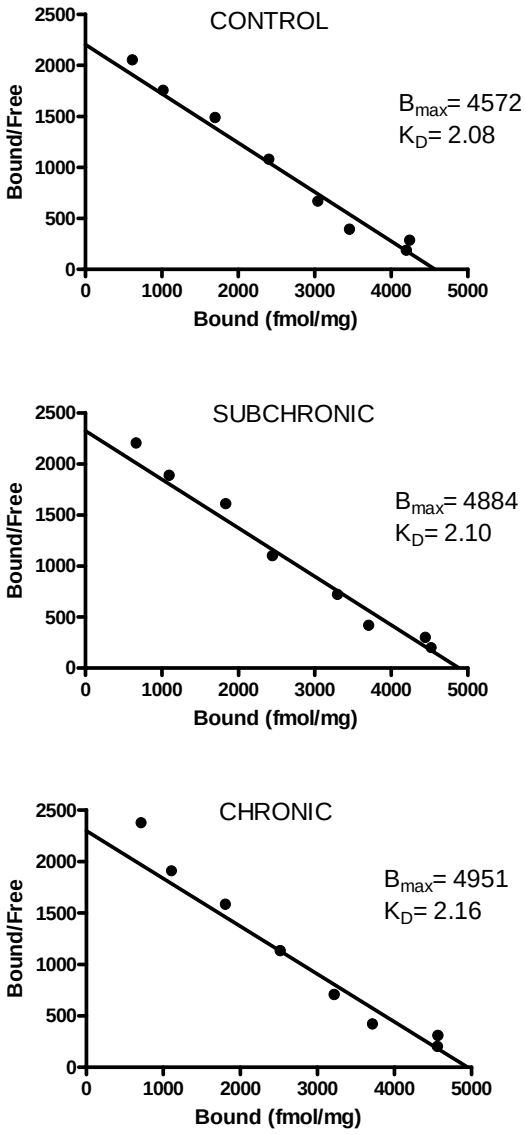

Fig. 4-14. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic desipramine 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand.Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Affinity Constants for Each Fraction of [^{3}H]-cAMP Binding for Desipramine Treatments (K_D in *nM*)

	Control		Subchronic		Chronic	
	Mean	SEM	Mean	SEM	Mean	SEM
Desipramine 1 mg/kg						
Cytosolic						
SD	3.50	0.70	2.63	0.26	4.32	0.81
WKY	5.43	0.74	9.09*	1.10	5.32$^{\#}$	0.30
Particulate						
SD	4.56	0.16	5.30	0.42	5.44	0.59
WKY	1.57	0.06	1.43	0.05	1.57	0.08
Desipramine 10 mg/kg						
Cytosolic						
SD	2.08	0.42	1.88	0.43	1.38	0.10
WKY	3.25	0.68	2.40	0.44	1.82	0.09
Particulate						
SD	5.49	0.28	5.59	0.19	6.70$^{**\#\#}$	0.32
WKY	2.22	0.05	2.24	0.03	2.32	0.07

Table 4-1. Affinity constants (K_D in *nM*) for [^{3}H]-cAMP binding. These were assessed (assay in triplicate) in both cytosolic and particulate fractions of frontal cortex of both SD and WKY rats. Effects of desipramine treatment were compared to strains' own control and also within strain subchronic and chronic treatment. Mean (+SEM) for n=6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons *, $p<0.05$, **<0.01 compared to control; #, $p<0.05$, ##<0.01 compared to subchronic.

Chapter 5
Effects of Bupropion on the Forced Swim Test and Protein Kinase A

Introduction

While the monoamine hypothesis of depression (Schildkraut, 1965; Bunney & Davis, 1965) does not separate the role of NE and 5-HT from DA (Willner, Muscat, Papp, & Sampson, 1991), monoamine oxidase inhibitors that increase brain concentrations of all three of these neurotransmitters are efficacious AD's (Stahl, 2000). Two reviews in the 1970's (Randrup *et al.*, 1975) and 1980's (Willner, 1983) addressed the overlooked neurotransmitter's potential role in the disease, thus creating interest in a DA hypothesis of depression. However, clinically available AD's that have a selective impact on DA remain elusive.

Bupropion is a widely prescribed first-line agent DA re-uptake inhibitor for the treatment of depression (Saddock & Saddock, 2001; Stahl, 2000; Arsana & Rosenbaum, 2000). It has weak re-uptake properties for DA and even weaker re-uptake properties for NE, but is more correctly described as a dual DA/NE re-uptake inhibitor (Ascher *et al.*, 1995; Stahl, 2000). The powerful impact on these transmitter systems have been difficult to explain based on the re-uptake properties alone (Stahl, 2000) and remain debatable (Ascher *et al.*, 1995). One explanation is that bupropion is broken down into a metabolite that is a more powerful NE re-uptake blocker than bupropion itself (Stahl, 2000). It

evidences a unique biochemical profile that differs greatly from all other AD's, with an unusual and not fully known NE link (Ascher *et al*., 1995).

Bupropion is known to be active in the FST for the heterozygous dopamine hydroxylase mouse, but not the dopamine hydroxylase deficient mouse (Cryan *et al*., 2001). In addition, it is active in the FST for the SD rat (Cooper, Hester, & Maxwell, 1980). Interestingly, it was determined that destruction of DA neurons eliminated that activity for bupropion in the FST (Cooper, Hester, & Maxwell, 1980). Those investigators thereby made the intriguing claim that bupropion acts as a selective DA re-uptake inhibitor *in vivo*, thus arguing against the biochemical data that diminishes the role of DA in bupropion's actions.

The hypothesis is here then that the two rat strains (SD, WKY) may differ in their response to bupropion, whether that be subchronically or chronically. In addition, the effect on PKA density could differ from that seen for other AD's, such as desipramine, since a unique biochemical profile has been claimed for bupropion.

Methods

Subjects

As in the general methods. Male WKY and SD rats 7.5-9 weeks of age at the start of the study.

Drug

The DA re-uptake inhibitor AD bupropion was used at two doses, 1 mg/kg and 10 mg/kg, ip. Bupropion HCl was purchased from Sigma Corporation. Preparation, dosing volume and dosing regimen was as in chapter four.

Tissue Preparation and Binding Assay

As in general methods for frozen tissue with a modified Rahman method (Rahman *et al*., 1977) except frontal cortices from individual rats were homogenized separately with a Caframo (Wiarton, Ontario) teflon stirrer. In order for the binding assay for each fraction to be completed in a single day, n=6 rats were selected randomly from n=10 per treatment group.

Data Analysis

As in chapter four.

Results

Forced Swim Test

Bupropion at a low dose of 1 mg/kg in the FST (see Figure 5-1) exhibited significant main effects for strain ($F_{(1,56)}$= 8.02, $p < 0.01$), but neither for treatment ($F_{(2,56)}$= 2.96, n.s.), nor for the strain X treatment interaction ($F_{(2,56)}$= 0.28, n.s.). Neither the vehicle control groups between the two strains differed nor the subchronic and chronic treatment groups differed significantly within strains.

Bupropion at a high dose of 10 mg/kg in the FST (see Figure 5-2) exhibited significant main effects for strain ($F_{(1,58)}$= 23.80, $p <0.001$) and treatment ($F_{(2,58)}$= 24.07, $p <0.001$), but not the strain X treatment interaction ($F_{(2,58)}$=0.69, n.s.). Significant decreases in immobility were demonstrated by both strains. This was shown subchronically (60% and 32%, $p<0.001$) and chronically (53%, $p<0.05$ and 38%, $p<0.01$) for SD and WKY rats, respectively. Neither the vehicle control groups between the two strains had differed nor the subchronic and chronic treatment groups differed within strains.

[^{3}H]-cAMP Binding

Bupropion at a low dose of 1 mg/kg in the cytosolic fraction of PKA exhibited main effects on B_{max} for strain ($F_{(1,35)}$= 124.90, p< 0.001), but neither for treatment ($F_{(2,35)}$= 0.02, n.s.), nor for the strain X treatment interaction ($F_{(2,35)}$= 0.03, n.s.). See Figure 5-3 for the mean results from individual hyperbolic analyses; Figures 5-4 and 5-5 depict scatchard plots of the same data as a single composite per treatment group. The vehicle control groups between strains differed significantly (p<0.001); however, the subchronic and chronic treatment groups did not differ within strains.

Significant main effects occurred for K_D for strain ($F_{(1,35)}$= 79.97, p<0.0001), but neither for treatment ($F_{(2,35)}$= 0.38, n.s.) nor the strain X treatment interaction ($F_{(2,35)}$= 1.16, n.s.). The control groups differed significantly between the two strains (p<0.0001). Neither strain differed within strain across treatment groups. See Table 5-1 for a summary of K_D results for all bupropion experiments.

In the particulate fraction, significant main effects occurred for strain ($F_{(1,35)}$= 343.22, p<0.001) and for treatment ($F_{(2,35)}$= 7.63, p<0.01), but not for the strain X treatment interaction ($F_{(2,35)}$= 0.61, n.s.). See Figure 5-6 for a composite view of the results from hyperbolic analyses; Figures 5-7 and 5-8 depict scatchard plots of the same data. Both strains evidenced a decrease (10%) in B_{max} following chronic injection, but this was significant (p< 0.05) only for the SD strain. The vehicle control groups between strains differed significantly (p<0.001); however, the subchronic and chronic treatment groups did not differ within strains.

Significant main effects occurred for K_D for strain ($F_{(1,35)}$= 65.34, $p<0.0001$), but neither for treatment ($F_{(2,35)}$= 1.78, n.s.) nor the strain X treatment interaction ($F_{(2,35)}$=0.81, n.s.). The control groups differed significantly between the two strains ($p<0.01$). Neither strain differed within strain across treatment groups.

Bupropion at a high dose of 10 mg/kg in the cytosolic fraction of PKA exhibited no main effects on B_{max} for strain ($F_{(1,35)}$= 4.14, n.s.) The effect for treatment was significant ($F_{(2,35)}$= 4.87, $p< 0.05$). The strain X treatment interaction was not significant: ($F_{(2,35)}$= 1.12, n.s.). The WKY strain evidenced a significant decrease in B_{max} (17%, $p<0.05$) following chronic injection. See Figure 5-9 for a composite view of the results from hyperbolic analyses; Figures 5-10 and 5-11 depict scatchard plots of the same data.

Neither the vehicle control groups between the two strains differed nor did the subchronic and chronic treatment groups differ within strains. Significant main effects occurred for K_D for strain ($F_{(1,35)}$= 4.44, $p<0.05$), but neither for treatment ($F_{(2,35)}$= 2.08, n.s.) nor the strain X treatment interaction ($F_{(2,35)}$=1.33, n.s.). The two strains did not differ in control group values nor were there any differences across treatment groups within strains.

In the particulate fraction significant main effects occurred for strain ($F_{(1,35)}$= 71.25, $p<0.001$), but not for treatment ($F_{(2,35)}$= 1.11, n.s.) nor the strain X treatment interaction ($F_{(2,35)}$= 0.09, n.s.). See Figure 5-12 for a composite view of the results from hyperbolic analyses; Figures 5-13 and 5-14 depict scatchard plots of the same data. The vehicle control groups between strains differed significantly ($p<0.001$); however, the subchronic and chronic treatment groups did not differ within strains.

Significant main effects occurred for K_D for strain ($F_{(1,35)}$= 14.42, $p<0.001$), but neither for treatment ($F_{(2,35)}$= 0.14, n.s.) nor the strain X treatment interaction ($F_{(2,35)}$=0.98, n.s.). The two strains did not differ in control group values nor were there any differences across treatment groups within strains.

Discussion

Previously Tejani-Butt *et al.* (2003) had found that while the WKY strain was sensitive to nomifensine, a NE and DA re-uptake inhibitor, the SD strain was not. The current laboratory had found that although both strains detect nomifensine, the WKY were more sensitive (Rogacki, Corbett & Wettstein, 2000). Due to lack of use of nomifensine clinically, in the present work the frequently prescribed DA re-uptake inhibitor bupropion was tested instead in the FST.

The low dose of bupropion was negative for both strains in the FST, but the SD strain demonstrated some decrease in immobility, more so chronically. At the high dose of bupropion, both strains (the SD strain highly so) evidenced an AD-like effect of that drug. While the immobility scores for the WKY controls in both swims were elevated as compared to the SD control values, these were not significantly different among strains. The magnitude of difference in immobility does not explain the larger effect for the SD strain with bupropion because that strain demonstrated lower control scores, but rather that strain may be more sensitive to DA as compared to the WKY strain.

Although the PKA density of the WKY strain was not affected significantly in either fraction by a low dose of bupropion, the SD strain demonstrated a decrease in the particulate fraction as a result of chronic treatment. In contrast, the SD strain was not

affected significantly in either fraction by a high dose of bupropion, while the WKY strain evidenced a decrease in the cytosolic fraction as a result of chronic treatment. This suggests the possibility of a differential regulation of DA by the two strains. At least at the second messenger level, the dose level of bupropion was interpreted differently. In addition, the fraction that demonstrated an impact of chronic drug treatment differed.

Neither strain evidenced a significant difference in subchronic and chronic treatments within the same strain in either fraction at either dose. The PKA density in both the cytosolic and particulate fractions for the control groups was usually lower, often in a highly significant manner, for the WKY strain as compared to the SD strain. This decrease was pronounced for the latter fraction.

No changes were demonstrated regarding affinity for [^{3}H]-cAMP as measured by K_D values. Bupropion had no effect on PKA function via this parameter.

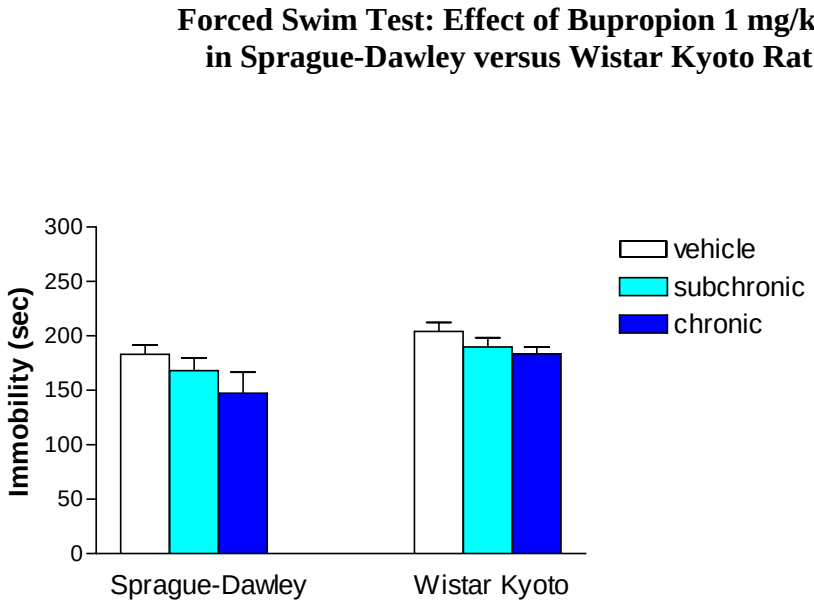

Fig. 5-1. Effect of bupropion at 1 mg/kg in the forced swim test:
SD vs. WKY rats. The vehicle and subchronic injection scheme included three ip drug injections: 24 hr, 5 hr, and 1 hr prior to the FST following 19 days of vehicle. The chronic group received one daily ip drug injection for 21 consecutive days. Immobility time in sec (+ SEM) was measured observationally. N=10 per treatment group.Two-way ANOVA followed by Bonferroni comparisons; nothing significant.

Forced Swim Test: Effect of Bupropion 10 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

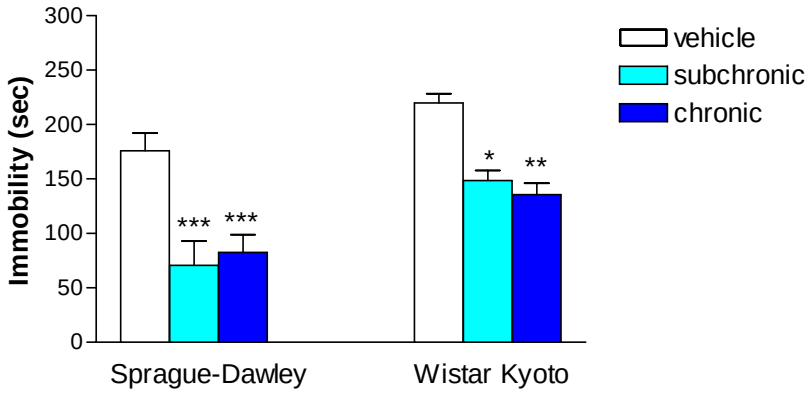

Fig. 5-2. Effect of bupropion at 10 mg/kg in the forced swim test: SD vs. WKY rats. The vehicle and subchronic injection scheme included three ip drug injections: 24 hr, 5 hr, and 1 hr prior to the FST following 19 days of vehicle. The chronic group received one daily ip drug injection for 21 consecutive days. Immobility time in sec (+ SEM) was measured observationally. N=10 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; *, $p<0.05$, **<0.01 vs. WKY control; ***<0.001 vs. SD control.

[^{3}H]-cAMP Binding in the Cytosolic Fraction of the Frontal Cortex: Effect of Bupropion 1 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

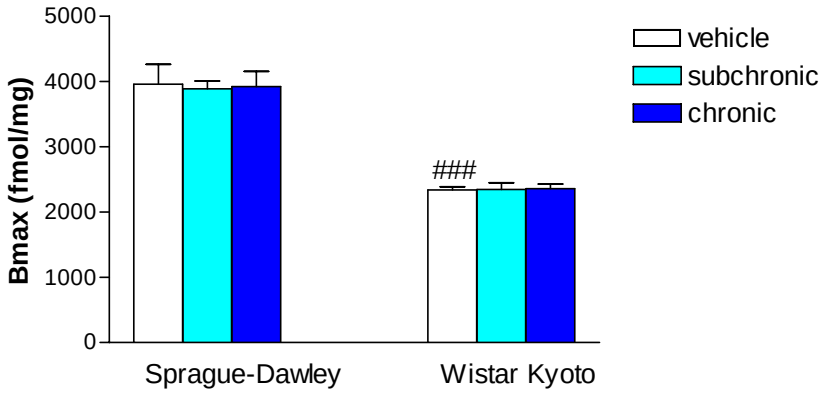

Fig. 5-3. Effect of bupropion at 1 mg/kg on cytosolic PKA binding: SD vs. WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; ###, $p<0.001$ vs. SD control.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Bupropion 1 mg/kg

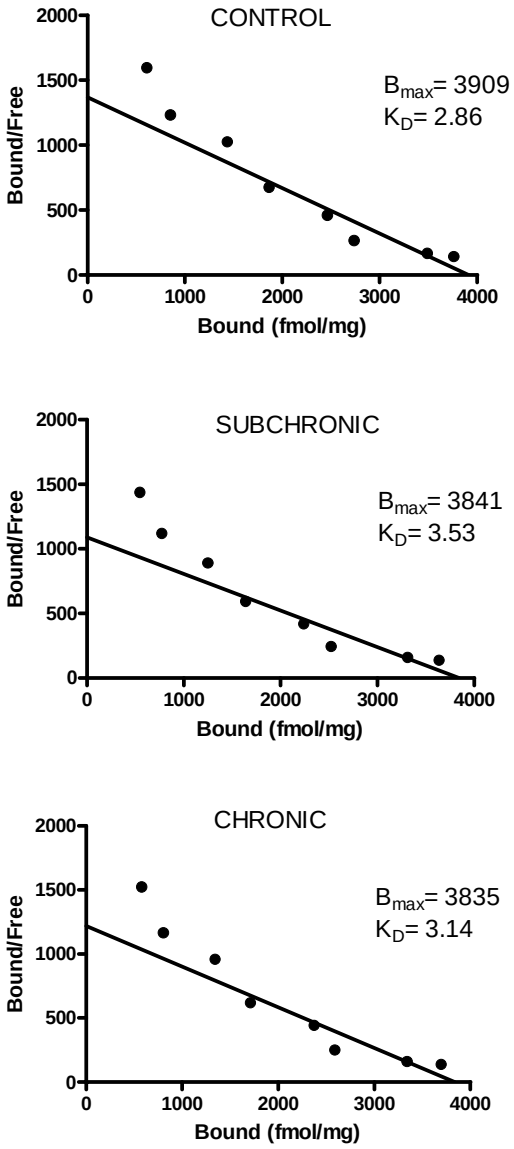

Fig. 5-4. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of SD rats. Control vs. subchronic or chronic bupropion 1 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein.
Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Bupropion 1 mg/kg

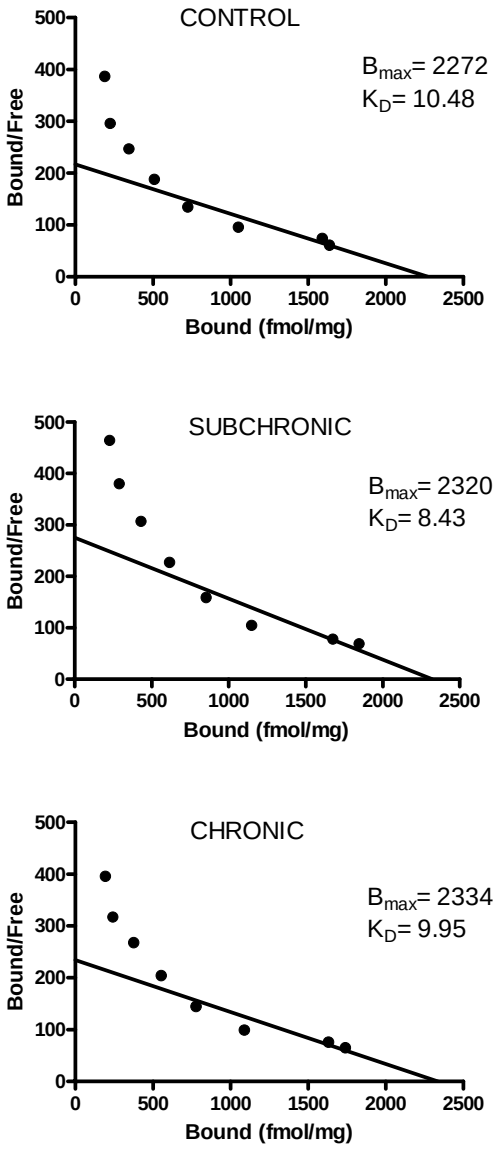

Fig. 5-5. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic bupropion 1 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein.
Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

[^{3}H]-cAMP Binding in the Particulate Fraction of the Frontal Cortex: Effect of Bupropion 1 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

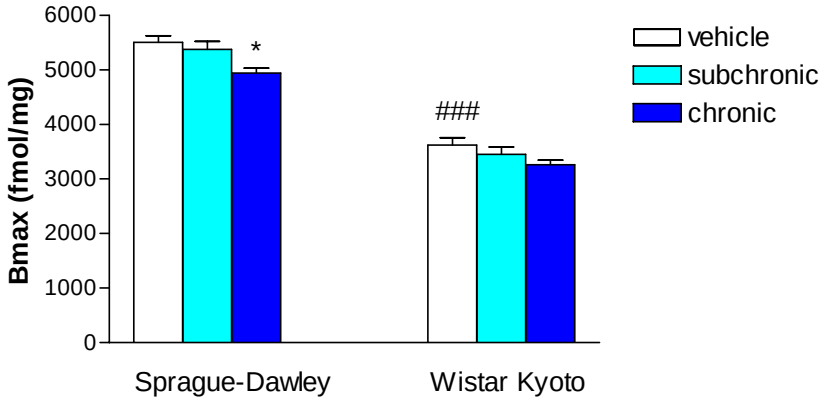

Fig. 5-6. Effect of bupropion at 1 mg/kg on particulate PKA binding: SD vs.WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; ###, $p<0.001$; *<0.05 both vs. SD control.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Bupropion 1 mg/kg

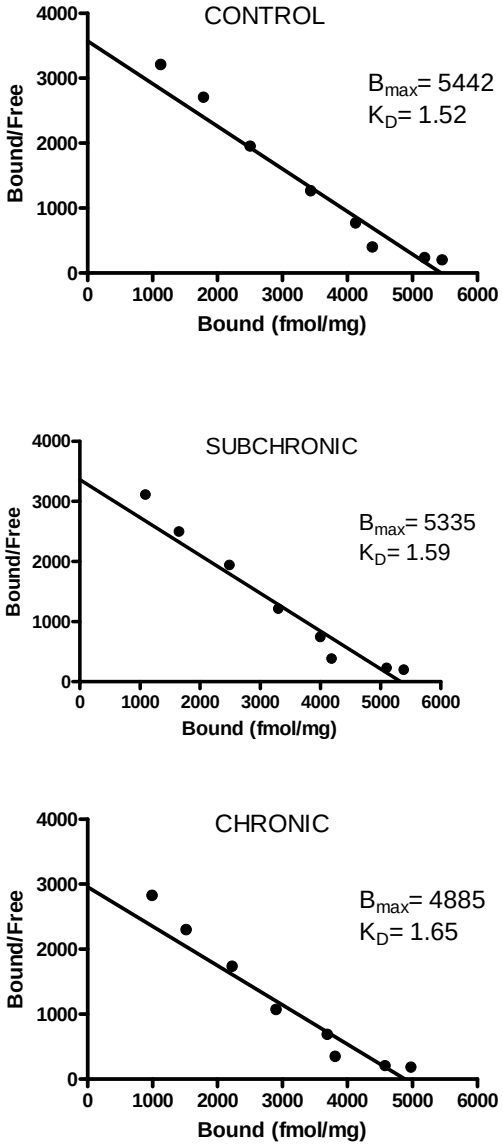

Fig. 5-7. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of SD rats. Control vs. subchronic or chronic bupropion 1 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Bupropion 1 mg/kg

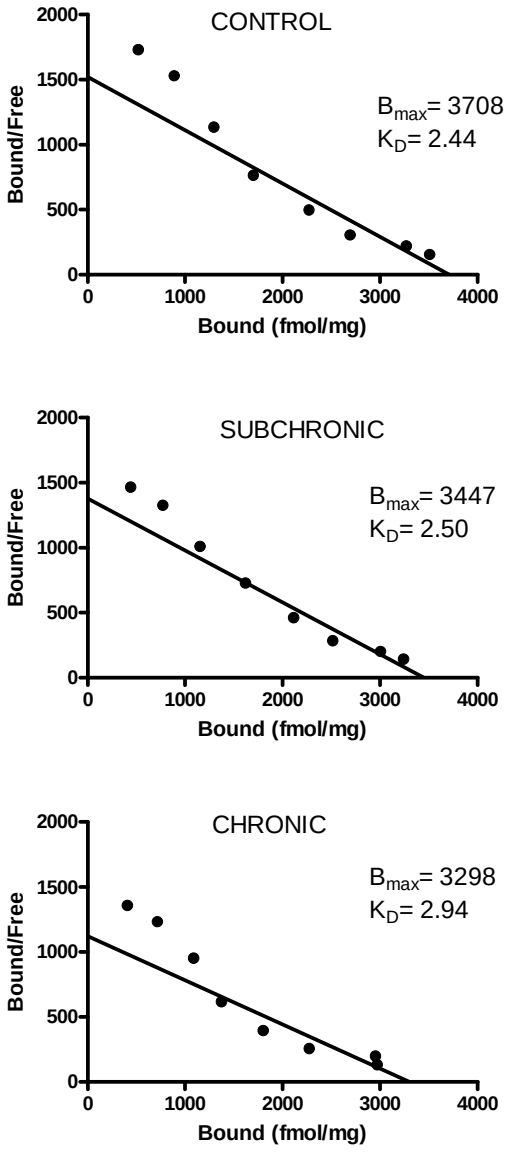

Fig. 5-8. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic bupropion 1 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

[^{3}H]-cAMP Binding in the Cytosolic Fraction of the Frontal Cortex: Effect of Bupropion 10 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

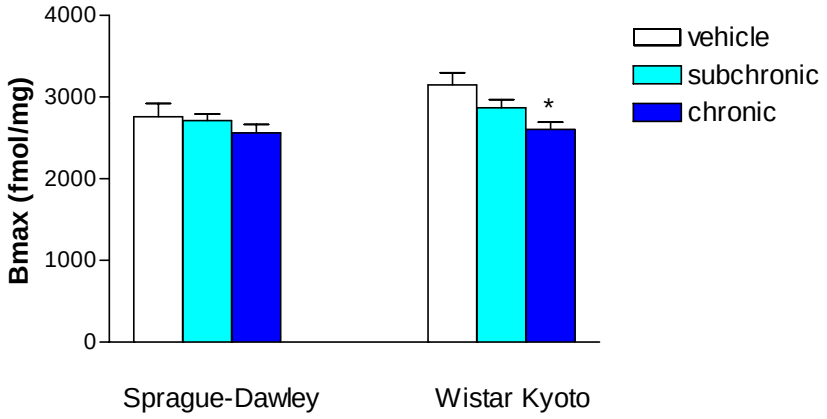

Fig. 5-9. Effect of bupropion at 10 mg/kg on cytosolic PKA binding: SD vs. WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; *, $p<0.05$ vs. WKY control.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Bupropion 10 mg/kg

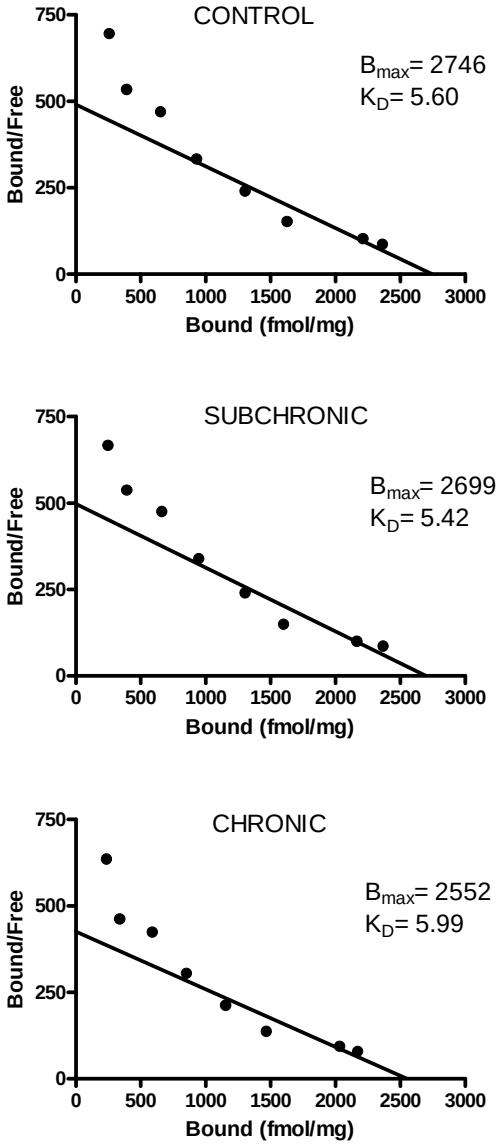

Fig. 5-10. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of SD rats. Control vs. subchronic or chronic bupropion 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Cytosolic Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Bupropion 10 mg/kg

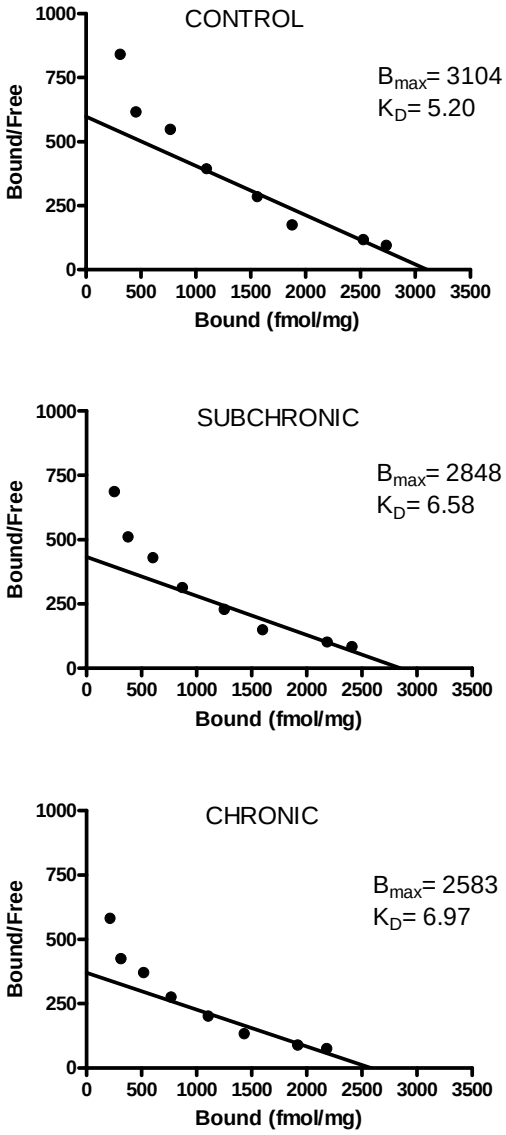

Fig. 5-11. Scatchard analysis of the saturation functions for the cytosolic fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic bupropion 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand.Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein.Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

[^{3}H]-cAMP Binding in the Particulate Fraction of the Frontal Cortex: Effect of Bupropion 10 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

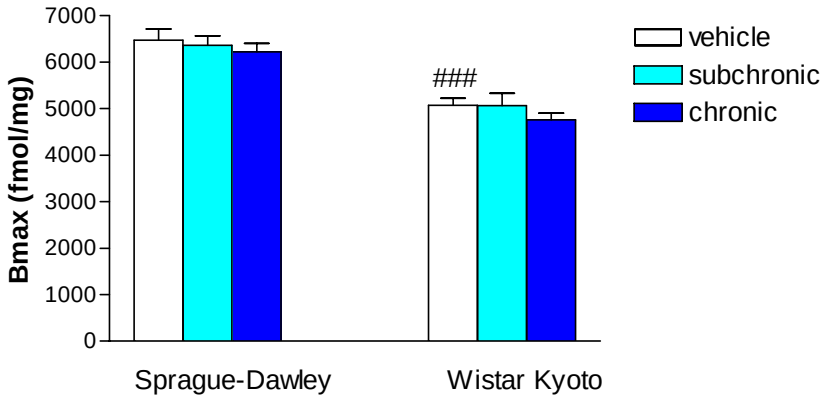

Fig. 5-12. Effect of bupropion at 10 mg/kg on particulate PKA binding: SD vs. WKY rats. Saturation binding assays were conducted with approximately 40 *ug* protein and various concentrations of [^{3}H]-cAMP (0.125-25 *nM*). Incubation was at 25 ^{o}C for 60 min followed by rapid filtration. N= 6 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; ###, $p<0.001$ vs. SD control.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Sprague-Dawley Rat: Effect of Subchronic and Chronic Bupropion 10 mg/kg

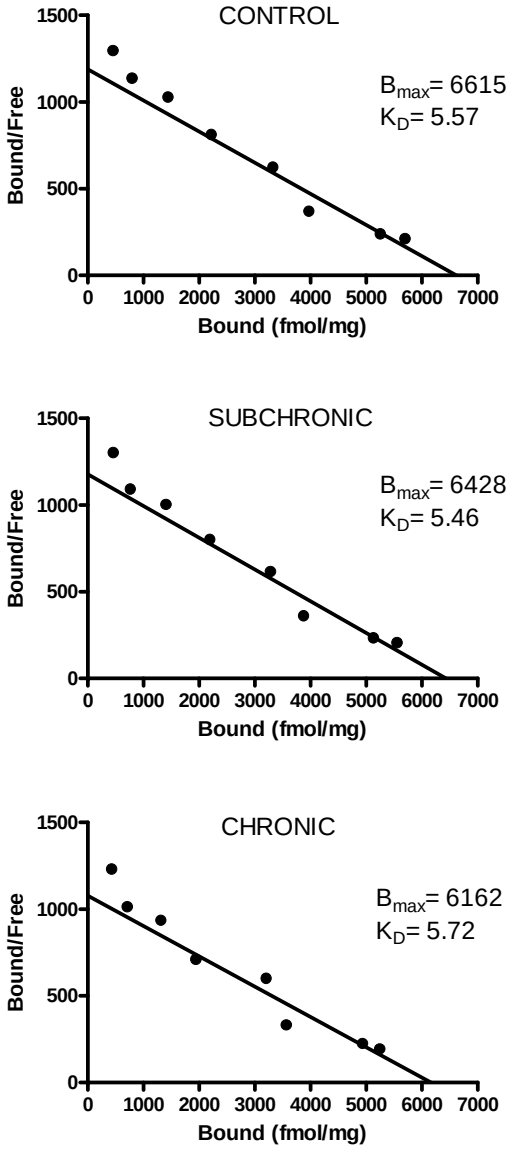

Fig. 5-13. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of SD rats. Control vs. subchronic or chronic bupropion 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Scatchard Analysis of the Particulate Fraction of Frontal Cortex from Wistar Kyoto Rat: Effect of Subchronic and Chronic Bupropion 10 mg/kg

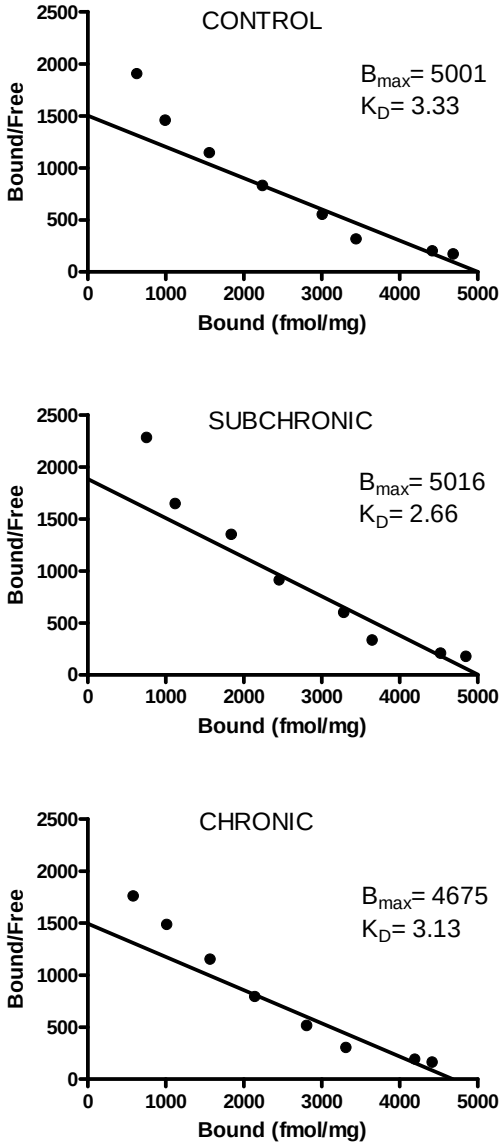

Fig. 5-14. Scatchard analysis of the saturation functions for the particulate fraction of frontal cortex of WKY rats. Control vs. subchronic or chronic bupropion 10 mg/kg injection (ip). X axis: Bound divided by free [^{3}H]-cAMP radioligand. Y axis: Bound [^{3}H]-cAMP expressed in fmol/mg of protein. Inset: B_{max} and K_D values obtained by a one site hyperbolic fit for each treatment group. Each line represents the pooled data from 6 separate rats within a given treatment group.

Affinity Constants for Each Fraction of [^{3}H]-cAMP Binding for Bupropion Treatments (K_D in *nM*)

	Control		Subchronic		Chronic	
	Mean	SEM	Mean	SEM	Mean	SEM
Bupropion 1 mg/kg						
Cytosolic						
SD	3.27	0.39	3.87	0.38	3.51	0.32
WKY	11.31	1.54	9.11	1.20	10.32	0.93
Particulate						
SD	1.72	0.08	1.74	0.08	1.84	0.06
WKY	2.86	0.35	3.02	0.14	3.49	0.31
Bupropion 10 mg/kg						
Cytosolic						
SD	5.80	0.29	5.60	0.31	6.14	0.32
WKY	5.73	0.65	6.92	0.48	7.29	0.59
Particulate						
SD	5.59	0.38	6.26	0.72	6.15	0.73
WKY	4.36	1.51	2.86	0.20	3.46	0.44

Table 5-1. Affinity constants (K_D in *nM*) for [^{3}H]-cAMP binding. These were assessed (assay in triplicate) in both cytosolic and particulate fractions of frontal cortex of both SD and WKY rats. Effects of bupropion treatment were compared to strains' own control and also within strain subchronic and chronic treatment. Mean (+SEM) for n=6 per treatment group. Two-way ANOVA followed by Bonferroni comparison, nothing significant.

Chapter 6
Effects of Alprazolam on the Forced Swim Test

Introduction

A link between bipolar disorder and GABA deficit led to the GABA theory of depression (Emrich, Zerssen, Kissling, Moller, & Windorfer, 1980). This was based on the clinical effectiveness of valproic acid, which elevates brain GABA, and alleviates manic-depression. Furthermore, low plasma levels of GABA have been indicated as a trait-like marker for bipolar disorder (Petty, Kramer, Fulton, Moeller, & Rush, 1993; Petty, 1994). Plasma GABA was found to be low in 40% of unipolar depressives, and remained low in either phase of bipolar disorder (Petty, Kramer, Fulton, Moeller, & Rush, 1993). In the case of unipolar depression, the GABA mimetics progabide (Morselli *et al*., 1986) and fengabine (Musch, 1986) were effective in most patients. Gamma amino butyric acid was thus proposed as a common link between unipolar and bipolar depression.

Besides the aforementioned indications regarding the $GABA_A$ (benzodiazepine receptor) and release of GABA by valproic acid, the $GABA_B$ receptor is implicated as a target of many AD drugs from different classes. Specifically, various AD's increase the number of $GABA_B$ receptors in rat frontal cortex (Lloyd, Thuret, & Pilc, 1985). In behavioral models such as learned helplessness and olfactory bulbectomy, imipramine

upregulates $GABA_B$ receptors only in those animals that respond behaviorally to the AD (Lloyd, Zivkovic, Scatton, Morselli, & Bartholini, 1989).

Therefore, it appears that both $GABA_A$ and $GABA_B$ receptors are implicated in the pharmacological treatment of depression. In the former case direct effects are noted. In the latter case it seems that while indirect action may occur as AD's from multiple classes impact $GABA_B$ receptors, there is currently no available AD that acts selectively at that receptor.

The anxiolytic alprazolam was used here due to both clinical reports of its AD efficacy and one report of effect in the FST. A review was conducted of the clinical effectiveness of three benzodiazepines that are widely prescribed for major depression (Petty, Trivedi, Fulton, & Rush, 1995). While two were not implicated as AD in action, one (alprazolam) was equivalent to the TCA's in the treatment of major depression, but with a faster onset of action. One group claimed AD effects for that compound in the FST, as subchronic alprazolam reduced immobility time in rats similar to that of desipramine (Flugy, Gagliano, Cannizzaro, Novara, & Cannizzaro, 1992).

The hypothesis here then was that alprazolam would yield an AD profile in the FST in one strain or the other and that this profile could differ subchronically versus chronically. If an AD-like effect could be demonstrated, then PKA binding would be conducted.

Methods

Subjects

As in the general methods. Male WKY and SD rats were 7.5-9 weeks of age at the start of the study.

Drug

The benzodiazepine anxiolytic alprazolam was used at two doses, 2 mg/kg and 20 mg/kg, ip. Alprazolam free base was purchased from Sigma Corporation. It was prepared as a suspension in distilled water via homogenzation in a 0.5% carboxy methylcellulose vehicle. Dosing volume and regimen was as in chapter four.

Results

Forced Swim Test

Alprazolam at a low dose of 2 mg/kg in the FST (see Figure 6-1) exhibited no significant main effects for strain ($F_{(1,59)}$= 0.48, n.s.), treatment ($F_{(2,59)}$= 1.38, n.s.) , nor for the strain X treatment interaction ($F_{(2,59)}$= 0.78, n.s.). The SD strain demonstrated a 22% increase in immobility with chronic treatment that was not significant, with no appreciable increase with the subchronic treatment. The WKY were consistent with immobility at approximately vehicle control level with either injection scheme. Neither the control groups between the two strains differed nor did the subchronic and chronic treatment groups differ within strains.

Alprazolam at a high dose of 20 mg/kg in the FST (see Figure 6-2) exhibited significant main effects for strain ($F_{(1,59)}$= 16.73, $p < 0.001$) and for treatment ($F_{(2,59)}$= 11.32, $p < 0.001$). The strain X treatment interaction was not significant ($F_{(2,59)}$= 2.09, n.s.). The SD strain demonstrated significant increases in immobility (32%, $p<0.05$ and 30%, $p<0.01$), respectively, with subchronic and chronic dosing. The increase (29%) in immobility that was demonstrated by the WKY strain subchronically was not significant.

Neither the vehicle control groups between the two strains differed nor did the subchronic and chronic treatment groups differ within strains.

Discussion

Regarding the benzodiazepine alprazolam, an interesting review of the clinical data had indicated that perhaps this anxiolytic possessed a dual indication as an AD, and that in fact, the onset of action of alprazolam as a treatment for major depression surpassed temporally that of the widely used AD's (Petty, Trivedi, Fulton, & Rush, 1995). In addition, one group found that subchronic alprazolam decreased immobility time in the FST comparable to desipramine, indicative of an efficacious AD effect (Flugy, Gagliano, Cannizzaro, Novara, & Cannizzaro, 1992).

The present work conducted with alprazolam at two doses with subchronic and chronic injection paradigms were in direct contrast with the results of Flugy and colleagues. Even at the lowest dose, the SD strain demonstrated a trend of increased immobility with chronically administered alprazolam. The WKY showed a trend of increased immobility at the high dose of alprazolam, while the SD evidenced significantly increased immobility with both the subchronic and chronic injection modes.

The most likely explanation for this was the clearly sedative effect of alprazolam, especially at the highest dose. The benzodiazepines are well-documented for that side effect (Sadock & Sadock, 2001). The rats appeared very relaxed throughout the FST in direct contrast to the stressed escape reaction that is seen characteristically at the onset of the test. Because alprazolam did not exhibited AD effects in the FST, the PKA saturation binding was not conducted.

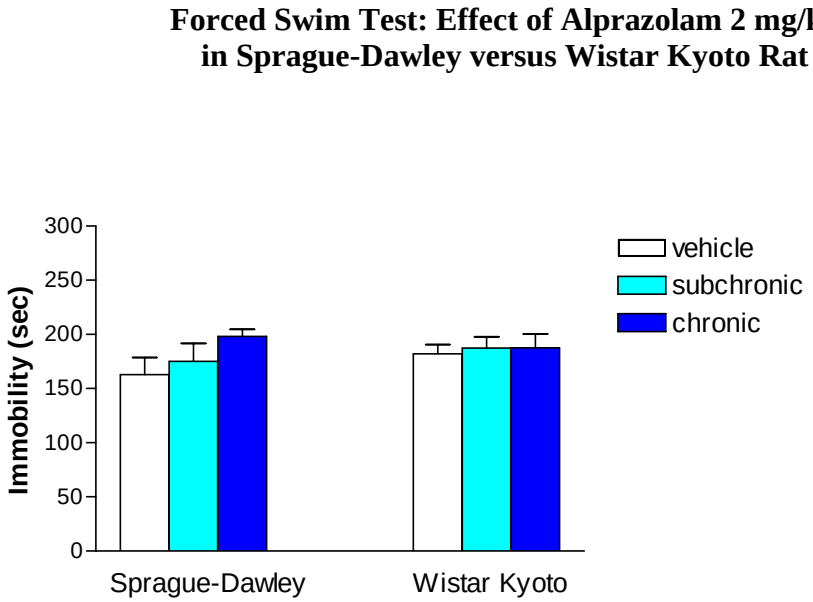

Fig. 6-1. Effect of alprazolam at 2 mg/kg in the forced swim test: SD vs. WKY rats. The vehicle and subchronic injection scheme included three ip drug injections: 24 hr, 5 hr, and 1 hr prior to the FST following 19 days of vehicle. The chronic group received one daily ip drug injection for 21 consecutive days. Immobility time in sec (+ SEM) was measured observationally. N=10 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; nothing significant.

Forced Swim Test: Effect of Alprazolam 20 mg/kg in Sprague-Dawley versus Wistar Kyoto Rat

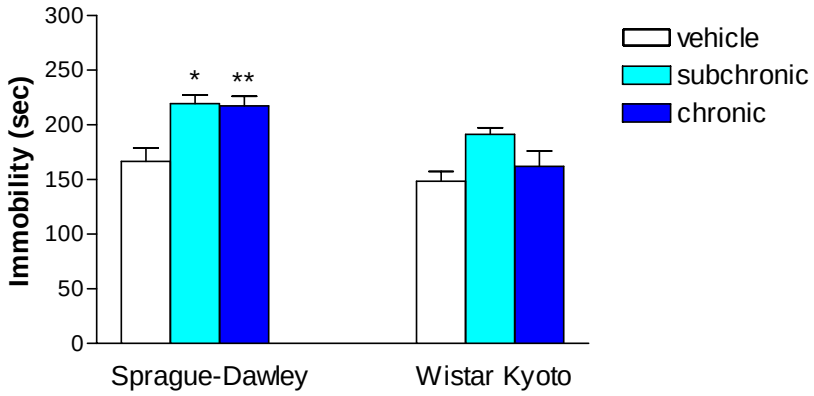

Fig. 6-2. Effect of alprazolam at 20 mg/kg in the forced swim test: SD vs. WKY rats. The vehicle and subchronic injection scheme included three ip injections: 24 hr, 5 hr, and 1 hr prior to the FST following 19 days of vehicle. The chronic group received one daily injection for 21 consecutive days. Immobility time in sec (+ SEM) was measured observationally. N=10 per treatment group. Two-way ANOVA followed by Bonferroni comparisons; *, $p<0.05$, **<0.01 vs. SD control.

Part Seven: Forced Swim Test and Protein Kinase A Control Levels

Chapter 7
Baseline Comparisons Across Studies

Introduction

As noted earlier, one group of investigators found lowered PKA densities in depressed suicide victims (Dwivedi, Conley, Roberts, Tamminga, & Pandey, 2002; Dwivedi *et al*., 2004). These 2002 findings were replicated in 2004 with two additional cohorts. Because the lowered densities were evidenced in both the cytosolic and the membrane fractions as compared to nonpsychiatric control subjects, these researchers concluded that abnormalities in PKA function are not specific to certain compartments, but rather generalized. Reduced [^{3}H]-cAMP binding without reduced affinity may indicate a lesser abundance of regulatory subunits present in prefrontal cortex of depressed suicides (Dwivedi *et al*., 2004).

Methods

As stated in the general methods and chapters four through six. These data are the combined control groups from those studies.

Data Analysis

Unpaired two-tailed *t* tests were conducted with GraphPad Prism version 4.03 software (GraphPad Software, Inc., San Diego, CA). Variability is reported as standard error of the mean.

Results

Forced Swim Test

An unpaired *t* test analysis between combined control group FST scores from six separate studies per strain (twelve studies total) revealed that the WKY rat evidenced a higher average immobility score (197.8 sec +/- 3.50) as compared to the SD rat (169.9 sec +/- 4.47); $t(1,18)=4.92$, $p<0.0001$. See Figure 7-1.

[^{3}H]-cAMP Binding

An unpaired *t* test analysis between combined control group cytsolic fraction $B_{max's}$ from four separate studies per strain (eight studies total) revealed that the WKY rat evidenced a lower average B_{max} value (2489 fmol/mg +/- 73.85) as compared to the SD rat (3185 fmol/mg +/- 138.5); $t(1,10)=4.43$, $p<0.01$. See Figure 7-2.

Additionally, an unpaired *t* test analysis was conducted for the K_D's produced by those same control groups. The WKY strain demonstrated a significantly lower affinity (6.34 +/- 0.59) in the cytosolic fraction as compared to the SD strain (3.66 +/- 0.16); $t(1,10)=4.51$, $p<0.01$.

An unpaired *t* test analysis between combined control group particulate fraction $B_{max's}$ from four separate studies per strain (eight studies total) revealed that the WKY rat evidenced a lower averaged B_{max} value (4365 fmol/mg +/- 51.30) as compared to the SD rat (5992 fmol/mg +/- 197.0); $t(1,10)=8.00$, $p<0.0001$. See Figure 7-3.

Additionally, an unpaired *t* test analysis was conducted for the K_D's produced by those same control groups. The WKY strain demonstrated a significantly higher affinity

(2.75 *nM* +/- 0.34) in the particulate fraction as compared to the SD strain (4.36 *nM* +/- 0.17); $t(1,10)=4.19$, $p<0.01$.

Discussion

The higher immobility control behavior demonstrated in the FST by the WKY rat as compared to the SD rat was anticipated by previous work (Lopez-Rubalcava & Lucki, 2000; Pare, 1989; Rogacki, Corbett, & Wettstein, 2000; Tejani-Butt, Kluczynski, & Pare, 2003). This higher immobility baseline in the FST is one hallmark of the WKY rat's "depressive" characteristics. This marker of "behavioral despair" in the FST has similarly been shown in mice (NMRI; Vaugeois, Passera, Zuccaro, & Costentin, 1997) and other rat strains (Brown Norway, Flinders Sensitive Line; Gomez, Lahmame, de Kloet, & Armario, 1996; Malkesman *et al.*, 2005) that reveal analogs of depression.

Regarding lowered PKA densities in both cytosolic and particulate fractions, the findings here for the SD rat as compared to the WKY rat align with those findings from the Dwivedi group for both fractions in the prefrontal cortex of normal controls as compared to depressed suicides (Dwivedi, Conley, Roberts, Tamminga, & Pandey, 2002; Dwivedi *et al.*, 2004). Similarly here, the WKY rat, an analog of human depression, demonstrated significant lowering of PKA densities in both fractions. It is difficult to ascertain from the clinical work mentioned whether this lowering has more to do with the disease state or agonal state, as the tissues were taken from suicide victims.

It should be noted, however, that another group found PKA density in the cytosolic fraction of nonpsychiatric controls to be the same as that in depressive suicides (Lowther,

et al., 1997). That group did assess different Brodmann areas of the cortex, perhaps indicative of regional variation of PKA density within the frontal cortex.

Although the K_D values were significantly different between the two strains for both fractions, the actual differences were less than two-fold in magnitude, probably not different enough to convey an alteration in the functionality of the receptor. While the changes are small, it is noteworthy that for the soluble fraction, both the K_D and B_{max} values move in the direction of making cAMP less capable of activating PKA for the WKY (higher K_D-lower affinity, and lower B_{max}). In contrast, in the particulate fraction, the two values move in opposite directions: still lower overall B_{max}, but a higher affinity (lower K_D).

Taken together, the WKY rat is confirmed again as an analog of human depression, not only in terms of past work regarding the “depressive” behavior demonstrated in the FST, but also here regarding PKA regulatory function. This is a crucial point as better understanding of PKA arises. The WKY rat may serve as a useful strain to investigate abnormalities of PKA function.

Forced Swim Test Control Level of Immobility: Sprague-Dawley versus Wistar Kyoto Rat

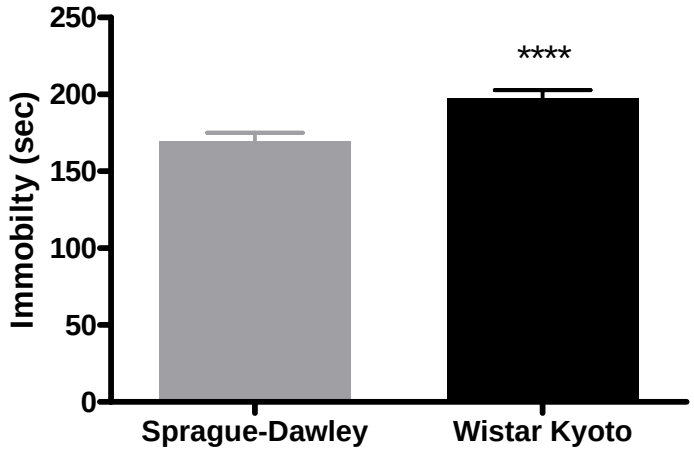

Fig. 7-1. Control levels of immobility for the SD and WKY strains in the FST. N=60 per strain. The WKY evidenced a much higher endogenous immobility baseline. Unpaired *t* test; ****, p<0.0001.

[^{3}H]-cAMP Binding in the Cytosolic Fraction of the Frontal Cortex: Control Levels in the Sprague-Dawley versus Wistar Kyoto Rat

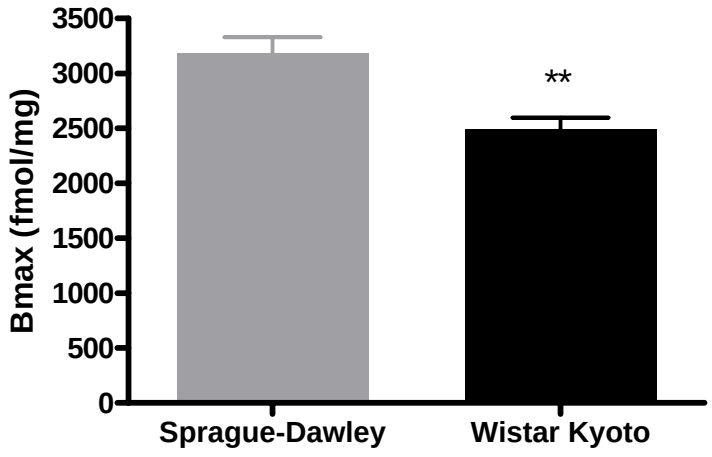

Fig. 7-2. Control levels of [^{3}H]-cAMP binding for the SD and WKY strains in the cytosolic fraction of the frontal cortex.Y axis: Receptor density measured as B_{max} in fmol/mg of protein. The WKY evidenced a comparatively lower PKA density. N=24 per strain. Unpaired *t* test; **, $p<0.01$.

[^{3}H]-cAMP Binding in the Particulate Fraction of the Frontal Cortex: Control Levels in the Sprague-Dawley versus Wistar Kyoto Rat

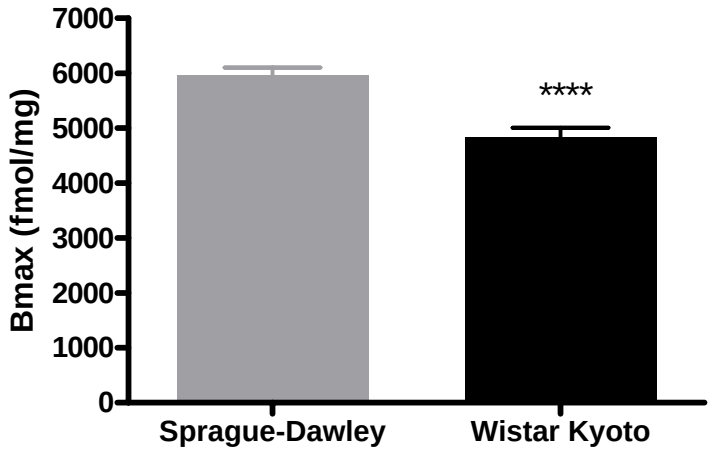

Fig. 7-3. Control levels of [^{3}H]-cAMP binding for the SD and WKY strains in the particulate fraction of the frontal cortex. Y axis: Receptor density measured as B_{max} in fmol/mg of protein. The WKY evidenced a comparatively lower PKA density. N=24 per strain. Unpaired *t* test; ****, $p<0.0001$.

Part Eight: General Discussion

Summary of Overall Findings

The cAMP system has been long proposed as dysregulated in depression, but it was not until the Lowther and colleagues' 1997 study that the role of cAMP's receptors, the PKA regulatory subunits, were addressed. While measuring cAMP levels in the brains of depressed patients is impossible, indirect methods such as cAMP assays conducted on skin fibroblasts and blood platelets had previously indicated that this second messenger may be dysregulated in depression. The various AD treatments that came about in the 60's and 70's (TCA's, SSRI's) created an almost exclusive focus on the monoamines, perhaps to a detrimental extent that persists until current times. Second messenger effects in this disease are still more proposed than researched, with a relatively small number of studies and hypotheses focusing on cAMP. The focus on monoamines continues to hinder elucidation of what may be cAMP's pivotal role in depression.

No study had addressed the effects of AD's on the PKA regulatory subunits until Lowther *et al.* (1997) showed that depressed suicides that had been chronically treated with AD's were found to have lowered PKA density in the cytosolic fraction of the frontal cortex. Since the clinical studies have many uncontrolled variables, the scope of this thesis was to determine in controlled studies in rodents whether effects of AD's on PKA density could be shown. One controllable variable would be use of strains since AD effects could be measured both in a "normal" control strain and one known to exhibit depressive characteristics, thus determining whether these systems respond differently to

AD treatment. If PKA density changes due to AD treatment were measurable, then many additional assessments of the cAMP-PKA system are possible. One utility would be the ability to screen novel compounds for AD effect if specific profiles of AD effects on PKA density are discriminable. Secondly, another utility is to understand how AD's affect the second messenger systems and whether differing mechanisms of action have their own profile of impact, which could explain why certain drugs work for certain patients. Thirdly, another utility would be elucidation of what underscores the need for chronic AD treatment to alleviate depressive symptomatology. This is the first work to assess AD effects on regulatory PKA in a controlled laboratory setting.

From these experiments the overall findings were as follows:

1. *Overall few correlations between behavior (FST scores) and biochemistry (PKA binding) were found.* Pearson's correlations were conducted using Sigmastat (SPSS, Inc., Chicago, IL). While some subchronic and chronic effects of desipramine appeared to alter both parameters, the findings are difficult to interpret as significant correlations were also found in only two out of sixteen control group comparisons. If there were correlations between the FST and PKA binding at control levels, then this finding should be consistent for a given strain. The small number of samples possible here (n=6 per treatment group) make correlational analysis difficult. See Table GD-1.

2. *The SD and WKY strains evidence different behaviors in the FST both at control levels (immobility baseline), as well as response to drug treatments.* While some of this

was previously reported (desipramine effects, immobility baseline) or seen in the current laboratory, some of the drugs had not been tested before (bupropion for the WKY strain, alprazolam for both strains). In addition, it was not known if these effects would differ subchronically (characteristic FST paradigm) and chronically. A hypothesis was that drug effects could alter behavioral effects subchronically versus chronically in the FST, and that perhaps this could underscore a change in the cAMP-PKA system, such as downregulation. Forced swim test behavior did not change for either strain due to injection scheme.

3. *Antidepressants affected PKA density. Strain differences were evidenced regarding drug, dose level, and injection scheme.* Both the SD and the WKY strains responded to desipramine by demonstrating increases and decreases in PKA density with despramine and bupropion, respectively. This may underlie the differing mechanism of action of these two drugs, one being a NE re-uptake inhibitor and the other a DA re-uptake inhibitor with a more mixed, unknown mechanism. While the WKY strain showed altered PKA density responses to only high doses of drug treatment, the SD strain also responded to low drug doses that were negative in the FST. In addition, these responses at low doses for the SD strain occurred only in the particulate fraction. In either strain, significant effects at high drug doses occurred only in the cytosolic fraction. These differing dose level effects may reflect the sensitivity of the SD strain to respond to low doses of AD's at the membrane level, while both strains may have demonstrated transcriptional processes (not measured here) due to high dose levels of AD's since the

cytosolic PKA regulatory receptor(s) are proposed to phosphorylate proteins that translocate to the nucleus to initiate transcription.

4. *PKA control levels are lower in both cytosolic and particulate fractions for the WKY strain as compared to the SD strain.* The WKY strain appears to have reduced PKA levels akin to that seen in depressed suicides as measured by reduced PKA density in both receptor fractions. This may be another one of the WKY strain's analogs of human depression in addition to those previously published for that strain, including many behavioral, neuroendocrine, and biochemical disturbances.

Overall Findings

This is the first work that has assessed a receptor that binds to cAMP and may be altered in density by AD treatment in a controlled laboratory paradigm. In addition, this was conducted in two strains of rats, one "normal" and one characterized to exhibit depressive characteristics. The purpose of the strain comparison was to determine whether these strains, previously found differentially sensitive to AD compounds administered subchronically in an animal model of depression (the FST), would also interpret AD's differently at the cAMP receptor level. Although it is frequently accepted that proper strain choice is crucial to demonstrating an effect in a behavioral model, biochemical studies most often overlook this parameter. Conclusions based on one specific strain for a given biochemical measure may be extrapolated to human populations although it may not even apply to another strain of the same species.

The current work addresses not only use of strain by which to draw overriding conclusions, but also the extrapolation of results from a given laboratory. Regarding the

FST, it had already been mentioned that the exact paradigm used to assess AD compounds can radically change the ability to detect drug effects. In the present laboratory, the standard Porsolt method and the WKY rat strain have been found as the most reliable to yield positive effects for AD drugs of varying mechanism of action. Although the SD rat did show some behavioral responsiveness to desipramine in the present work, the WKY rat was found to be more sensitive. In contrast, at the receptor level, the SD strain had appeared to be the more sensitive one to detect changes in PKA density. This work therefore emphasizes the need to exhibit caution about strain choice for chosen measures. The use of a single strain may be very misleading.

Also misleading can be the assumption that various groups working with a receptor that binds to [^{3}H]-cAMP are all assessing the same receptor and that one or all of these groups are actually measuring PKA. A few of the groups had published a minimal pharmacological profile of the examined receptor, but some groups have shown no such profile. The majority have mentioned a one site fit in one or both of the fractions tested, but which PKA receptor subtype(s) is (are) being measured, if it is indeed a PKA receptor ?

In the present work, the measured K_D's for [^{3}H]-cAMP saturation binding are in reasonable agreement with most groups, with the exception of the Dwivedi group. The densities tend to vary more across investigators, with the extremes of low density being that of the Dwivedi group or of high density being that shown here. Considering that the second messenger cAMP is a pathway utilized by numerous G protein-coupled receptors,

it would make intuitive sense that the density of PKA receptors would be correspondingly very high.

Throughout the published work on [^{3}H]-cAMP binding, the B_{max}'s tend to correspond with the density, or just a fewfold higher, of most individually known G-protein coupled receptors found in the brain. Any assessment done to characterize the receptor measured here confirmed that the high magnitude of binding was attributed to a single receptor that had bound labeled cAMP and displaced unlabeled cAMP.

Since no publications exist that have shown as extensive a binding profile as the present work, it can only be assumed that probably the same receptor measured by the majority of groups is the same receptor assessed here although the measured density is a several fold higher than the highest reported. Is this really a PKA receptor ?

PKA is not the only cAMP-binding protein in existence. Several putative such proteins were recently studied, with the Epac proteins (exchange protein directly activated by cAMP) among them (Dremier, Kopperud, Doskeland, Dumont, & Maenhaut, 2003). Both purified EpacI and PKA RIα bound highly to [^{3}H]-cAMP and that binding was displaced by unlabeled cAMP. This was not the case for a few other putative cAMP binding proteins tested . This suggests that PKA-independent pathways exist regarding cAMP-dependent effects. The dogma that PKA is the main intracellular receptor in mammalian cells has permitted the perhaps false assumption by several groups that they have definitively measured a PKA receptor simply on the basis of labeling and displacing cAMP.

While it is acknowledged here that the receptor measured cannot be termed PKA solely on the basis of its cAMP binding properties, the scope of the present work was not to investigate whether or not it is a PKA receptor that has been bound here, but whether the receptor measured had differed in two rat strains and would it be influenced by AD treatment. The foundation for this work was the initial study by Lowther and colleagues (1997) that had shown that while unmedicated human depressives did not differ from normals in PKA receptor density, chronic AD's did lower that measure. The current work contrasted with these findings (but concurred with later findings by the Dwivedi group using tissue from depressed suicides) in that the abnormal rat strain evidenced a comparatively lower PKA density in untreated conditions.

In addition, chronic AD treatment with a TCA was found to increase, not decrease, PKA density. This agrees with recent work shown in preliminary form by the Dwivedi group with desipramine at the same high dose (Shukla *et al*., 2003), although the experimental paradigm in that case involved the reversal of a CORT-induced lowering of PKA density. The same direction of effect was found in the SD rat here at the low desipramine dose subchronically, but no work has been published for effects on PKA with any AD treatment at a low dose or for acute or subchronic AD effects on the regulatory PKA subunit.

Dwivedi *et al.* (2004) had claimed that lowered PKA density was found in depressed suicides, whether or not they had been medicated. In the drug-treated suicides assessed by either the Lowther or Dwivedi groups, most depressives had been treated with TCA's and a few with MAO inhibitors, but not with DA-reuptake inhibitors. It is therefore not

possible to make any comparison to published work regarding DA impact on PKA regulatory in either a clinical or preclinical setting.

A possible explanation for the difference in strain response in both fractions regarding bupropion treatment at two dose levels and two injection paradigms may be due to the SD and WKY strains demonstrating a differential distribution of DA transporter sites (Jiao, Pare, & Tejani-Butt, 2003). This study had demonstrated that significant comparative decreases and increases existed between the strains in DA transporter density in various brain regions involved in depressive behavior. These differences in density and distribution were thought to produce possible changes in DA levels in the cell body and mesolimbic regions that may contribute to the depressive characteristics found in the WKY strain. Since it appears that the SD and WKY rats may regulate DA in a dissimilar fashion, it could be anticipated that the two strains may have interpreted bupropion differently at a biochemical level.

Apparently the summated output from several brain regions as measured by the FST evidences no true pattern of correlation with the impact of AD treatment on PKA density in the frontal cortex. Although the SD rats, for example, had demonstrated a highly significant AD effect in the FST as a result of the high dose of bupropion, the PKA measure was unaffected in either fraction tested. While the high dose of desipramine is highly significant in the WKY strain in the FST, the increase in cytosolic PKA for that strain is not of the magnitude of that for the SD strain, which demonstrated a smaller decrease in immobility to desipramine as compared to the WKY strain.

Shukla *et al.* (2004) had found that 10 mg/kg of desipramine administered chronically in the SD rat not only had increased the PKA density of the cytosolic fraction but also that of the particulate component, although not to the same magnitude as in the former fraction. In the current work, the particulate fraction was not impacted in any condition except for the low dose of bupropion in the SD rat. Perhaps the present work is characterizing a different receptor than the aforementioned investigators, which is also supported by the high affinity K_D's and very low Bmax's reported for both fractions by that group. A high affinity receptor would be predicted to demonstrate greater effect in response to drug treatment.

The present work may also be assessing an endogenous PKA deficit as the WKY rat had demonstrated lowered PKA densities in the control conditions. Perhaps it may be inferred here that the WKY rat has a lowered level of cAMP in the frontal cortex that correlates with a proposed lower cAMP level in human depressives (Abdullah & Hamadah, 1970). The strain's endogenously sustained CORT release at early dark (Solberg, Turek, Olson, & Redei, 1998), analogous to a human depressives' cortisol release at awakening, is a more native approach to modeling the disease state than the introduction of a tonic release pellet.

Shukla *et al.* (2004) described their findings with desipramine as "normalization" of the CORT-induced lowering of PKA density in the prefrontal cortex. These investigators state that clinically effective therapy with AD's normalizes the disturbed HPA activity present in major depression. However, in the present work no induction of PKA lowering was necessary to demonstrate increased PKA density with desipramine treatment;

therefore, this refutes any hypothesis by that group regarding HPA axis normalization as the SD rat had demonstrated this same increase in a “normal” state.

Originally it had been proposed in the current work that a rat strain exhibiting depressive characteristics may be the advantageous one in which to model AD effects on PKA. During the time this work was conducted, a study was published that described an analogous hypothesis in the area of cognitive decline (Ramos et al., 2003). While this group states that there is a highly consistent literature indicating that PKA activation enhances long-term memory function, their own findings indicated in contrast that in aged rats and monkeys, PKA inhibition was the correct strategy by which to restore prefrontal cortical cognitive abilities in the elderly.

The same conclusion cannot be drawn in the current study regarding the area of depression. Similar responses were found in normal (SD) and abnormal (WKY) rats for the NE-reuptake inhibitor desipramine at a high dose level. The differences in response to the DA re-uptake inhibitor bupropion was described earlier as a potential indication of the differential DA transporter density found in the two strains.

Perhaps the differences between cognition and depression at the receptor level should not be unexpected. In the FST a number of “normal” strains do demonstrate AD effects to a variety of clinically utilized AD’s in that animal model of depression. While the swim test is a widely used animal model and it is therefore easily validated for many laboratories, in contrast, it is quite difficult to show memory enhancements in animal models, particularly if deficits are not induced. Simple environmental manipulation, the

stressful event of being in a swim tank, is sufficient to provide the setting in which to show a positive effect with AD's.

The direct correlation of an effect on PKA and performance in an animal model was not the case here. One hypothesis of the current work was that the FST measures cAMP; this was suggested by some past work with effect of PDE inhibitors (Saccomano *et al*., 1991). In the current laboratory, work with rolipram has indicated heavy sedation that yields increased, rather than decreased, levels of immobility (personal observation). It remains unsolved regarding what the FST actually measures. Although a component of it could be cAMP, it may have to be driven by certain receptors, with noradrenergic and dopaminergic drugs the most easily measured across laboratories in the FST. This may not be directly translatable to a measurable receptor change, especially not subchronically, at least not in the cortex. The current work did concur with clinical findings in that in most cases, an effect on PKA density required a chronic administration of an AD.

The effect of compounds in the FST is highly correlated with the effect of drugs in the clinic. Can the measurement of effects on PKA density by AD's make the same claim ? A paucity of studies exists regarding AD impact on PKA with little progress made since Lowther and colleagues published the first study on [^{3}H]-cAMP binding and AD effect in 1997. The current findings and the Dwivedi group's Society for Neuroscience presentation (Shukla *et al*., 2004) remain as the only such work in existence. In comparison to the clinical work with TCA's, it appears that both groups contradict clinical findings, with Lowther's work indicating decreased PKA density due to AD

treatment in depressed humans. The Dwivedi group's rodent studies contradict those researchers own work with human tissues that indicate no effect of chronic AD treatment in human depressives.

In conclusion, the current work indicated some strain concurrences, as well as differences, in effect with both the FST and the PKA density measurements. The FST results appeared generally similar whether a drug was injected subchronically or chronically. In contrast, the PKA measurements were always altered, with one exception, by a high dose of an AD injected chronically. The caveat of extrapolations of data from certain strains remains, but this is also true regarding extrapolations of rodent data to clinical populations.

Both the topics of how the FST measures AD activity with clinical drug effect concordance and if AD effect can be fingerprinted via changes in PKA density remain unsolved. Although cAMP has been researched for decades in regards to depression, relevance of alterations of its receptor PKA in response to AD treatment remains a primarily unexplored topic for future investigations.

Pearson's Correlational Analysis (*r* Values) Between FST Scores and Bmax Values from Frontal Cortex

	Control	Subchronic	Chronic
Desipramine 1 mg/kg			
Cytosolic			
SD	0.31	-0.70	0.40
WKY	0.61	-0.29	0.15
Particulate			
SD	0.03	-0.07	0.24
WKY	-0.26	0.34	0.82*
Desipramine 10 mg/kg			
Cytosolic			
SD	-0.90*	-5.55	-0.86*
WKY	0.24	-0.75	0.50
Particulate			
SD	-0.33	0.26	0.09
WKY	0.30	-0.86*	-0.10
Bupropion 1 mg/kg			
Cytosolic			
SD	0.29	-0.75	-0.53
WKY	-0.15	-0.50	-0.54
Particulate			
SD	0.01	0.10	-0.73
WKY	0.07	0.63	-0.22
Bupropion 10 mg/kg			
Cytosolic			
SD	-0.90*	0.23	-0.40
WKY	0.50	0.20	-0.50
Particulate			
SD	0.60	-0.32	-0.60
WKY	-0.10	-0.30	-0.04

Table GD-1. Pearson's correlational analysis (*r* values) were determined for SD and WKY strains between FST scores and B_{max} values from cytosolic and particulate fractions of frontal cortex. Rats were treated with a vehicle control, subchronic drug or chronic drug. Each *r* value represents a comparison of n=6 for each measure; *, $p < 0.05$.

References

Abdullah, Y.H., & Hamadah, K. (1970). 3',5' cyclic adenosine monophosphate in depression and mania. Lancet, 1, 378-81.

Arana, G.W., & Rosenbaum, J.F. (2000). Handbook of Psychiatric Drug Therapy. Philadelphia, PA: Lippincott Williams & Wilkins.

Ascher, J.A., Cole, J.O., Colin, J-N., Feighner, J.P., Ferris, R.M. Fibiger, H.C., Golden, R.N., Martin, P., Potter, W.Z., Richelson, E., & Sulser, F. (1995). Bupropion: A review of its mechanism of antidepressant activity. Journal of Psychiatry, 56, 395-401.

Avissar, S., & Schreiber, G. (1992). The involvement of guanine nucleotide binding proteins in the pathogenesis and treatment of affective disorders. Biological Psychiatry, 31, 435-59.

Bobon, D., Breulet, M., Gerard-Vandenhove, M.A., Guiot-Goffioul, F., Plomteux, G., Sastre y Hernandez, M., Schratzer, M., Troisfontaines, B., von-Frenckell, R., & Wachtel, H. (1988). Is phosphodiesterase inhibition a new mechanism of antidepressant action? A double blind double-dummy study between rolipram and desipramine in hospitalized major and/or endogenous depressives. European Archives of Psychiatry and Neurological Sciences, 238 (1), 2-6.

Borsini, F. (1995). Role of the serotonergic system in the forced swimming test. Neuroscience and Biobehavioral Reviews, 19(3), 377-95.

Borsini, F., & Meli, A. (1988). Is the forced swimming test a suitable model for revealing antidepressant activity ? Psychopharmacology, 94, 147-60.

Borsini, F., Volterra, G., & Meli, A. (1986). Does the behavioral "despair" test measure "despair" ? Physiology and Behavior, 38, 385-6.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-52.

Brandon, E.P., Idzerda, R.L., & McKnight, G.S. (1997). PKA isoforms, neural pathways, and behaviour: Making the connection. Current Opinion in Cell Biology, 7, 397-403.

Bunney, W.E., & Davis, J.M. (1965). Norepinephrine in depressive reactions. Archives of General Psychiatry, 13, 483-94.

Cheng, Y.C., and Prusoff, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. Biochemical Pharmacology, 22, 3099-3108.

Chen, Y-W., & Dilsaver, S.C. (1996). Lifetime rates of suicide attempts among subjects with bipolar and unipolar disorders relative to subjects with other Axis I disorders. Biological Psychiatry, 39(10), 896-9.

Cooper, B.R., Hester, T.J., & Maxwell, R.A. (1980). Behavioral and biochemical effects of the antidepressant bupropion (Wellbutrin): Evidence for selective blockade of dopamine uptake *in vivo*. The Journal of Pharmacology and Experimental Therapeutics, 215(1), 127-34.

Cowburn, R.F., Marcusson, J.O., Eriksson, A., Wiehager, B., & O'Neill, C. (1994). Adenylyl cyclase activity and G-protein subunit levels in postmortem frontal cortex of suicide victims. Brain Research, 633, 297-304.

Cryan, J.F., Dalvi, A., Jin, S-H., Hirsch, B.R., Lucki, I., & Thomas, S.A. (2001). Use of dopamine-β-hydroxylase-deficient mice to determine the role of norepinephrine in the mechanism of action of antidepressant drugs. The Journal of Pharmacology and Experimental Therapeutics, 298(2), 651-7.

De Pablo, J.M., Parra, A., Segovia, S., & Guillamon, A. (1989). Learned immobility explains the behavior of rats in the forced swimming test. Physiology and Behavior, 46, 229-37.

Dremier, S., Kopperud, R., Doskeland, S.O., Dumont, J.E., & Maenhaut, C. (2003). Search for new cyclic AMP-binding proteins. Federation of European Biochemical Societies Letters, 546, 103-7.

Dugovic, C., Solberg, L.C., Redei, E., Van Reeth, O., & Turek, F.W. (2000). Sleep in the Wistar-Kyoto rat, a putative genetic animal model for depression. Neuroreport, 28(3), 627-31.

Duman, R.S., Heninger, G.R., & Nestler, E.J. (1997). A molecular and cellular theory of depression. Archives of General Psychiatry, 54, 597-606.

Duman, R.S., Malberg, J., Nakagawa, S., & D'Sa, C. (2000). Neuronal plasticity and survival in mood disorders. Biological Psychiatry, 48, 732-9.

Durand, M., Berton, O., Aguerre, S., Edno, L., Combourieu, I., Mormede, P., & Chaouloff, F. (1999). Effects of repeated fluoxetine on anxiety-related behaviours, central

serotonergic systems, and the corticotropic axis axis in SHR and WKY rats. Neuropharmacology, 38(6), 893-907.

Dwivedi, Y., Conley, R.R., Roberts, R.C., Tamminga, C.A., & Pandey, G.N. (2002). [3H]cAMP binding sites and protein kinase A activity in the prefrontal cortex of suicide victims. American Journal of Psychiatry, 159(1), 66-73.

Dwivedi, Y., Mondal, A.C., Shukla, P.K., Rizavi, H.S., & Lyons, J. (2004b). Altered protein kinase A in brain of learned helpless rats: Effects of acute and repeated stress. Biological Psychiatry, 56, 30-40.

Dwivedi, Y. & Pandey, G.N. (2000). Adrenal glucocorticoids modulate [3H]cyclic AMP binding to protein kinase A (PKA), cyclic AMP-dependent PKA activity, and protein levels of selective regulatory and catalytic subunit isoforms of PKA in rat brain. The Journal of Pharmacology and Experimental Therapeutics, 294(1), 103-116.

Dwivedi, Y., Rizavi, H.S., Shukla, P.K., Lyons, J., Faludi, G., Palkovits, M., Sarosi, A., Conley, R.R., Roberts, R.C., Tamminga, C.A., & Pandey, G.N. (2004a). Protein kinase A in postmortem brain of depressed suicides victims: Altered expression of specific regulatory and catalytic subunits. Biological Psychiatry, 55, 234-43.

Emrich, H.M., Zerssen, D.V., Kissling, W., Moller, H-J, & Windorfer, A. (1980). Effect of sodium valproate on mania. Archives fur Psychiatrie und Nervenkrankheiten,229, 1-16.

Flugy, A., Gagliano, M., Cannizzaro, C., Novara V., & Cannizzaro , G. (1992). Antidepressant and anxiolytic effects of alprazolam versus the conventional antidepressant desipramine and the anxiolytic diazepam in the forced swim test in rats. European Journal of Pharmacology. 214(2-3), 233-8.

Gomez, F., Lahmame, A., de Kloet, E.R., & Armario, A. (1996). Hypothalamic-pituitary-adrenal response to chronic stress in five inbred strains: differential responses are mainly located at the adrenocortical level. Neuroendocrinology, 63(4), 327-37.

Greenberg, S.M., Castelluci, V.F., Bayley, H., & Schwartz, J.H. (1987). A molecular mechanism for long-term sensitization in Aplysia. Nature, 329, 62-65.

Holsboer, F., Lauer, C.J., Schreiber, W., & Krieg, J.C. (1995). Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for affective disorders. Neuroendocrinology, 62, 340-7.

Horowski, R., & Sastre Y Hernandez, M. (1985). Clinical effects of the neurotropic selective cAMP inhibitor rolipram in depressed pateints: Global evaluation of the preliminary reports. Current Therapeutic Research, 38(1), 23-29.

Jiao, X., Pare, W.P., & Tejani-Butt, S. (2003). Strain differences in the distribution of dopamine transporter sites in rat brain. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 27, 913-9.

Jordan, S., Kramer, G.L., Zukas, P.K., & Petty, F. (1994). Previous stress increases in vivo biogenic amine response to swim stress. Neurochemical Research, 19(12), 1521-5.

Karege, F., Lambercy, C., Schwald, M, Steimer, T., & Cisse, M. (2001). Differential changes of cAMP-dependent protein kinase activity and [^{3}H]-cAMP binding sites in rat hippocampus during maturation and aging. Neuroscience Letters, 315, 89-92.

Kirby, L.G., Chou-Green, J.M., Davis, K., & Lucki, I. (1997). The effects of different stressors on extracellular 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Brain Research, 760, 218-30.

Klimek, V., Stockmeier, C., Overholser, J., Meltzer, H.Y., Kalka, S., Dilley, G., & Ordway, G.A. (1997). Reduced levels of norepinephrine transporters in the locus coeruleus of major depression. Journal of Neuroscience, 17(21), 8451-8.

Lahmame, A., & Armario, A. (1996). Differential responsiveness of inbred strains of rats to antidepressants in the forced swim test: Are Wistar Kyoto rats an animal model of subsensitivity to antidepressants ? Psychopharmacology, 123, 191-8.

Lahmame, A., del Arco, C., Pazos, A., Yritia, M., & Armario, A. (1997). Are Wistar-Kyoto rats a genetic animal model of depression resistant to antidepressants ? European Journal of Pharmacology, 337, 115-23.

Leiser, M., Rubin, C.S., & Erlichman, J. (1986). Differential binding of the regulatory subunit (RII) of cAMP-dependent protein kinase II from bovine brain and muscle to RII-binding proteins. Journal of Biological Chemistry, 261, 1904-8.

Leonard, B.E. (2000). Noradrenaline and depression. Reviews in Contemporary Pharmacotherapy, 11, 257-66.

Leonard, B.E. (1991). Antidepressants: Current concepts of mode of action. Encephale, 17, 127-31.

Lloyd, K.G., Thuret, F., & Pilc, A. (1985). Upregulation of gamma-aminobutyric acid B ($GABA_B$) binding sites in rat frontal cortex: A common action of repeated administration

of different classes of antidepressants and electroshock. Journal of Pharmacology and Experimental Therapeutics, 235, 191-9.

Lloyd , KG., Zivkovic, B., Scatton , B., Morselli, PL., & Bartholini, G. (1989). The gabaergic hypothesis of depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 13(3-4), 341-51.

Lopez-Rubalcalva, C., & Lucki, I. (2000). Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. Neuropsychopharmacology, 22(2), 191-99.

Louis, W.J., & Howes, L.G. (1990). Genealogy of the spontaneously hypertensive rat and Wistar-Kyoto rat strains: Implications for studies of inherited hypertension. Journal of Cardiovascular Pharmacology, 16(Supplement 7), 51-55.

Lowther, S., Crompton, M.R., Katona, C.L.E., and Horton, R.W. (1996). GTPγS and forskolin-stimulated adenylyl cyclase activity in post-mortem brain from depressed suicides and controls. Molecular Psychiatry, 1, 470-7.

Lowther, S., Katona, C.L.E., Crompton, M.R., & Horton, R.W. (1997). Brain [3H]cAMP binding sites are unaltered in depressed suicides, but decreased by antidepressants. Brain Research, 758, 223-228.

Malkesman, O., Braw, Y., Maayan, R., Weizman, A., Overstreet, D.H., Shabat-Simon, M., Kesner, Y., Touati-Werner, D., Yadid, G., & Weller, A. (2006). Two different putative genetic animal models of childhood depression. Biological Psychiatry, 59(1), 17-23.

Manji, H.K., Potter, W.Z., & Lenox, R.H. (1995). Signal transduction pathways. Molecular targets for lithium's actions. Archives of General Psychiatry, 52, 531-43.

Morselli, P.L., Fournier, V., Nacker, J.P., Orofiama, B., Bottin, P., & Huber, J.P. (1986). Therapeutic action of progabide in depressive illness: A controlled clinical trial. In: GABA and mood disorders. G. Bartholini, K.G. Lloyd, and P.L. Morselli (Eds.), pp. 118-25. New York: Raven Press.

Moyer, J.A., Sigg, E.B., & Silver, P.J. (1986). Antidepressants and protein kinases: Desipramine treatment affects pineal gland cAMP-dependent kinase activity. European Journal of Pharmacology, 121(1), 57-64.

Musch, B. (1986). The antidepressant activity of fengabine: A critical overview of the present results in open clinical studies. In: GABA and mood disorders. G. Bartholini, K.G. Lloyd, and P.L. Morselli (Eds.), pp. 171-7. New York: Raven Press.

Nestler, E.J., & Greengard, P. (9183). Protein phosphorylation in the brain. Nature, 305, 583-8.

Nestler, E.J., Terwilliger, R.Z., & Duman, R.S. (1989). Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. Journal of Neurochemistry, 53(5), 1644-7.

Nishino, N., Kitamura, N., Hashimoto, T., Kajimoto, Y., Shirai, Y., Murakami, N., Nakai, T., Komure, O., Shirakawa, O., Mita, T., & Nakai, H. (1993). Increase in [3H]cAMP binding sites and decrease in Gia and Goa immunoreactives in left temporal cortices from patients with schizophrenia. Brain Research, 615, 41-9.

Pandey, G.N. & Davis, J.M. (1979). Cyclic AMP and adenylate cyclase in psychiatric illness. In: Neuropharmacology of cyclic nucleotides. G.C. Palmer (Ed.), pp. 112-151. Baltimore: Urban & Schwarzenberg.

Pare, W.P. (1989). Stress ulcer susceptibility and depression in Wistar Kyoto (WKY) rats. Physiology and Behavior, 46, 993-8.

Pare, W.P. (1994a). Hyponeophagia in Wistar Kyoto (WKY) rats. Physiology and Behavior, 55(5), 975-8.

Pare, W.P. (1994b). Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. Physiology and Behavior, 55(3), 433-9.

Pare, W.P., Kluczynski, J., & Tejani-Butt, S.M. (1999). Strain differences in the open field test and the Porsolt forced swim test following antidepressant treatment. Society for Neuroscience Abstract, 25: 635.19.

Pare, W.P., & Redei, E. (1993). Depressive behavior and stress ulcer in Wistar Kyoto rats. Journal of Physiology, 87, 229-38.

Pare, W.P., & Tejani-Butt, S.M. (1996). Effect of stress on the behavior and the 5-HT system in Sprague-Dawley and Wistar Kyoto rat strains. Integrative Physiological and Behavioral Science, 31(2), 112-21.

Petty, F. (1994). Plasma concentrations of gamma-aminobutyric acid (GABA) and mood disorders: A blood test for manic depressive disease ? Clinical Chemistry, 40(2), 296-302.

Petty, F., Jordan, S., Kramer, G.L., Zukas, P.K., & Wu, J. (1997). Benzodiazepine prevention of swim stress-induced sensitization of cortical biogenic amines: An *in vivo* microdialysis study. Neurochemical Research, 22(9), 1101-4.

Petty, F., Kramer, G.L., Fulton, M., Moeller, F.G., & Rush, A.J. (1993). Low plasma GABA is a trait-like marker for bipolar illness. Neuropsychopharmacology, 9(2), 125-132.

Petty, F., Trivedi, M.H., Fulton, M., & Rush, A.J. (1995). Benzodiazepines as antidepressants: Does GABA play a role in depression ? Biological Psychiatry, 38(9), 578-91.

Porsolt, R.D. (2000). Animal models of depression: Utility for transgenic research. Reviews in the Neurosciences, 11(1), 53-8.

Porsolt, R.D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioral despair in rats: A new model sensitive to antidepressant treatments. European Journal of Pharmacology, 47, 379-91.

Porsolt, R.D.,Bertin, A., Blavet, N., Deniel, M., & Jalfre, M. (1979). Immobility induced by forced swimming in rats: Effects of agents which modify central catecholamine and serotonin activity. European Journal of Pharmacology, 57, 201-10.

Porsolt, R.D., Bertin, A., & Jalfre, M. (1977). Behavioral despair in mice: A primary screening test for antidepressants. Archives of International Pharmacodynamics, 229, 327-36.

Porsolt, R.D., Le Pichon, M, & Jalfre, M. (1977). Depression: A new animal model sensitive to antidepressant treatments. Nature, 266, 730-2.

Price, M.L., Kirby, L.G., Valentino, R.J., & Lucki, I. (2002). Evidence for corticotropin-releasing factor regulation of serotonin in the lateral septum during acute swim stress: Adaptation produced by repeated swimming. Psychopharmacology, 162(4), 406-14.

Racagni, G., Brunello, N., Tinelli, D., & Perez, J. (1992). Biochemical hypotheses on the mechanism of action of antidepressant drugs: cAMP-dependent phosphorylation system. Pharmacopsychiatry, 25, 51-5.

Rahman, S., Li, P.P., Young, L.T., Kofman, O., Kish, S.J., & Warsh, J.J. (1997). Reduced [3H]-cyclic AMP binding in postmortem brain from subjects with bipolar affective disorder. Journal of Neurochemistry, 68(1), 297-304.

Ramos, B.P., Birnbaum, S.G., Lindenmayer, I., Newton, S.S., Duman, R.S., & Arnsten, A.F.T. (2003). Dysregulation of protein kinase A signaling in the aged prefrontal cortex: New strategy for treating age-related cognitive decline. Neuron, 40(4), 835-45.

Randrup, A., Munkvad, I., Fog, R., Gerlach, J., Molander, L., Kjellberg, B., & Scheel-Kruger, J. (1975). Mania, depression, and brain dopamine. In: Current developments in psychopharmacology. W.B. Essman and L.Valzelli (Eds.), Volume 2, pp. 206-48. New York: Spectrum.

Redei, E., Pare, W.P., Aird, F. & Kluczynski, J. (1994). Strain differences in hypothalamic-pituitary-adrenal activity and stress ulcer. American Journal of Physiology, 266(2 Part 2), R353-60.

Rogacki, N., Corbett, R., & Wettstein, J.G. (2000). Strain differences in response to antidepressant drugs in the forced swim test. The Faseb Journal,14(8), A1321.

Saccomano, N.A., Vinick, F.J., Koe, B.K., Nielsen, J.A., Whalen, W.M., Meltz, M., Phillips, D., Thadieo, P.F., Jung, S., & Chapin, D.S. (1991). Calcium-independent phosphodiesterase inhibitors as putative antidepressants: [3-(bicycloalkyloxy)-4-methoxyphenyl]-2-imidazolidinones. Journal of Medicinal Chemistry, 34(1), 291-298.

Sadock, B.J. & Sadock, V.A. (2001). Kaplan and Sadock's Pocket Handbook of Clinical Psychiatry. New York: Lippincott, Williams and Wilkins.

Schildkraut, J.J. (1967). The catecholamine hypothesis of affective disorders: A review of supporting evidence. International Journal of Psychiatry, 4(3), 203-17.

Scott, A.I., Perini, A.F., Shering, P.A., & Whalley, L.J. (1991). In-patient major depression: Is rolipram as effective as amitriptyline? European Journal of Clinical Pharmacology, 40 (2),127-9.

Shukla, P.K., Mondal, A.C., Rizavi, H.S., Payappagoudar, G., Prakasam, S., Pandey, G.N., & Dwivedi, Y.(2004). Antidepressant effects on HPA axis-mediated changes in protein kinase A in rat brain. Poster Presentation to the Society for Neuroscience, San Diego.

Soderpalm B. (1989). The SHR exhibits less "anxiety" but increased sensitivity to the anticonflict effect of clonidine compared to normotensive controls. Pharmacology and Toxicology. 65(5), 381-6.

Solberg, L.C., Turek, F.W., Losee Olson, S., & Redei, E. (1998). Sustained increase of the ACTH and corticosterone diurnal peak in Wistar Kyoto rats. Society for Neuroscience Abstract, 24: 543.8.

Stahl, S.M. (2000). Essential Psychopharmacology: Neuroscientific Basis and Practical Applications. New York: Cambridge University Press.

Tejani-Butt, S., Kluczynski, J., & Pare, W.P. (2003). Strain-dependent modification of behavior following antidepressant treatment. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27, 7-14.

Tejani-Butt, S.M., Pare, W.P., & Yang, J. (1994). Effect of repeated novel stressors on depressive behavior and brain norepinephrine receptor system in Sprague-Dawley and Wistar Kyoto (WKY) rats. Brain Research, 649, 27-35.

Vaugeois, J.M., Passera, G., Zuccaro, F., & Costentin, J. (1997). Individual differences in response to imipramine in the mouse tail suspension test. Psychopharmacology, 134(4), 387-91.

Wachtel, H. (1983). Potential antidepressant activity of rolipram and other selective cyclic adenosine 3',5'-monophosphate phosphodiesterase inhibitors. Neuropharmacology, 22(3A), 267-72.

Wachtel, H. (1989). Dysbalance of neuronal second messenger function in the aetiology of affective disorders: A pathophysiological concept hypothesising defects beyond first messenger receptors. Journal of Neural Transmission, 75, 21-9.

Weber, B., Lewicka, S., Deuschle, M., Colla, M., Vecsei, P., & Heuser, I. (2000). Increased diurnal plasma concentrations of cortisone in depressed patients. Journal of Clinical Endocrinology and Metabolism, 85(3), 1133-6. .

Weiss, J.M., Goodman, P.A., Losito, B.A., Corrigan, S., Charney, J.M., & Bailey, W.H. (1981). Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. Brain Research Reviews, 3, 167-205.

Willner, P. (1984). The validity of animal models of depression. Psychopharmacology, 83, 1-16.

Willner, P. (1983). Dopamine and depression: A review of recent evidence. Brain Research Reviews, 6, 211-224.

Willner, P. Muscat, R., Papp, M., & Sampson, D. (1991). Dopamine, depression and antidepressant drugs. In: The mesolimibic dopamine system: From motivation to action. P. Willner & J. Scheel-Kruger (Eds.), pp. 387-410. New York: John Wiley & Sons.

Zimmerman, M., McDermut, W., & Mattia, J.I. (2000). Frequency of anxiety disorders in psychiatric outpatients with major depressive disorder. American Journal of Psychiatry, 157(8), 1337-1340.