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The neurobiology and preclinical evaluation of $GABA_B$ agonists for the treatment of cocaine addiction

by

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A dissertation submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of the requirement of the degree of
Doctor of Philosophy

Department of Psychology
Specialization in Neurosciences

Carleton University
Ottawa, Ontario
March, 2001

The undersigned recommend to the
Faculty of Graduate Studies and Research
Acceptance of the thesis:

"The Neurobiology and Preclinical Evaluation of $GABA_B$ Agonists for the Treatment of Cocaine Addiction"

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in Partial fulfillment of the requirements for

the degree of Doctor of Philosophy

Chair

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Carleton University
March, 2001

Abstract

Recent experiments suggest that γ-aminobutyric acid (GABA) compounds produce a clinically relevant modulation of cocaine reinforcement. Several of these studies report that $GABA_B$ receptors in particular may be critically involved in this mediating this effect. The following series of experiments were aimed at examining how the $GABA_B$ agonists baclofen and CGP 44532 influence cocaine self-administration in the rat. A number of schedules of reinforcement, including fixed-ratio, progressive-ratio and discrete trials, were used to model various aspects of cocaine-reinforcement. $GABA_B$ receptor stimulation produced marked reductions in cocaine self-administration that did not appear to be accounted for by a general disruption of behaviour. The results further indicated that baclofen's anti-cocaine effects were likely mediated by $GABA_B$ receptors in the ventral tegmental area. Additional studies showed that the baclofen-induced disruption of cocaine self-administration was attenuated by the $GABA_B$ antagonist CGP 56433A further supporting the involvement of $GABA_B$ receptors. Baclofen was also found to dramatically reduce heroin intake, although it is possible that sedative or other non-specific effects contributed to these results. The demonstration that baclofen's effect was dependent on the unit injection dose of cocaine and the response requirements of the schedule of reinforcement predict that, in a clinical setting, any potential therapeutic effect will interact with the cost and availability of cocaine.

Acknowledgments

I would like to thank Dave for being the being the best supervisor a person could hope to work for. I cannot begin to thank him for all he has done for me, both in Ottawa and in North Carolina. The last two years have been quite the adventure, and he and Nadine have done more for me than I could ever hope to repay.

To all the people at Carleton with whom I started my graduate career, and my friends in Ottawa, I would like to say thank you for helping me through the good times and bad, and for not forgetting about me after I left. It is nice to feel like I still belong in the LSRB even though I crossed over the border. To Monique Andrews, my thanks for your assistance with the CGP 44532 studies. To everyone at Wake Forest who welcomed me into their country and their labs, I am grateful for the opportunity to have met and worked with you.

To Dr. Jennifer Arnold, I am forever indebted to you for convincing me to work with Dave, and for your friendship and advice over the years. You helped me stay sane and focused, and always manage to make me laugh when I was sure I was on the edge.

To my family and friends, who for 6 years remembered to ask me how my rats were doing, I am grateful for your support and encouragement. It always helped knowing that you thought I was doing something worthwhile.

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List of Abbreviations

5-HT: Serotonin

DA: Dopamine

FR: Fixed-ratio

GABA: Gamma-aminobutyric acid

IC: Intracerebral

IP: Intraperitoneal

NAC: Nucleus Accumbens

NE: Norepinephrine

PFC: Prefrontal Cortex

PR: Progressive Ratio

STR: Striatum

VTA: Ventral Tegmental Area

List of Publications

Hayley, S., Brebner, K., Lacosta, S., Merali, Z. & Anisman, H. (1999). Sensitization to the effects of tumor necrosis factor-a: Neuroendocrine, central monoamine and behavioral variations. *Journal of Neuroscience*, 19, 5654-5665.

Brebner, K., Froestl, W., Andrews, M., Phelan, R. & Roberts, D.C.S. (1999). The $GABA_B$ agonist CGP 44532 decreases cocaine self-administration in rats: demonstration using a progressive ratio and discrete trials procedure. *Neuropharmacology*, 38, 1797-1804.**

Brebner, K., Hayley, S., Zacharko, R., Merali, Z. & Anisman, H. (2000). Synergistic effects of interleukin-1b, interleukin-6, and tumor necrosis factor-a: Central monamine and behavioral variations. *Neuropsychopharmacology*, 22, 566-580.

Brebner, K., Phelan, R. & Roberts, D.C.S. (2000). Effect of baclofen on cocaine self-administration reinforced under FR1 and progressive ratio schedules. *Psychopharmacology*, 148, 314-321.**

Brebner K., Phelan, R. & Roberts, D.C.S. (2000). Intra-VTA baclofen attenuates cocaine self-administration in rats on a progressive ratio schedule of reinforcement. *Pharmacology, Biochemistry & Behavior*, 66, 857-862.**

Roberts, D.C.S. & Brebner, K. (2000). GABA modulation of cocaine self-administration. *Annals of the New York Academy of Sciences*, 909, 145-158.**

**publications derived from the experiments contained within this thesis

This thesis will examine the neurobiological basis of cocaine reinforcement. Specifically, it will address how the inhibitory neurotransmitter γ–amino-butyric acid (GABA) can modulate cocaine reinforcement. The experiments that will be conducted are aimed primarily at demonstrating that pharmacological manipulations at $GABA_B$ synapses can disrupt cocaine self-administration in rats. One assumption is that the knowledge gained from these studies will have implications for understanding cocaine addiction and its treatment.

The past 30 years has seen a transition in attitudes towards drug addiction. Prior to the 1960s the generally held belief was that cocaine addiction was a purely psychological disorder (Musto, 1996). For example, the lack of overt withdrawal symptoms following cocaine use was taken as evidence that users were not physically dependent on cocaine, so addiction must therefore be a result of some underlying psychopathology. While the behavioural and social components of drug use have long been recognized, there has only recently been an appreciation of the fact that addiction has a biological, or more specifically a neurological, basis. In clinical terms, drug addiction is now classified as a disease that is characterized by compulsive drug craving, loss of control over drug-taking behaviour, chronic relapses and continued use even in the face of negative consequences (American Psychiatric Association, 1994). The emphasis on the chronic nature of the disease reflects the growing belief that while acute drug use causes acute changes in brain function, prolonged drug use causes brain changes that are long lasting, so that an 'addicted' brain is fundamentally different from a non-addicted brain (Peris et al., 1990; Volkow, 1990; Volkow, 1992).

Paralleling the changes in attitude regarding the biobehavioural nature of drug addiction were tremendous advances in technology that helped define the site of action and neurological consequences of cocaine use. Early investigators were limited to observing the behavioural effects of systemic drug administration for clues as to how drugs were affecting the brain. Now it is possible to quantify and in some cases actually visualize the ways in which brain activity is altered in the presence of drugs. These techniques have enabled researchers to move beyond indirect measurements of brain function in response to drugs by determining what cocaine does at the molecular and cellular levels. Animal self-administration studies can be used in conjunction with these techniques to determine the functional implications of cocaine-induced brain changes.

The study of cocaine addiction has been a multipdisciplinary effort that has included experimental techniques from all areas of neuroscience (Leshner, 1999). A search of the literature reveals that there are approximately 17,000 publications that deal with the effects of cocaine. These range from molecular studies in cell culture to real-time brain imaging of drug craving in human addicts. Our approach has been to use the rat self-administration model to studying the neurobiology of cocaine addiction. Although some may question the appropriateness of using rats to study what has long been considered a uniquely human phenomenon, there are several reasons why rat self-administration studies are considered a legitimate experimental context in which human substance abuse disorders can be studied. First, rats voluntarily consume most of the same drugs that humans abuse (Headlee et al., 1955; Schuster & Thompson, 1969; Koob et al., 1998). Second, the patterns of drug self-administration that can be engendered in rats are reminiscent of the patterns of drug intake

observed in human drug addicts (Gawin & Kleber, 1985; Gawin, 1991). Third, the reinforcing effects of drugs like cocaine are mediated by structures that are common to all mammalian brains and not peculiar to humans (See Woolverton & Johnson, 1992; Wise, 1996a; 1996b; Leshner & Koob, 1999 for review). Thus, the convergence of evidence states that there are common structures in the brain that make rats an appropriate species for studying human addiction.

The following literature review describes those aspects of the neurobiology of cocaine that relate to the behavioural pharmacology of drug addiction. While it is obvious that the field of cocaine research is far larger than can be reviewed in this thesis, the overwhelming evidence from these studies indicates that the reinforcing, or addictive properties of cocaine are related to alterations in dopamine (DA) levels in a small number of brain structures that are collectively referred to as the mesolimbic DA system.

1.1 Cocaine and Dopamine

At the cellular level, cocaine binds to monoamine transporters and thereby acts as an indirect agonist for DA, serotonin (5-HT), and norepinephrine (NE). By blocking transmitter reuptake into the presynaptic terminals, the net effect of cocaine is to increase the levels of these neurotransmitters in the synaptic cleft. The precise contribution of each of these transmitter systems to the development and maintenance of cocaine addiction remains to be determined; however, the idea that the DA transporter is a critical site of action for the reinforcing effects of cocaine has overwhelming support (see Koob et al., 1998; Leshner & Koob, 1999 for review).

Much of the evidence supporting a role for DA in the behavioural effects of cocaine

comes from animal self-administration studies. The earliest self-administration studies used a simple fixed-ratio (FR) schedule of reinforcement to show that systemic administration of DA receptor antagonists such as haloperidol, sulpiride, and spiperone disrupted cocaine self-administration behaviour by shifting the cocaine dose-response curve to the right, producing increases in the rate of cocaine intake at all supra-threshold doses (DeWit & Wise, 1977; Roberts & Vickers, 1984; Roberts et al., 1989b; Hubner & Moreton, 1991; Caine & Koob, 1994). This increase in rate of drug intake is similar to that observed when the unit-injection dose of cocaine is reduced, and has been interpreted as a compensatory response to a diminished drug effect (Yokel & Wise, 1975). In studies that used a progressive ratio (PR) schedule of reinforcement to measure an animal's motivation to self-administer cocaine, pretreatment with DA antagonists decreased self-administration, indicating that the drug was less reinforcing (Roberts et al., 1989b). Pretreatment with indirect DA receptor agonists on the other hand, shifted the cocaine dose-response curve to the left (Depoortere et al., 1993; Caine & Koob, 1993; Roberts & Ranaldi, 1995).

There are several different DA receptor subtypes, with unique anatomical and functional characteristics, that may be differentially involved in mediating the reinforcing effects of cocaine. The DA receptors are classified into 2 subfamilies based on their pharmacological profiles: the D_1-like receptor subtypes which include D_1 and D_5, and the D_2-like subtypes which include D_2, D_3 and D_4 receptors (Sibley & Monsma, 1992). There is increasing evidence that both D_1, and D_2 receptors are important in mediating the reinforcing properties of cocaine (Caine & Koob, 1993; Caine & Koob, 1994; Caine et al., 1995; Self et al., 1996; Caine et al., 2000b; Self et al., 2000). Several studies have reported that

specific D_1 and D_2 antagonists attenuate self-administration behaviour in rats (Hubner & Koob, 1990; Hubner & Moreton, 1991; McGregor & Roberts, 1993; Caine & Koob, 1994; Caine et al., 1995). In addition, some D_1 agonists appear to have some use in preventing drug-seeking behaviour in animal models of relapse and suppress the initiation of cocaine self-administration (Self & Stein, 1992; Self et al., 2000). Studies investigating the discriminative stimulus effects of cocaine have also implicated D_1 and D_2 receptors as the primary mediators of cocaine's subjective effects. Like other DA reuptake inhibitors, D_1 and D_2 receptor agonists can also substitute for cocaine (Callahan et al., 1991; Spealman et al., 1991; Self & Stein, 1992; and see Pulvirenti & Koob, 1994; Roberts & Ranaldi, 1995 for review). The evidence regarding the role of D_3 receptors in cocaine reinforcement is somewhat less clear cut. Early studies showed that the preferential D_3 agonist 7-OH-DPAT substituted for cocaine at high doses (Caine & Koob, 1995). Recent evidence indicates that these effects were likely due to activation of D_2 receptors, and that D_3 receptors may actually play an inhibitory role in cocaine reinforcement. Low doses of 7-OH-DPAT are not self-administered and D_3 partial agonists have been reported to inhibit cocaine-seeking behaviour (Caine & Koob, 1993; Pilla et al., 1999).

Much of the past 20 years of research into the neurobiology of addiction has been aimed at locating the specific brain structures that are most critically involved in mediating the reinforcing properties of cocaine (see Koob & Bloom, 1988; Kalivas et al., 1990; Bardo, 1998 for review). For reasons that remain to be determined, increases in DA levels in a small set of structures that are collectively referred to as the mesolimbic system appear to play a primary role in the phenomenon of reinforcement, which has lead some, correctly or

incorrectly, to refer to the mesolimbic DA system as a "reward pathway" (Engel et al., 1998; Murphy et al., 1999; Comings & Blum, 2000).

The mesolimbic DA system projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAC), prefrontal cortex (PFC), olfactory tubercule and amygdala. It is one of three midbrain DA systems, and has been postulated to function primarily as a gateway for modulating limbic system signals that mediate basic biological drives (e.g. food, water and sex). Several authors have speculated that one of the reasons drugs of abuse are habit forming is because of their actions at these sites that subserve biologically significant rewards (see Wise, 1996a; 1996b for review).

The majority of studies indicate that cocaine-induced increases in DA levels in the NAC are primarily responsible for the behavioural effects of cocaine. Perhaps the most compelling evidence comes from studies demonstrating that 6-hydroxydopamine lesions of the NAC decrease or completely block cocaine self-administration behaviour (Roberts et al., 1977; 1980). Lesions of VTA DA cell bodies that send projections to the NAC also disrupt cocaine self-administration (Roberts & Koob, 1982), as do kainic acid lesions of the NAC (Zito et al., 1985). Dopaminergic manipulations within other mesolimbic structures, including the PFC and amygdala also have an impact on cocaine self-administration, likely by increasing DA turnover in the NAC (Goeders & Smith, 1986; Schenk et al., 1991; McGregor & Roberts, 1993; McGregor et al., 1996; but see Martin-Iverson et al., 1986). In contrast, lesions to brain regions outside the mesolimbic system, such as the caudate, produce no change in cocaine self-administration (Roberts & Zito, 1987).

More recent studies show that microinjection of DA antagonists directly into the

NAC disrupts self-administration (Maldonado et al., 1993). Furthermore intra-NAC administration of pertussis toxin, which selectively inactivates G_i and G_o proteins involved in signal transduction, also reduces cocaine-reinforcement (Self et al., 1994).

Microdialysis, electrochemical and electrophysiological studies demonstrate changes in cellular activity in the presence of cocaine. *In vivo* microdialysis studies have provided additional evidence that NAC DA levels play a central role in cocaine reinforcement, confirming that extracellular DA levels in the NAC are elevated following cocaine self-administration (Pettit & Justice, 1989; 1991). The increase in NAC DA levels appears to mirror the pattern of cocaine self-administration, although it is important to note that these results are not sufficient to conclude that the cocaine-induced changes in DA are responsible for initiating or maintaining the self-administration behaviour. Electrochemical and electrophysiological studies have likewise reported that several different groups of neurons in the NAC increase firing during specific portions of the cocaine self-administration session, and that the firing rate of these neurons is DA-dependent and reflects the changes in self-administered cocaine levels (Gratton & Wise; 1994; Gratton, 1996; Nicola & Deadwyler, 2000).

The evidence reviewed thus far leaves little doubt as to the critical role that NAC DA levels play in cocaine-reinforcement. However, there are several lines of evidence that suggest that increases in the reinforcing effects of cocaine cannot be understood simply in terms of accumbens DA levels. For example, there are pharmacological agents (e.g. some types of antidepressants) that, like cocaine, block presynaptic uptake of DA, yet do not produce euphoric-effects, and are not addictive (Rothman & Glowa, 1995; Tella et al.,

1997). Furthermore, cocaine acts as an indirect agonist at 5-HT and NE transporters, which suggests that neurotransmitter systems other than DA might also have a direct impact on reinforcement. Roberts et al. (1999) recently demonstrated that self-administration of a number of cocaine analogues was predicted more by the ratio of selectivity for DA/5-HT transporters than by the binding affinity for the DA transporter alone. The authors speculate that in addition to having a high affinity for the DA transporter, drugs must also have a relatively lower affinity for the 5-HT transporter in order to be self-administered. Even more compelling are studies that have used recombinant DNA techniques to create DA transporter knockout mice. If the interaction of cocaine with the DA transporter, and the resultant increases in extracellular DA in the NAC is absolutely necessary to induce cocaine-reinforcement, then cocaine would not be expected to serve as a reinforcer in these animals. DA transporter knockout mice show high basal levels of extracellular DA that are unaffected by cocaine and, although cocaine reinforcement in these mice is reduced compared to wild-type mice, they nevertheless do acquire cocaïne self-administration behaviour (Rocha et al., 1998a).

1.2 Cocaine and 5-HT

The actions of cocaine are not restricted to the DA transporter, making it likely that other neurotransmitter systems contribute to cocaine's behavioural profile. Cocaine binds to the 5-HT transporter with greater affinity than it binds to the DA transporter, inhibiting 5-HT uptake up to 4 times more potently than DA uptake (Ritz & Kuhar, 1989). The majority of midbrain 5-HT neurons originate in the raphe nuclei, and both the VTA and NAC receive 5-HT projections from the raphe nuclei (Vertes, 1991). Systemic cocaine produces an

elevation of extracellular 5-HT in the VTA, which suggests that 5-HT levels in mesolimbic structures may contribute to the behavioural effects of cocaine.

Behavioural studies have indicated that 5-HT antagonizes the reinforcing effects of cocaine. Depletion of forebrain 5-HT levels using the selective neurotoxin 5,7 DHT increases cocaine-reinforced break points (Loh & Roberts, 1990; Roberts et al., 1994), while pretreatments with the selective 5-HT reuptake inhibitors fluoxetine or sertraline have been reported to attenuate cocaine self-administration in rats and monkeys, respectively (Carroll et al., 1990; Richardson & Roberts, 1991; Kleven & Woolverton, 1993; Peltier & Schenk, 1993). Enhancing central 5-HT concentrations by pretreatment with the 5-HT precursor *l*-tryptophan has also been reported to reduce cocaine-reinforced break points (McGregor et al., 1993).

There are at least 14 different 5-HT receptor subtypes (Hoyer et al., 1994), and the results of several studies that have investigated specific receptor involvement in cocaine self-administration have been somewhat inconsistent (see Walsh & Cunningham, 1997 for review). For example, the partial 5-HT_{1A} agonist buspirone increases responding for cocaine (Nader & Barrett, 1990) while the specific 5-HT_{1A} agonist 7-OHDPAT and the partial 5-HT_{1A} agonist gepirone either have no effect on, or decrease cocaine self-administration (Gold & Balster, 1990; Nader & Barrett, 1990; Peltier & Schenk, 1993). In addition, the 5-HT_{1A} antagonist spiroxetrine has also been reported to decrease rates of cocaine self-administration in monkeys (Nader & Barrett, 1990).

Data from studies with 5-HT_2 receptors are equally inconclusive. The 5-HT_2 receptor agonist quipazine has been reported to decrease cocaine self-administration. The 5-HT_2

antagonist ketanserin increases cocaine self-administration in some studies (Howell & Byrd, 1995), while in others it appears to have no effect (Lacosta & Roberts, 1993). One possible explanation for the lack of consistency in these studies has to do with the questionable selectivity of these agents. For example the partial 5-HT_{1A} agonist buspirone also has DA antagonist properties which may explain why it increases cocaine-reinforced responding. The partial 5-HT_{1A} agonist gepirone, on the other hand, is a more specific agonist that has no effect on DA levels. The lack of selectivity of these compounds makes it difficult to interpret the exact influence of 5-HT receptor subtype activity on cocaine self-administration.

Several recent studies have indicated that 5-HT_{1B} receptors may be particularly important in mediating the cellular, neurochemical and behavioral effects of cocaine. 5-HT_{1B} agonists have been shown to mediate cocaine-induced reductions in VTA GABA release (Cameron & Williams, 1994), and modulate the effect of cocaine on striatal *c-fos* expression (Lucas et al., 1997). 5-HT_{1B} receptor agonists also lower the threshold dose of cocaine that supports self-administration, and increase break points for responding on a PR schedule of reinforcement, indicating that 5-HT_{1B} receptor stimulation potentiates the reinforcing effects of cocaine, possibly by facilitating cocaine-induced elevations in NAC DA levels (Parsons et al., 1998b). However, it has also been demonstrated that mice lacking the 5-HT_{1B} receptor show increased basal- and cocaine-evoked extracellular DA levels in the NAC (Shippenberg et al., 2000), and an increased motivation to self-administer cocaine compared to wild-type mice. These data demonstrate that cocaine may be more reinforcing in these animals (Rocha et al., 1998b).

It has been suggested that 5-HT may help to mediate cocaine's effect on mesolimbic DA levels via a GABAergic mechanism. Systemic cocaine increases 5-HT levels (Parsons & Justice, 1993) and decreases GABA levels in the VTA (Kalivas & Duffy, 1995). The reduction in VTA GABA levels is not blocked by D_1 or $D_{2/3}$ antagonists, indicating that the effect is not due to cocaine-induced increases in DA. GABAergic striatal neurons that project to the substantia nigra and VTA express 5-HT_{1B} receptors. It is possible that cocaine activates presynaptic 5-HT_{1B} receptors to inhibit GABA release and thereby disinhibit VTA DA neurons to potentiate the action of cocaine (Cameron & Williams, 1994; Parsons et al., 1998a). In mice that lack the 5-HT_{1B} receptor it has been suggested that a compensatory decrease in VTA GABAergic transmission may contribute to the increased basal and cocaine-evoked DA levels, although this possibility has not been confirmed (Rocha et al., 1998b).

1.3 Cocaine and NE

Although cocaine also binds to the NE transporter there is little evidence that NE contributes significantly to the reinforcing effects of cocaine. No effect on cocaine self-administration has been reported following administration of NE receptor blockers (De Wit & Wise, 1977), NE reuptake blockers (Woolverton, 1987; Tella, 1995) or selective lesions of NE systems (Roberts et al., 1977). More recent studies have reported that although some NE reuptake blockers enhance the discriminative stimulus effects of some doses of cocaine, the effect could not be blocked by pretreatment with an alpha-1 adrenergic antagonist (Kleven & Koek, 1998), nor does the NE reuptake inhibitor desipramine substitute for cocaine in discrimination studies (Cunningham & Callahan, 1991). These results suggest that

NE does not play a strong role in modulating the discriminative stimulus effects of cocaine, and that the inhibition of NE reuptake is not a critical modulator of the reinforcing properties of cocaine.

2. γ-amino-butyric-acid (GABA)

In the past 5 years several investigators have begun to examine how the inhibitory neurotransmitter GABA, which is known to interact with and modulate DA neurotransmission, may contribute to cocaine reinforcement (Klitenick et al., 1992; Santiago et al., 1993a; 1993b; Wise, 1996a; 1996b; Ling et al., 1998; Morgan & Dewey, 1998). Several recent studies have indicated that drugs specific to GABA synapses have the potential to modulate the neurochemical and behavioral effects of cocaine (Goeders et al., 1989; Santiago et al., 1993a; Roberts et al., 1996; Roberts & Andrews, 1997; Dewey et al., 1997; 1998; Campbell et al., 1999; Kushner et al., 1999; Shoaib et al., 1999).

2.1 GABA Receptors

GABA, the major inhibitory neurotransmitter in the brain, acts on 3 different receptor subtypes–$GABA_A$, $GABA_B$ and $GABA_C$. Both $GABA_A$ and $GABA_C$ receptors are ionotropic. $GABA_A$ receptors are made up of 5 subunits belonging to different families (α1-6, β1-4, γ1-4, δ, ε and π), which combine to form 16 known heterooligomeric subtypes, each containing α- β- and γ- subunits. The differential expression of the subunit isoforms determines the pharmacological and physiological properties of each of the receptor subtypes. $GABA_A$ receptors are coupled with chloride (Cl^-) channels, and mediate most of the fast synaptic inhibition in the brain (MacDonald & Olsen, 1994). They are selectively blocked by

bicuculline and are modulated by benzodiazepines, steroids and barbiturates (Froestl et al., 1995; Chebib & Johnston, 1999; Bormann, 2000).

Although the $GABA_C$ receptor subunits (ρ1-3) that have been cloned appear to act very much like $GABA_A$ receptors, they share only 30-38% sequence homology with $GABA_A$ receptors, and are pharmacologically, biochemically and physiologically distinct. In 1998, the International Union of Pharmacology committee provisionally recommended that $GABA_C$ receptors should be considered a subset of $GABA_A$ receptors (Humphrey & Barnard, 1998), however there has been some resistance to this suggestion (Bormann, 2000). $GABA_C$ receptors are linked to Cl^- channels and like $GABA_A$ receptors mediate fast synaptic inhibition; however, they are insensitive to both bicuculline and the $GABA_B$ agonist baclofen. Furthermore, GABA is an order of magnitude more potent at $GABA_C$ than at $GABA_A$ synapses (Bormann & Feigenspan, 1995; Johnston, 1996). $GABA_C$ receptors are activated selectively by the GABA analogue CACA (cis-4-amino-crotonic acid), and blocked by TMPA [(1,2,5,6-tetrahydropyridine-4-yl) methylphosphinic acid].

$GABA_B$ receptors are more closely related to members of the metabotropic glutamate receptor family than to $GABA_A$ or $GABA_C$ receptors. $GABA_B$ receptors have seven transmembrane domains, an extended N-terminal extracellular domain and an intracellular C terminal. The $GABA_B$ receptor subtypes ($GABA_B$-R1a, $GABA_B$-R1b and $GABA_B$-R2) are metabotropic G-protein coupled receptors that are thought to modulate their effector systems through the inhibitory G-proteins $G\alpha_i$ and $G\alpha_o$ (Couve et al., 2000). $GABA_B$ receptors mediate slow inhibitory signalling, by modulating neurotransmitter (e.g. DA, NE, 5-HT) release through presynaptic G-protein-coupled inhibition of Ca^{++} currents,

or hyperpolarization of postsynaptic membranes through activation of K^+ conductances (Hill & Bowery, 1981; Mott & Lewis, 1994; Misgeld et al., 1995; Kaupmann et al., 1997; Kaupmann et al., 1998; Takahashi et al., 1998; White et al., 1998; Bowery & Enna, 2000; Couve et al., 2000).

$GABA_B$ receptors function only in the form of a heterodimer of $GABA_B$-R1a/b and $GABA_B$-R2 proteins linked in a 1:1 ratio through coiled domains at the C terminus (Kammerer et al., 1999). The two $GABA_B$-R1 splice variants differ in the N-termini, and share approximately 35% sequence homology. Recently, two additional splice variants have been identified in the rat–$GABA_B$-R1c and -R1d (Isomoto et al., 1998) which may ultimately account for pharmacological variability among the receptors. $GABA_B$-R1 receptors are blocked by $GABA_B$ receptor antagonists and show similar agonist binding affinity as wild-type receptors when expressed as a part of a heterodimer. No ligand binding has been demonstrated for the $GABA_B$-R2 component. It has been suggested that the presence of the $GABA_B$-R2 component is necessary for the maturation and transport of $GABA_B$-R1 from the endoplamsic reticulum to the plasma membrane and enables the dimer to function as a $GABA_B$ receptor (Kaupmann et al., 1997; Couve et al., 1998; Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Couve et al., 2000).

$GABA_B$ receptors are widely distributed throughout the CNS. In both human and rat brain, the thalamic nuclei, the cerebellum and cerebral cortex show the highest levels of $GABA_B$ binding sites. The two $GABA_B$-R1 splice variants appear to show different patterns of distribution. $GABA_B$-R1a is predominant in the striatum, hippocampus, dentate gyrus, hypothalamus, the granule cell layer of the cerebellum and the brainstem, while $GABA_B$-R1b

is most highly expressed in the superficial cortical layers, interpeduncular nucleus, molecular layer of the cerebellum and the spinal cord (Bischoff et al., 1999; Billinton et al., 2000; Margeta-Mitrovic et al., 2000). Furthermore, $GABA_B$-R1a subtypes are more often associated with presynaptic receptors, whereas the $GABA_B$-R1b variant is more concentrated postsynaptically (Bischoff et al., 1999; Yamada et al., 1999; Fritschy et al., 1999). $GABA_B$ receptors are antagonized by phaclofen, saclofen, and CGP56433A, and are activated by baclofen (β-(aminomethyl)-p-chlorohydrocinnamic acid) and methylphosphinic acid analogues of GABA such as CGP44532 and CGP35024. Because $GABA_B$ receptor activation results in slow inhibition of synaptic transmission, the processes that are mediated by these receptors are particularly susceptible to compounds that induce changes in receptor function (Couve et al., 2000).

2.2 GABA and Mesolimbic DA

Manipulation of GABA systems has been shown to have direct effects on DA pathways, causing dose-dependent decreases in extracellular DA concentrations in the CNS of primates and rodents (Dewey et al., 1992; Klitenick et al., 1992; Santiago et al., 1993a; 1993b; Dewey et al., 1997; Morgan & Dewey, 1998; Gerasimov et al., 2000). In the mesolimbic DA system, the majority of neurons in the NAC are GABAergic neurons that project to DA cells within the VTA and regulate their activity (Kita & Kitai, 1988). The VTA contains two major cell types: the primary dopaminergic neurons which release DA in the NAC and PFC, and the secondary GABAergic neurons which function as inhibitory interneurons to control the firing of principal DA neurons (Kalivas, 1993; White, 1996; Van Bockstaele & Pickel, 1995; Steffensen et al., 1998).This arrangement suggests that GABA

projection neurons and GABA-interneurons within the NAC and VTA have the capacity to powerfully influence DA release and DA cell firing (Klitenick et al., 1992; Cameron & Williams, 1994; Churchill & Kalivas, 1994; Kalivas & Duffy, 1995). Indeed, intracerebral administration of the $GABA_B$ agonist baclofen has been shown to reduce DA release from the terminals in the VTA, substantia nigra, PFC cortex and striatum and inhibit DA cell firing in the VTA (Kalivas, 1993; Santiago et al., 1993a; 1993b; Kabuto et al., 1995; Westerink et al., 1998).

2.3 GABA Modulation of Cocaine Self-administration

As the anatomical and physiological interaction between dopaminergic and GABAergic neurons in the mesolimbic system became more clearly defined, the hypothesis that GABA neurotransmission may play a role in mediating the reinforcing effects of drugs such as cocaine emerged. Several studies have shown that GABA-related compounds such as the gamma-transaminase inhibitor gamma-vinyl-GABA (GVG), the benzodiazepines alprazolam and chlordiazepoxide, the GABA uptake inhibitor 1-(2-(((diphenylmethylene)amino)oxy)ethyl-1,2,5,6-tetrahydro-3-pryidinecarboxylic acid hydrocholoride (NNC-711) and the $GABA_B$ agonist baclofen can influence the physiological and behavioral effects of cocaine (Goeders et al., 1989; 1993; Roberts et al., 1996; Dewey et al., 1997; Roberts & Andrews, 1997; Dewey et al., 1998; Morgan & Dewey, 1998; Shoaib et al., 1998; Campbell et al., 1999; Kushner et al., 1999, Gerasimov et al., 2000).

It is not yet entirely clear whether $GABA_A$ or $GABA_B$ receptors are more critically involved in mediating the effect of GABAergic drugs on cocaine-reinforcement. For example, GVG, which inhibits cocaine-induced increases in NAC DA levels and attenuates

cocaine self-administration (Dewey et al., 1997; Kushner et al., 1997; Dewey et al., 1998; Morgan & Dewey, 1998) causes a general elevation in GABA levels which would affect both GABA receptor subtypes. Recently, however, it has been shown that $GABA_B$ antagonists block GVG's inhibition of cocaine-induced increases in DA release (Ashby et al., 1999). These results suggest that stimulation of $GABA_B$ receptors may be essential to the effect of GVG on cocaine self-administration. There is, however, some evidence that GVG also decreases responding for food reinforcement, suggesting that its effects on cocaine self-administration may be the result of a generalized decrease in operant responding, or a non-specific effect on central reward systems (Kushner et al., 1999; Caine et al., 2000a).

A number of recent publications have provided evidence that $GABA_B$ receptors may be critically involved in GABAergic modulation of cocaine reinforcement. Several investigations have reported that the systemic administration of the $GABA_B$ agonist baclofen is particularly effective at attenuating cocaine self-administration in rodents (Roberts et al., 1996; Roberts & Andrews, 1997; Campbell et al., 1999; Shoaib et al., 1999). The neural substrates that underlie baclofen's impact on cocaine self-administration are unknown. Although the VTA contains both $GABA_A$ and $GABA_B$ receptors (Bowery et al., 1987; Kalivas et al., 1990), *in vivo* studies have demonstrated that microinjection of baclofen into the VTA decreases the release of extracellular DA in the NAC (Kalivas et al., 1990; Yoshida et al., 1994; Westerink et al., 1997). Manipulation of GABAergic transmission in the VTA attenuates the rewarding properties of intracranial self-stimulation (Willick & Kokkinidis, 1995), morphine-induced place preference (Tsuji et al., 1996), and heroin- and cocaine-reinforced responding (Shoaib et al., 1998; Xi & Stein, 1999). Taken together, these results

suggest that the VTA is an important site of potential GABA/DA interaction and that VTA $GABA_B$ receptors in particular may have an inhibitory effect on reward function.

Summary

Historically, much of the research that explored the basic neurobiology of cocaine addiction focused on the dopaminergic theory of reinforcement. These studies have uncovered an enormous amount of information about how cocaine affects the brain. Substantial evidence gathered from electrochemical, microdialysis, lesion and behavioural studies indicates that the addictive properties of cocaine and other psychostimulant drugs are associated with increased activation of the mesolimbic DA system (see Koob & Bloom, 1988; Kalivas et al., 1990; Bardo, 1998 for review). However, the mesolimbic DA system has extensive anatomical and physiological interactions with other neurotransmitter systems, and several recent investigations have begun to look beyond the DA hypothesis in order to further identify the primary pathways and receptor mechanisms involved in cocaine reinforcement.

A growing body of evidence indicates that GABAergic compounds have the ability to modify cocaine reinforcement, although the magnitude and specificity of these effects remains to be determined. The following series of experiments are aimed at examining how $GABA_B$ agonists influence cocaine self-administration. The experiments will take into account how environmental factors such as the schedule of reinforcement and the unit dose of cocaine can interact with pharmacological variables to affect an animal's self-administration behaviour.

Series 1: To characterize the effects of systemic baclofen on cocaine self-administration

It is becoming increasingly apparent that drugs that are specific to GABA synapses have the potential to affect cocaine self-administration. A number of recent studies have shown that the $GABA_B$ agonist baclofen attenuates cocaine reinforcement. Baclofen is a GABA analogue that was first synthesized in 1962. It crosses the blood-brain-barrier, and has a 1000 fold higher affinity for $GABA_B$ than $GABA_A$ receptors. The (R)-(-) enantomer is thought to be the active compound; (+) baclofen is approximately 100 fold less potent than (-) baclofen in binding assays.

The magnitude and specificity of baclofen's effect on cocaine self-administration appears to depend on the schedule of reinforcement used. Under FR schedules, animals are required to complete a set number of lever responses in order to obtain each infusion of cocaine. This type of schedule is typically used to explore the rate of cocaine intake. The typical dose-response function for cocaine self-administration is an inverted U-shaped curve, with low to moderate doses producing higher rates of responding than higher doses. The increased rate of intake of the lower dose of the drug has traditionally been interpreted as a compensatory response to the decreased reinforcing efficacy of the low dose of cocaine (Yokel & Wise, 1975; DeWit & Wise, 1977).

Baclofen's effect on cocaine self-administration under FR schedules appears to be somewhat inconsistent. Roberts et al. (1996) reported that relatively high doses of intraperitoneal (IP) baclofen (2.5 or 5.0 mg/kg) did not disrupt i.v. cocaine self-administration (1.5 mg/kg per injection) on an FR1 schedule, although a similar dose did

attenuate responding for 0.66 mg/kg of cocaine on an FR5 schedule (Shoaib et al., 1998). Very recently, Campbell et al. (1999) reported that baclofen pretreatment (2.5 and 5.0 mg/kg, IP) disrupts responding for low doses of cocaine (0.2 or 0.4 mg/kg) on an FR1 schedule. While these reports would appear to be contradictory, differences in both the response requirement and the unit dose of cocaine in these studies may account for the disparate results. In order to address this possibility, one of the goals of the present investigation was to obtain complete dose-response curves for both baclofen and cocaine on an FR1 schedule.

In order to compare our results with previously published attenuations of cocaine self-administration that resulted from treatment with DA antagonists, a dose-response curve was also established for the effects of haloperidol on FR1 responding. The prediction was that baclofen and haloperidol would have different effects on patterns of responding under FR1 conditions.

Under PR schedules of cocaine-reinforcement the dependent measure, break point, is affected by changes in the unit injection dose of cocaine, and the ascending limb of the dose response curve has previously been identified (Arnold & Roberts, 1997; Stafford et al., 1998). The 0.18 mg/kg per injection dose of cocaine is near threshold and usually supports a total of 30-40 responses/session. The highest dose (1.5 mg/kg per injection) can be expected to support about 300-400 responses/session which is equivalent to a final ratio of about 77. A growing literature illustrates that the dose response curve is sensitive to pharmacological, hormonal and neurotoxic manipulations (Roberts et al., 1989a, 1989b; Roberts & Bennett, 1993; McGregor et al., 1994; Roberts et al., 1994; McGregor et al.,

1996).

Roberts et al. (1996) demonstrated that baclofen reduced break points across a series of unit-injection doses of cocaine, under a PR schedule of reinforcement, without producing a corresponding change in the rate of cocaine intake under an FR1 schedule. Because the response requirements on a PR schedule of reinforcement increase with each drug infusion, the question as to whether attenuation of cocaine self-administration is a real effect, or merely a result of a non-specific suppression in operant responding must be addressed (Zarrindast et al., 1989; Grech & Balster, 1993). Several papers have examined the effect of baclofen on food-reinforced responding in an attempt to address the specificity issue. Shoaib et al. (1998) used a multiple schedule to show that baclofen decreased cocaine-reinforced responding, while responding during the food reinforced-component was only marginally affected. Roberts et al. (1996) demonstrated that baclofen had little effect on food-reinforced responding on a PR schedule identical to the one used in cocaine self-administration experiments. The present study used concurrent access to food and cocaine reinforcement in order to determine whether animals that decline to self-administer cocaine following baclofen retain the capacity to respond at high rates.

Materials and Methods

Subjects

Subjects in this Series of experiments were male Wistar rats (Charles River Farms, Quebec) weighing 275-300g at the start of the experiments. All animals were placed under quarantine for 1 week following arrival at the facility, were housed according to Canadian

Council on Animal Care standards, and were maintained on a 12-h reversed light/dark cycle (lights off at 3:00 am). Following quarantine, animals were food deprived for 18-h then trained to press a lever for food reinforcement on a FR1 schedule. Thereafter, Purina® Rat Chow was available *ad libitum*, except as noted below. Water was available *ad libitum* throughout all phases of the experiment. Each rat was implanted with a chronically indwelling Silastic® jugular cannula that exited through the skin on the dorsal surface in the region of the scapulae (Roberts & Goeders, 1989). Following cannulation, rats were individually housed in a 25 x 25 x 25 cm testing chambers. The cannula was connected through a stainless steel protective spring to a counterbalanced swivel apparatus that allowed free movement within the chamber.

Procedure

Beginning the day after surgery animals were given access to a response lever that controlled the delivery of cocaine injections on an FR1 schedule. Concurrent with the start of each cocaine injection (1.5 mg/kg per injection in 0.12 ml saline) a stimulus light located above the lever was activated to signal a 20-s post-infusion time-out period, during which responses produced no programmed consequence. Rats received daily 6-h test sessions (9 am-3 pm) that began with one non-contingent injection of cocaine. After the animals had established a stable daily pattern of cocaine intake (>30 injections/6-h and regular post-infusion pauses) on an FR1 schedule, they were randomly assigned to experimental groups.

Cocaine Dose-Response Curve

Rats (N = 8-12) were given access during daily 3-h sessions to a response lever that delivered cocaine under an FR1 schedule. The effect of baclofen pretreatment (3.2 mg/kg

IP, - 30 min) on self-administration of four unit injection doses of cocaine (0.19, 0.38, 0.75 or 1.5 mg/kg per injection) was assessed using a repeated measures design. At least four baseline sessions preceded the baclofen test day, after which the unit injection dose of cocaine was adjusted according to a Latin square design.

Baclofen Dose-Response Curves

Two groups of rats (n = 5 per group) were trained to self-administer either 0.75 or 1.5 mg/kg per injection of cocaine under an FR1 schedule of reinforcement. Following at least three days of stable responding, rats received baclofen (1.8, 3.2 or 5.6 mg/kg, IP) 30 min prior to the test session. The order of baclofen injections was counterbalanced according to a Latin square design and test sessions were separated by at least three days. The time between each cocaine infusion (inter-injection interval) was also measured in order to determine the precise impact of baclofen on the pattern, or rate, of responding. For comparison, a separate group of rats trained to self-administer 0.75 or 1.5 mg/kg per injection of cocaine (N = 6) was pretreated with haloperidol (32, 56 or 100 μg/kg, IP) 60 min prior to the test session. The order of haloperidol doses was counterbalanced across subjects and test sessions were separated by at least 3 days.

Concurrent Access Conditions

The effect of baclofen on concurrent cocaine- and food-reinforced responding was examined in a separate group of rats (N = 6). Rats were initially trained on an FR1 schedule of cocaine reinforcement. Once stable daily patterns of intake were established (see above), a PR schedule was introduced. Cocaine infusions (1.5 mg/kg per injection) were contingent upon an increasing number of responses incremented through the following progression: 1,

2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603.This progression is calculated by the equation: Response ratio = $(5 \times e^{(0.2 \times \text{infusion number})})-5$ rounded to the nearest integer. Final ratios, the last ratio successfully completed before the animals failed to complete a response within a one-hour period were recorded daily (procedure described in detail in Richardson & Roberts, 1996).Under this schedule, stable performance was defined as three consecutive days of cocaine self-administration with break points within a range of 4 increments. Once stable responding on the PR schedule had been established, testing included a concurrently available food reinforced lever. Rats were 16-h food-deprived at the beginning of the session and received access to Purina® Rat chow for 2 hours immediately following the session. The session began with the introduction of a food-reinforced lever; the cocaine-reinforced lever was introduced 5 min later. All animals received a single administration of non-contingent food and cocaine at the start of each daily session. Initially a single response on the food lever resulted in the delivery of a 45 mg nutritionally balanced food pellet (Noyes Inc.). The schedule was incremented from FR1 to FR5 during the first four sessions. Daily baseline testing continued for at least four days before the effect of baclofen (1.0, 1.8, 3.2, or 5.6 mg/kg, IP, 30 min prior to the session) was investigated. All injections were administered according to a Latin square design, and at least 3 days separated test days.

Drugs

Cocaine HCl was supplied by the National Institute on Drug Abuse (Research Triangle, NC). (±)-Baclofen was purchased from Research Biochemicals International. Baclofen was dissolved in pyrogen-free sterile saline. Haloperidol (ICN Biochemicals Inc,

Ohio) was dissolved in glacial acetic acid and then diluted in sterile saline. The pH of the haloperidol solution was adjusted to between 5.6 and 6.0 using NaOH. Dosages are expressed as the salt.

Data Analysis

In the FR1 experiments, the dependent measure was the number of cocaine injections/3-h session. Baseline (BL) rates were calculated by averaging the number of cocaine injections self-administered on the day before each test session. In the PR experiment, the dependent measure was break point (defined as the number of completed increments prior to a 1-h period during which no cocaine infusions were obtained). Baseline rates were calculated as described in the FR1 experiments. The total number of food reinforced responses was collected for the entire 6 hours although statistical analysis was restricted to the first two hours of the session, when the effect of baclofen on cocaine-reinforced responding was observed. Data were analyzed by repeated measures ANOVA with *post hoc* Newman Keuls multiple comparisons.

Results

Cocaine Dose-Response Curve

Animals that failed to complete testing on at least 3 of the 4 unit injection doses of cocaine were excluded from the analysis. Baclofen pretreatment (3.2 mg/kg, IP) was found to significantly attenuate cocaine self-administration under an FR1 schedule. The ANOVA revealed a significant Treatment x Dose interaction [$F = 6.038$, $df = 3,12$, $p<0.01$], and multiple comparisons indicated that baclofen reduced cocaine self-administration at all but

the highest unit dose (1.5 mg/kg per injection) of cocaine. Baclofen had a proportionally greater effect at the lower unit doses of cocaine and little if any effect at the highest doses (1.5 mg/kg per injection–See Fig 1a). The typical response patterns following baclofen pretreatment are included for the 0.75 and 1.5 mg/kg per injection doses). Decreases in self-administration of cocaine, when they were observed, were accounted for by a period of non-responding at the start of the test-session, rather than a reduction in the rate of responding over the course of the session (see Figure 1b).

Baclofen Dose-Response Curves

The effect of baclofen (1.8, 3.2 or 5.6 mg/kg, IP) on cocaine-reinforced responding (0.75 or 1.5 mg/kg per injection) under an FR1 schedule is illustrated in Figure 2. In assessing the baclofen dose-response curves, only data from animals that received all three doses of baclofen were included in the analysis. ANOVA revealed a significant effect of baclofen at both unit doses of cocaine [0.75 mg/kg per injection: $F = 7.13$, $df = 3,16$, $p<0.01$; 1.5 mg/kg per injection: $F = 4.55$, $df = 3,16$, $p<0.01$]. Baclofen produced a greater attenuation of cocaine self-administration at the lower unit injection doses. Multiple comparisons revealed that while 3.2 mg/kg of baclofen significantly attenuated responding for the low unit-dose of cocaine, a 5.6 mg/kg dosage was required to affect responding for 1.5 mg/kg of cocaine.

The distribution of inter-injection intervals was found to be dose-dependent, with the time between infusions increasing as the unit dose of cocaine increases (Figure 3). Although reductions in the total number of responses were evident following some doses of baclofen, they were accounted for by periods of non-responding, usually at the beginning of the test

Figure 1 (A): Effect of baclofen (3.2 mg/kg, IP) on self-administration of various doses of cocaine. Rats (N = 8-12) were trained to respond for several doses of cocaine (0.19, 0.38, 0.75 or 1.5 mg/kg/infusion) under an FR1 schedule of reinforcement. Points represent the mean (± SEM) number of responses during baseline (BL) days and over the 3-h test session. Animals were pretreated with baclofen 30 min prior to the test session. At all but the highest unit dose of cocaine baclofen produced a significant reduction in responding ($p<0.05$). Reductions in cocaine intake were accounted for by periods of non-responding at the beginning of the test session (see **B**), which resulted in a decrease in the total number of injections.

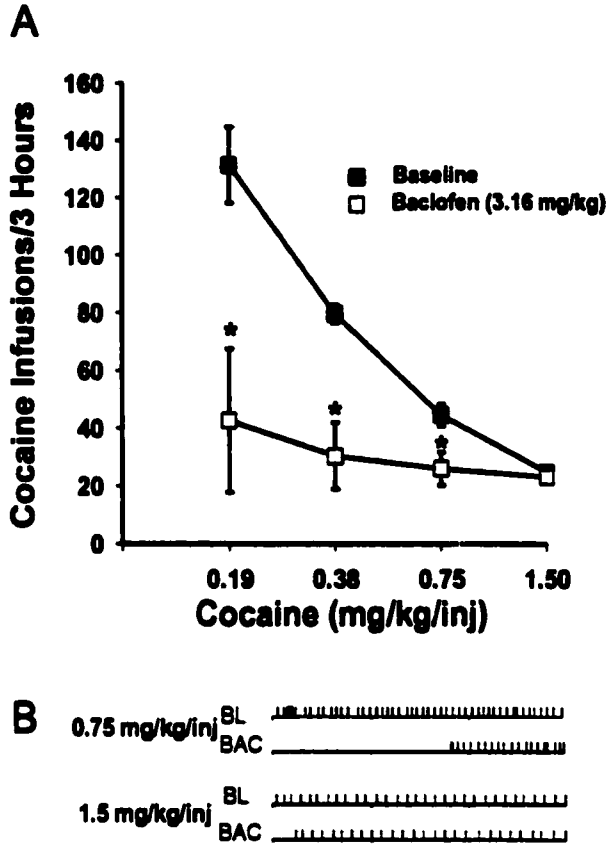

A
Cocaine Infusions/3 Hours
160
140
120
100
80
60
40
20
0
Baseline
Baclofen (3.16 mg/kg)
0.19
0.38
0.75
1.50
Cocaine (mg/kg/inj)
B
0.75 mg/kg/inj
BL
BAC
1.5 mg/kg/inj
BL
BAC

Figure 2: Effect of baclofen (1.8, 3.2 or 5.6 mg/kg, IP) on cocaine self-administration on an FR1 schedule. Points represent the mean (± SEM) number of injections self-administered during a 3-h baseline (BL) or test session. Separate groups (N = 5 per group) of animals were trained to respond for either 0.75 or 1.5 mg/kg per injection of cocaine and were pretreated with baclofen 30 min before the session. Baclofen produced a greater attenuation of cocaine self-administration at the lower unit injection doses. Self-administration of the lower unit dose (0.75 mg/kg per injection) was significantly affected by both 3.2 and 5.6 mg/kg of baclofen ($p<0.05$), whereas the high unit injection dose (1.5 mg/kg) was significantly reduced only by 5.6 mg/kg baclofen ($p<0.05$).

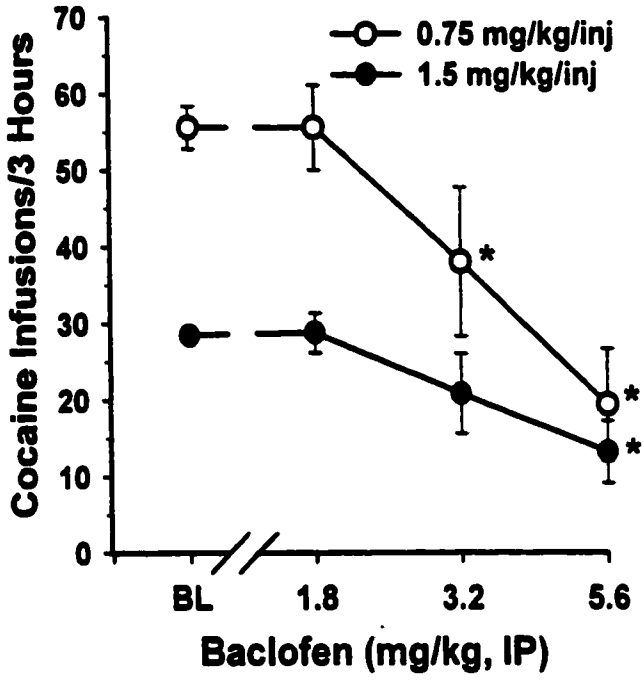
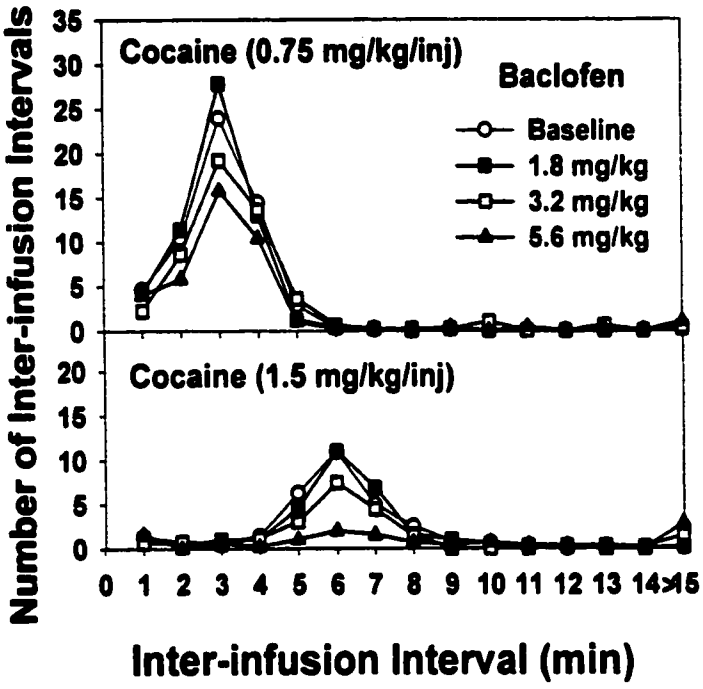

0.75 mg/kg/inj
1.5 mg/kg/inj
Cocaine Infusions/3 Hours
70
60
50
40
30
20
10
0
BL
1.8
3.2
5.6
Baclofen (mg/kg, IP)
*
*
*

Figure 3: Effect of baclofen (1.8, 3.2 or 5.6 mg/kg, IP) on the distribution of inter-injection intervals during cocaine self-administration on an FR1 schedule. Points represent the mean number of injections of cocaine that occurred during the indicated intervals for animals trained to respond for either 0.75 mg/kg per injection (**top**) or 1.5 mg/kg per injection (**bottom**). Curves represent the effect of pretreatment with various doses of baclofen. While baclofen produced a dose-dependent reduction in the total number of infusions, it failed to affect the distribution of inter-injection intervals at either concentration of cocaine. Reductions in self-administration were accounted for by periods of non-responding at the beginning of the test sessions. Note that an hour long pause in responding would result in only a single entry in the >15 min interval and would account for a substantial decrease in the total number of injections.


Cocaine (0.75 mg/kg/inj)
Baclofen
Baseline
1.8 mg/kg
3.2 mg/kg
5.6 mg/kg
Cocaine (1.5 mg/kg/inj)
Number of Inter-infusion Intervals
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14>15
Inter-infusion Interval (min)

sessions. Baclofen pretreatment failed to shift the peak inter-injection intervals at any of the doses administered, indicating that the rate of cocaine self-administration was identical to baseline patterns once the rats began responding.

Haloperidol pretreatment (32, 56 or 100 μg/kg, IP) increased responding for both 0.75 and 1.5 mg/kg per injection of cocaine over the three hour session. Examination of the inter-injection intervals revealed that haloperidol caused a dose-dependent shift to the left (see Figure 4).

Concurrent Access Conditions

The typical baseline pattern of responding for concurrently available cocaine and food reinforcement is illustrated in Figure 5 (top). With concurrent access, cocaine-reinforced responding on the PR schedule predominated until a break point was reached. The pattern of cocaine-self-administration under these conditions was indistinguishable from that previously observed on a PR schedule (e.g. Roberts et al. 1989b;1996). That is, the addition of a food reinforced lever did not appear to change the response characteristics or the break points from previous observations. During the first five minutes of the session when only the food lever was available, all animals responded at high rates. However, when the cocaine lever was introduced, four of the six animals responded almost exclusively on the cocaine lever until the break point was reached, at which time responding was redirected toward the food lever. The remaining two animals alternated between the cocaine and the food-reinforced levers after the introduction of the cocaine lever, and did not begin to respond exclusively on the food-lever until after the break point for cocaine was reached. Regardless of individual response patterns, the number of food pellets delivered over the entire 6 hour

Figure 4: Effect of haloperidol (32, 56 or 100 μg/kg, IP) on the distribution of inter-injection intervals during cocaine self-administration on an FR1 schedule. Points represent the mean number of injections of cocaine that occurred during the indicated intervals for animals (N = 6) trained to respond for 0.75 or 1.5 mg/kg per injection. Curves represent the effect of pretreatment with various doses of haloperidol. Haloperidol produced a dose-dependent shift to the left of the modal inter-injection interval; this effect is qualitatively different from the effect of baclofen.

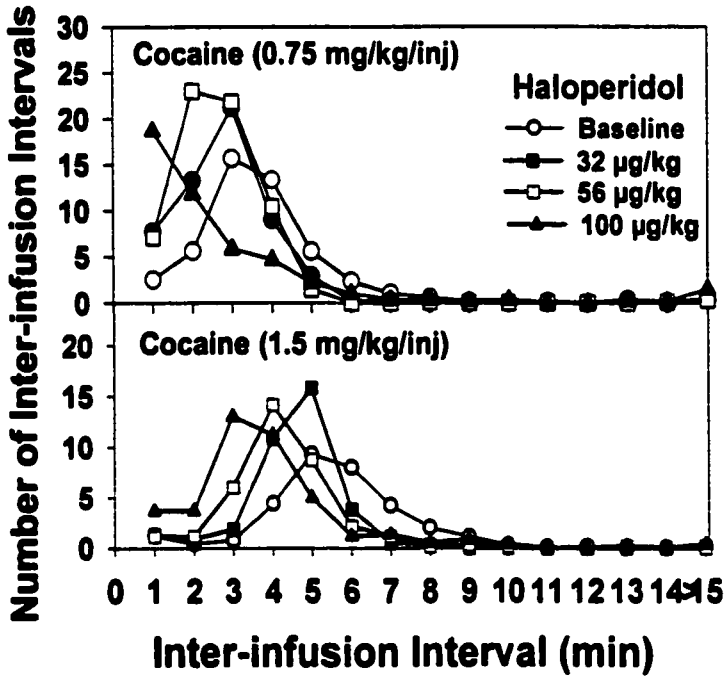
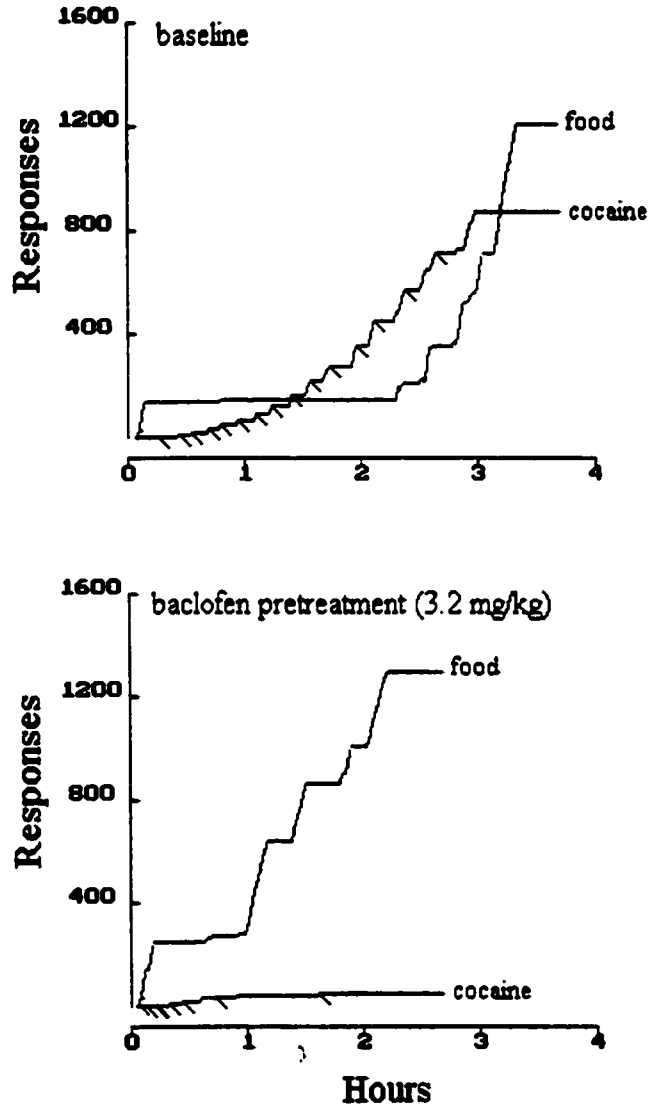

Cocaine (0.75 mg/kg/inj)
Haloperidol
Baseline
32 µg/kg
56 µg/kg
100 µg/kg
Cocaine (1.5 mg/kg/inj)
Number of Inter-infusion Intervals
Inter-infusion Interval (min)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 >15

Figure 5: Representative records illustrating the effect of baclofen (3.2 mg/kg, IP) on cocaine self-administration reinforced on a PR schedule. Food reinforcement was concurrently available on an FR5 schedule. During the first five minutes of the session, only the food reinforced lever was available; thereafter both food and cocaine levers were concurrently available for the duration of the session. Short downward marks represent cocaine infusions while upward increments in the record represent lever responses. Under baseline testing conditions (**top**), this animal demonstrated high rates of responding on the food-reinforced lever until the cocaine lever was introduced; thereafter it responded almost exclusively on the cocaine-reinforced lever while the response requirements were relatively low. Food-reinforced responding re-emerged as the cocaine break point was approached. Following baclofen (**bottom**), responding on the cocaine-reinforced lever was observed only at low ratios and responding switched exclusively to the food-reinforced lever very early in the session. This pattern of results does not suggest a generalized disruption of operant responding.


1600
1200
800
400
Responses
baseline
food
cocaine
0
1
2
3
4
baclofen pretreatment (3.2 mg/kg)
food
cocaine
Hours

session was relatively consistent across animals (approx. 400).

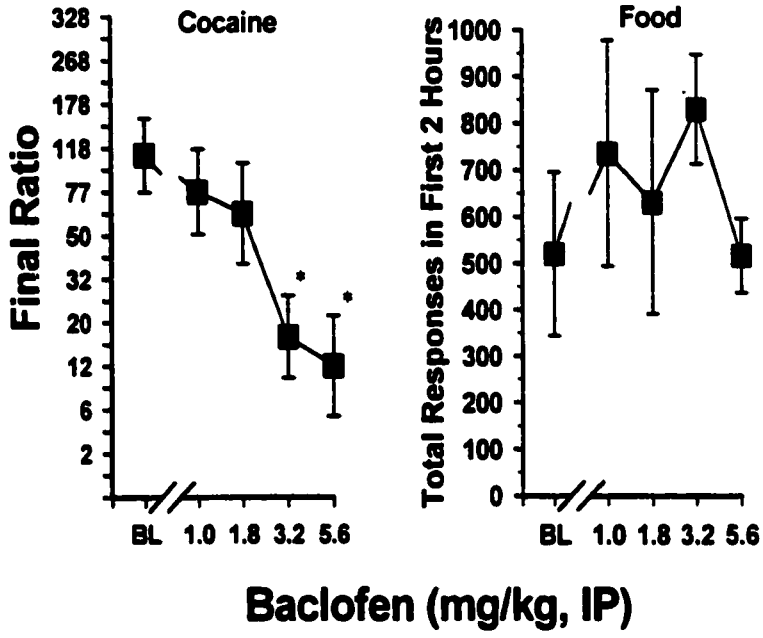
Baclofen pretreatment (3.2 mg/kg, IP) dramatically reduced cocaine-reinforced break points and altered the pattern of responding for concurrently available cocaine or food reinforcement (Figure 5, Bottom). Responding was not suppressed, but instead redirected towards the food-reinforced lever.

Baclofen was found to have a differential effect on food- and cocaine-reinforced responding (Figure 6, Left) under concurrent access conditions. One way repeated measures ANOVA revealed a significant effect of Dose [$F = 5.9$, $df = 4,20$, $p<0.01$] and *post-hoc* tests indicated that the two highest doses of baclofen produced a significant decrease in cocaine-reinforced break-points. Figure 6 (Right) shows the effect of baclofen pretreatment on food-reinforced responding on an FR5 schedule. Statistical analysis of the total responses during the first two hours (when the effects of baclofen on cocaine self-administration were observed) failed to reveal a statistically significant effect.

Discussion

The effect of baclofen pretreatment on cocaine self-administration was shown to be dependent on the unit injection dose of cocaine and on the response requirements of the schedule. Previous results have demonstrated that, on an FR schedule, baclofen suppresses intake of low (0.2 and 0.4 mg/kg per injection: Campbell et al., 1999) and medium (0.66 mg/kg per injection: Shoaib et al., 1998) but not high (1.5 mg/kg per injection: Roberts et al., 1996) unit injection doses of cocaine. The present data confirm these reports and demonstrate this dose-response relationship in a single experiment. The effect of baclofen

Figure 6: Effect of baclofen on responding for concurrently available cocaine and food reinforcement. **Left:** Points represent the mean (± S.E.M.) break points on a PR schedule of cocaine reinforcement (1.5 mg/kg per injection) under baseline conditions (BL) or following various doses of baclofen. **Right:** Points represent mean (± S.E.M.) food-reinforced responses (on an FR5 schedule) during the first two hours of the sessions represented in the left panel. See Figure 5 for details. Baclofen pretreatment caused a dose dependent decrease in break points for cocaine (*$p<0.05$). The right panel illustrates that during the first two hours, when cocaine self-administration was suppressed, animals emitted more than 500 responses (on average) on the food-reinforced lever. These results do not support the hypothesis that baclofen produces a non-specific disruption of operant responding.


Cocaine
Food
Final Ratio
328
268
178
118
77
50
32
20
12
6
2
Total Responses in First 2 Hours
1000
900
800
700
600
500
400
300
200
100
0
BL 1.0 1.8 3.2 5.6
BL 1.0 1.8 3.2 5.6
Baclofen (mg/kg, IP)

was also shown to be dependent on the schedule of reinforcement. At the same unit dose of cocaine (1.5 mg/kg per injection), responding on an FR1 was not affected by baclofen pretreatment whereas responding on a PR schedule was significantly decreased. These data are consistent with a previous demonstration (Roberts et al., 1996) that baclofen (2.5 mg/kg, IP) decreased break points across a broad range of unit-injection doses of cocaine (0.18-1.5 mg/kg per injection). The entire data set is compatible with the behavioral economic interpretation offered by Campbell et al. (1999). They argue that dose and response requirements are both constituents of unit price (Bickel et al., 1990), and that baclofen appears to have greater efficacy as the unit price (responses/mg/kg) increases. Other drugs, such as fluoxetine, L-tryptophan and carbamazepine show a similar profile (Carroll et al., 1990a; 1990b; 1990c).

Baclofen and haloperidol are similar in that they both reduce cocaine-reinforced break points on a PR schedule (Richardson et al., 1994; Roberts & Ranaldi, 1995; Roberts et al., 1996); however, the FR data indicate that each drug affects cocaine self-administration in a fundamentally different way. Analysis of the inter-injection intervals in the present study showed, as expected, that haloperidol shifted the peak inter-injection intervals to the left, indicating a uniform and dose-dependent increase in rate of drug intake. By contrast, baclofen shifted the cocaine intake curve downward on the FR1 schedule; at no point in the dose-response curve was there any indication of an increase in cocaine intake. Baclofen-induced reductions in cocaine self-administration were found to be due to periods of non-responding at the beginning of the test session, or long pauses between responses, rather than a decrease in the rate of responding over the course of the test sessions. If both baclofen and

haloperidol decrease the reinforcing effects of cocaine, it is unclear why they have fundamentally different effects on FR1 responding.

Consistent with previous reports (Roberts et al., 1996), the present results demonstrate that baclofen produced a dose-dependent reduction in break-points for responding for cocaine on a PR schedule. In order to rule out the possibility that baclofen produced a decrease in cocaine self-administration by interfering with the capacity of the rats to complete the operant response, the present experiment re-examined the effect of baclofen in animals given concurrent access to cocaine and food reinforcement. Analysis of food-reinforced responding (restricted to the first two hours following baclofen administration when the drug effect would be strongest) showed that responding on the food-reinforced lever was not significantly reduced from baseline, regardless of the dose of baclofen administered. Indeed, at all but the highest dose of baclofen, the number of food-reinforced responses was somewhat increased, suggesting that baclofen produced a redirection from cocaine-directed, toward food-seeking behavior. The results clearly demonstrate that animals were capable of completing several hundred responses on the food-reinforced lever during the time when cocaine-reinforced responding was suppressed. These data indicate that baclofen's effect on cocaine self-administration is not attributable to a generalized disruption of operant responding, and rule out the potential that a cocaine-baclofen combination produced a non-specific or toxic reaction.

Roberts et al. (1996) previously demonstrated that baclofen had little effect on food-reinforced responding on a PR schedule identical to the one used in cocaine self-administration experiments. In that study, the food- reinforced group was cocaine-naïve.

This may have been an important factor, since it has recently been shown that prolonged exposure to cocaine may alter the physiology of GABAergic systems (Zeigler et al., 1991; Pecins-Thompson & Peris, 1993; Bonci & Williams, 1996; Peris, 1996; Shoji et al., 1997; Kushner & Unterwald,, 1999), and potentially, the behavioral response to GABA drugs. It is therefore possible that the cocaine self-administration group was more sensitive than the food-reinforced (cocaine-naïve) group to the sedative, disruptive, or other non-specific effects of baclofen. Thus, the observed differential effects may have been due to the disparate drug histories, rather than a selective effect on cocaine. In this investigation the use of a concurrent access schedule of reinforcement allowed us to examine cocaine and food-reinforced responding in the same animal, thus controlling for drug history. The results confirm and extend previous observations that baclofen selectively attenuates the reinforcing effects of cocaine in rats (Roberts et al., 1996; Roberts & Andrews, 1997; Shoaib et al., 1998).

Series 2: Localization of baclofen's anti-cocaine effects

The demonstration that systemic baclofen reduced the break-point for responding for cocaine suggests that $GABA_B$ agonists have the potential to modulate reward processes. However, these data do not address the anatomical site of action, and the neural substrates that underlie baclofen's impact on cocaine self-administration are, as yet, unknown.

Our current working hypothesis is that baclofen's impact on cocaine self-administration is mediated through $GABA_B$ receptors on dopaminergic neurons within the VTA. GABA has extensive interactions with the mesolimbic DA system. The majority of

neurons in the NAC are GABAergic neurons that project to DA cells within the VTA and regulate their activity (Kita & Kitai, 1988). The VTA contains two major cell types: the primary dopaminergic neurons which release DA in the NAC and PFC, and the secondary GABAergic neurons which function as inhibitory interneurons to control the firing of primary DA neurons (Kalivas, 1993; Van Bockstaele et al., 1995; White, 1996; Steffenson et al., 1998). The DA cells of the VTA project principally to the NAC, thus forming a feedback loop that is presumably sensitive to cocaine-induced alterations in DA signals. Microinjection of baclofen into the VTA decreases extracellular DA in the NAC and the PFC (Kalivas et al., 1990; Klitenick et al., 1992; Yoshida et al., 1994; Westerink et al., 1997; Westerink et al., 1998). Manipulation of GABAergic transmission in the VTA also attenuates the rewarding properties of intracranial self-stimulation (Willick & Kokkinidis, 1995), morphine-induced place preference (Tsuji et al., 1996), and heroin- (Xi & Stein, 1999) and cocaine-reinforced responding (Shoaib et al., 1998).

At present, only one other report has examined the effect of intracerebral (IC) baclofen on cocaine self-administration. Shoaib et al. (1998) demonstrated that under an FR5 schedule of reinforcement, microinjection of 200 ng of baclofen into the NAC, or 300 ng into the VTA produced a significant decrease in the rate of cocaine intake. They concluded that $GABA_B$ receptors in both the NAC and in the VTA modulate the reinforcing properties of cocaine.

As demonstrated in the previous Series of experiments there is evidence that doses of baclofen that have little or no effect on cocaine intake under FR schedules markedly decrease break points on the PR schedule. The present investigation used a PR schedule of

reinforcement in order to clarify whether $GABA_B$ receptors in the VTA or NAC are more important in mediating the effect of baclofen on cocaine self-administration. Separate groups of rats received bilateral microinjections of various doses baclofen into the VTA, NAC or striatum (STR). As in Series 1, rats were given concurrent access to a food-reinforced lever during cocaine self-administration sessions in order to demonstrate that baclofen did not interfere with the capacity to respond during the period when cocaine-reinforced responding was suppressed.

Method

Subjects and Procedure

The care and training of the animals and the data analysis was identical to that described in Series 1, except as noted below.

Intracerebral cannulation

After animals showed stable rates of responding on the concurrent access schedule of food- (FR5) and cocaine (PR)-reinforcement for at least four days they were stereotaxically implanted with a bilateral cannula for IC injections under halothane anaesthesia. Rats were randomly assigned to one of three groups for surgery: VTA (n = 5), NAC (n = 6) and STR (n = 6). Cannulae were implanted into the VTA (A = -4.8, L = ±1.0, V = -7.2), the NAC (A = +1.0, L = 1.0, V = -5.9) or the STR (A = +0.7, L = ±2.5. V = -4.0) with the incisor bar set 5 mm above the interaural line using co-ordinates from Paxinos & Watson (1982). The tip of the injector was made to extend beyond the cannula by 1 mm. Delivery of baclofen was from a Hamilton syringe mounted in an infusion pump, connected

to the injector by Silastic tubing.

Various doses of baclofen were administered IC 30 minutes before the session, according to a latinized design. The doses were 32, 56 or 100 ng/0.5 µl/side into the VTA, and 100, 180 or 320 ng/0.5 µl/side into the NAC and STR. (Note: A vehicle injection was included in the VTA group, to control for the injection procedure. The lowest dose of baclofen administered to animals in the NAC or STR groups failed to produce a significant decrease from baseline responding, and therefore served as a control). At least 3 days of baseline separated baclofen test days. Following completion of testing animals were deeply anesthetized with sodium pentobarbital and transcardially perfused with saline and formalin. Brains were removed, sliced, and stained with cresyl violet in order to confirm cannula placement.

Results

The histological localizations of the bilateral injection cannulae in the different brain regions are shown in Figure 7. A total of 5 animals were included in the VTA group, and the NAC and STR groups contained 6 animals each. In all regions studied, intracerebral injections of baclofen produced dose-dependent decreases in cocaine-reinforced break points.

Figure 8 (Left) shows the effect of several doses of baclofen on responding under a PR schedule of reinforcement. Although baclofen significantly decreased cocaine-reinforced break points in all three groups of animals [VTA: $F = 10.49$, $df = 4,16$, $p<0.01$; NAC: $F = 36.16$, $df = 3,15$, $p<0.01$ and STR: $F = 4.05$, $df = 3,15$, $p<0.05$] the doses of baclofen

Fig 7: Histological diagrams showing cannula placement in the three different brain regions. Each point reflects the location of the baclofen infusion. Sections are modified from Paxinos and Watson (1982).

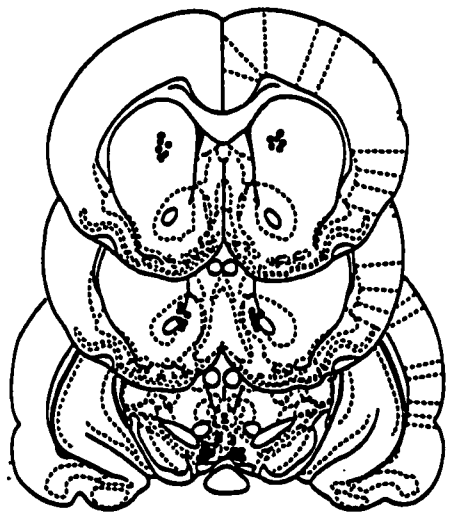
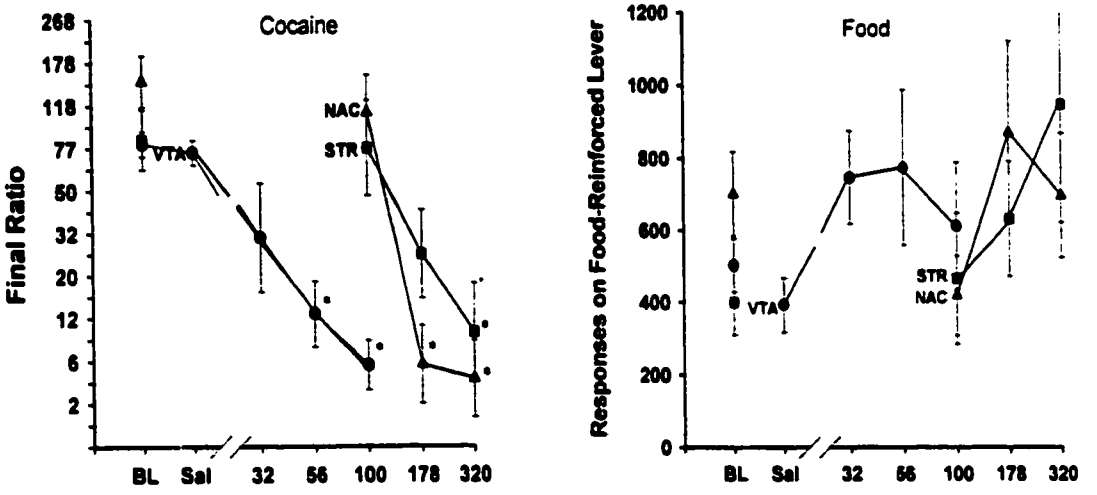


Figure 8: (**Left**) Effect of intracerebral baclofen on cocaine self-administration reinforced under a PR schedule. Points represent the mean (± S.E.M.) break points on a PR schedule of cocaine reinforcement following microinjection of various doses of baclofen (-30 min) into the VTA (Closed circles, n=5), NAC (Closed triangle, n=6) or STR (Closed square, n=6). Animals in the VTA group also received microinjections of saline. In all three groups, baclofen pretreatment caused a dose-dependent decrease in cocaine-reinforced breakpoints compared to baseline (*$p<0.05$). Although significant reductions in cocaine intake were observed after intra-NAC or intra-STR baclofen, the doses required to produce this effect were three fold higher than those that produced similar reductions in the VTA. (**Right**) Effect of intra-VTA, NAC or STR baclofen on operant responding under an FR5 schedule of food reinforcement. Points represent the mean (± S.E.M.) number of responses on a concurrently available food-reinforced lever during the cocaine self-administration session. Baclofen pretreatment did not affect responding on the food-reinforced lever in any of the groups of animals.

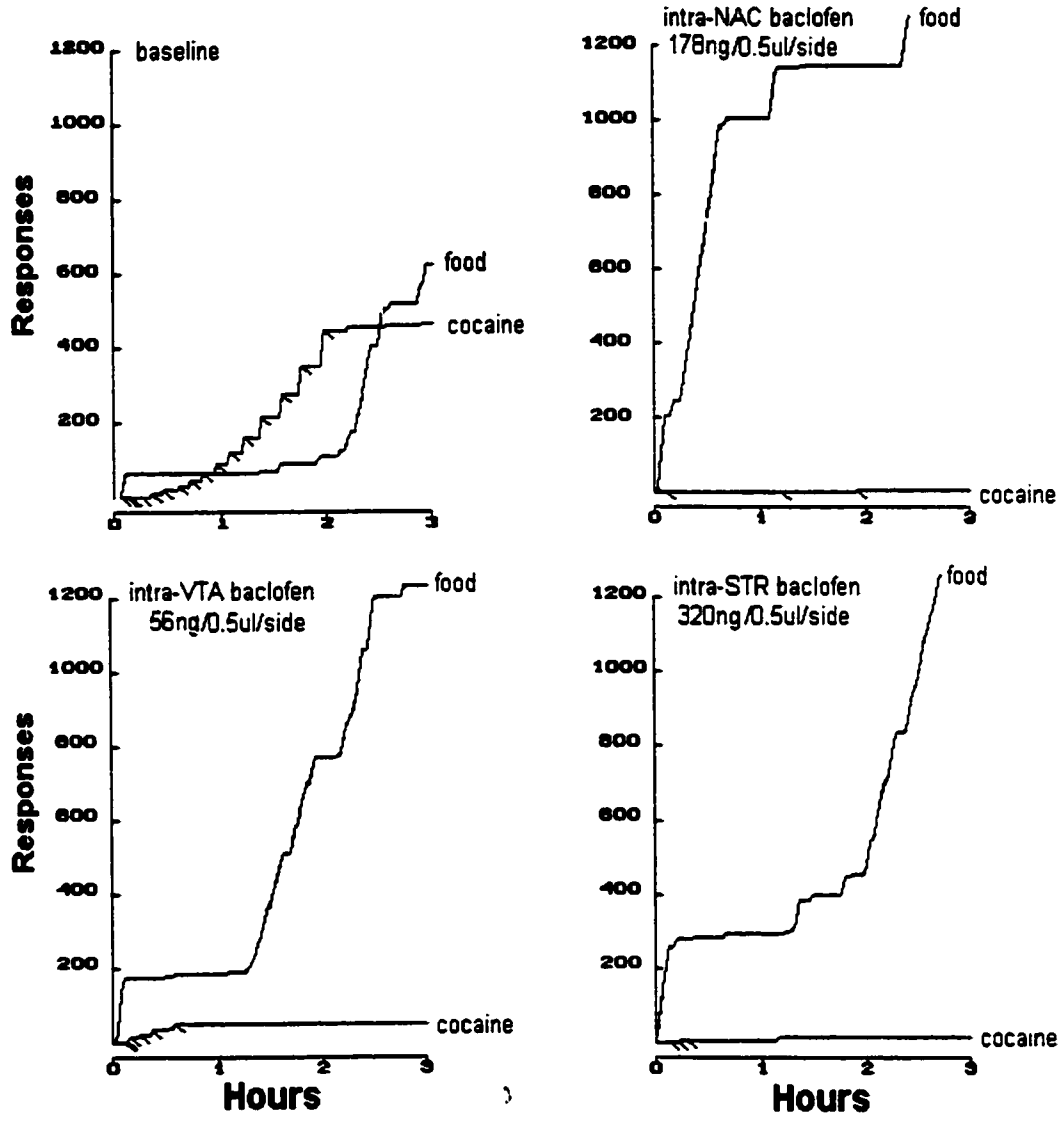

Cocaine
Final Ratio
268
178
118
77
50
32
20
12
6
2
VTA
NAC
STR
BL
Sal
32
56
100
178
320
Food
Responses on Food-Reinforced Lever
1200
1000
800
600
400
200
0
VTA
STR
NAC
BL
Sal
32
56
100
178
320
Baclofen (ng/0.5 µl/side) IC

required to produce a significant effect were substantially lower when injections were made into the VTA. Multiple comparisons demonstrated that the 56 and 100 ng doses of intra-VTA baclofen produced a significant decrease in cocaine self-administration ($p<0.05$). In the NAC group, larger doses of baclofen (180 and 320 ng) were required to produce a similar impact on break-points. In the STR group, a significant decrease in cocaine self-administration was only evident after the highest dose of baclofen (see Fig 8-Left).

The effect of IC baclofen pretreatment on responding for food reinforcement on an FR5 schedule is shown in Figure 8 (Right). Separate repeated measures ANOVAs indicated that during the first two hours of the test session when baclofen reduced cocaine self-administration, there was no significant effect on responding for food. Animals in all three groups completed several hundred responses on the food-reinforced lever, after even the largest doses of baclofen, indicating that they retained their capacity to complete the operant response.

Figure 9 shows the effect of baclofen on the patterns of responding for concurrently available food- or cocaine-reinforcement in a representative animal from each group of rats. The doses of baclofen shown represent the lowest dose at which a significant decrease in cocaine-reinforced responding was observed in the group of rats. At the beginning of the daily sessions, the food-reinforced lever was introduced into the cage 5 min before the cocaine lever. Baseline responding (top left) was characterized by vigorous responding on the food-reinforced lever until the cocaine lever was introduced, at which point rats switched to responding almost exclusively on the cocaine-reinforced lever. Responding on the food lever was reinstated as the animal approached the break point for cocaine. Each session

Figure 9: Representative records from each of the 4 groups illustrate the effect of baclofen on responding for concurrently available cocaine under a PR schedule, and food under an FR5 schedule of reinforcement. Short downward lines represent cocaine infusions while upper increments in the record represent lever responses. Baseline conditions are represented in the upper left, intra-VTA injections are shown lower left. Upper right illustrates the pattern of responding after baclofen was injected into the NAC and injections into the STR are shown in the lower right panel.


baseline
food
cocaine
Responses
1200
1000
800
600
400
200
0
1
2
3
intra-NAC baclofen
178ng/0.5ul/side
food
cocaine
intra-VTA baclofen
56ng/0.5ul/side
food
cocaine
Responses
Hours
intra-STR baclofen
320ng/0.5ul/side
food
cocaine
Hours

continued until the animal failed to obtain an infusion for a one hour period. Following baclofen pretreatment responding for food was initiated much earlier and was sustained throughout the first two hours of the test period. Cocaine responding, however, was markedly reduced and abruptly stopped early in the session. Baclofen pretreatment produced the same patterns of responding regardless of the site of the microinjection. Significant reductions in cocaine self-administration were observed at the 56 ng dose in the VTA (lower left), after 180 ng in the NAC (upper right), and after 320 ng in the STR (lower right).

Discussion

The present results demonstrate that IC injections of the $GABA_B$ agonist baclofen into the VTA, NAC or STR reduce cocaine self-administration under a PR schedule of reinforcement. Microinjections of baclofen into the VTA produced the most potent modulation of cocaine self-administration, decreasing cocaine-reinforced break-points at doses that were three times lower than those required to produce comparable reductions from injections into the NAC or STR. Several other studies have demonstrated an apparently specific effect of systemic pretreatments of $GABA_B$ agonists on cocaine self-administration (Roberts et al., 1996; Roberts & Andrews, 1997; Shoaib et al., 1998; Campbell et al., 1999). The results of the present investigation are consistent with previous reports showing that baclofen selectively attenuates the reinforcing effects of cocaine in rats, and suggests that the site of action for this effect includes $GABA_B$ receptors within the region of the VTA.

Shoaib et al. (1998) were the first to investigate the effects of IC injections of baclofen on cocaine self-administration. They reported that microinjections of 200 and 300

ng baclofen into the NAC or VTA (respectively) were required to produce decreases in cocaine self-administration on an FR5 schedule. In the present study we showed that doses as low as 56 ng into the VTA were statistically effective in reducing break points on a PR schedule. The different schedules used probably contributed to the differential sensitivity within regions, with the large number of responses required by the PR schedule (or high unit price) accounting for the increased sensitivity to the low doses of baclofen in the present investigation.

As was the case in the experiments in Series 1, data from the concurrent schedule showed that animals pretreated with baclofen were capable of responding several hundred times on the food-reinforced lever at a time when cocaine-reinforced responding was suppressed. These data would seem to rule out a generalized effect on responding and demonstrate that IC baclofen produced a redirection of responding from the cocaine- to the food-reinforced lever. This schedule offers an additional advantage in experiments that require repeated IC injections, in that it allows for the demonstration of the capacity to perform in each animal following every injection. Other alternatives are to assess potentially disruptive effects in a different situation and/or in a separate group of rats; however, since slight variations in cannula placement can have a significant impact on drug effects and the number of intracerebral injections through each guide cannula is limited, these options have their own drawbacks.

Under concurrent access conditions intracerebral injections of baclofen produced a redirection of responding from the cocaine-reinforced lever towards the food-reinforced lever. Stratford & Kelly (1997) have reported increased feeding in sated animals following

intra-NAC baclofen, raising the possibility that the reduction in cocaine self-administration in the present investigation was an indirect result of an increased motivation to respond for food. However, this seems unlikely, since pilot data collected for these studies showed that baclofen reduced cocaine self-administration in the absence of a concurrently available food-reinforced lever. Furthermore, according to Stratford & Kelly (1997) the effect of baclofen on feeding was restricted to the shell of the nucleus accumbens, and the redirection of responding observed in the present investigation was evident in all three groups of rats.

The results of the present investigation demonstrate that microinjection of low doses of baclofen into the VTA attenuates cocaine self-administration. GABA terminals are distributed throughout the brain, and it has been suggested that increased GABA levels may play a major role in attenuating centrally mediated reward mechanisms through interactions with the VTA/NAC DA system (Morgan & Dewey, 1998; Kushner et al., 1999; McBride et al., 1999). Microdialysis studies have confirmed that intra-VTA baclofen decreases extracellular DA in the NAC, and the medial PFC (Yoshida et al., 1994; Enrico et al., 1998; Westerink et al., 1997; Westerink et al., 1998).Thus $GABA_B$ receptors within the VTA may be critical to modulating the rewarding properties of cocaine.

Series 3: To characterize the effect of the $GABA_B$ agonist CGP 44532 on cocaine self-administration

Animal models of cocaine self-administration have provided considerable evidence to indicate that the mesocortical-mesolimbic DA system is critically involved in cocaine reinforcement (Roberts, 1992; Koob, 1992; Woolverton & Johnson, 1992), and it has been

suggested that a conditioned or rebound reaction within this system may be involved in relapse to drug use in humans (Koob, 1996). However, clinical trials with DA agonists or antagonists have not resulted in the development of an effective medication that promotes abstinence from cocaine use. Despite some promising reports from open trials, double blind studies have generally failed to confirm significant therapeutic effects (Kleber, 1995).

The experiments conducted in Series 1 and 2 of this thesis show that baclofen attenuates cocaine self-administration in rats. However, the pharmacological profile of baclofen may not be optimally suited to the treatment of cocaine addiction. Baclofen has a variety of behavioral effects in animals including striatal muscle relaxation, sedation and analgesia (Paredes & Agmo, 1989; Jelen et al., 1994). The muscle relaxant effects have been used to advantage in the treatment of spasticity in multiple sclerosis and spinal cord injured patients, although sedation is regarded as a limiting side effect. Since baclofen has, until recently, been the only specific $GABA_B$ agonist available, it has been difficult to assess the generality and specificity of these behavioral effects within this very limited drug class. Froestl et al. (1993; 1995) have now characterized a number of phosphinic acid analogues of GABA that are more selective and potent than baclofen at $GABA_B$ receptors. With this new series of compounds it may be possible to dissociate the various behavioral effects. For example, one of these compounds, CGP 44532, appears to have a larger therapeutic window than baclofen, showing stronger muscle relaxant properties without the occurrence of sedation or reduced vigilance in rhesus monkeys (Froestl et al., 1995). In addition, Enna et al. (1998) have recently shown that CGP 44532 has substantially more potent analgesic properties than baclofen in a rodent model of chronic pain, and is less likely to produce

tolerance to this effect. CGP 44532 binds to $GABA_B$ receptors with high affinity, yet is virtually inactive at many other binding sites including $GABA_A$, benzodiazepine and twelve other neurotransmitter receptor sites found in the central nervous system (Froestl et al, 1995).

The previous series of experiments used a concurrent access schedule to demonstrate that the anti-cocaine effect of baclofen appears to be separate from the muscle-relaxant effects of the drug. In the present report, we examined the effect of the novel $GABA_B$ receptor agonist, CGP 44532, on cocaine self-administration in rats in order to assess whether a drug with less sedative effects might also attenuate the reinforcing effects of cocaine.

Methods

Subjects and Procedure

Subjects and procedures were identical to those previously described, except as noted below.

Cocaine-reinforced responding on a PR schedule

Animals that showed stable performance on the PR schedule were used to test the effect of pretreatment with CGP 44532 (N = 6). CGP 44532 (0.063, 0.125, 0.25 or 0.5 mg/kg, IP) was administered 30 min prior to the self-administration session. The testing order of the doses of the $GABA_B$ agonist was counterbalanced according to a latinized design. At least three days of baseline cocaine self-administration separated test days.

The effect of CGP 44532 pretreatment on cocaine self-administration across four

doses of cocaine was examined in a separate group of rats (N =9). Four unit injection doses of cocaine (0.18, 0.38, 0.75, 1.5 mg/kg per injection) were examined with the order of testing being counterbalanced according to a latinized design. After a stable baseline was established on the PR schedule, the effect of pretreatment with CGP 44532 (0.125 mg/kg) was examined.

Food-reinforced responding on a PR schedule

Subjects (N = 6-8) were food restricted and trained to respond on a food-reinforced PR schedule (45 mg Noyes pellets). Animals were given access to food (Purina Rat Chow) for one hour following each training session. Rats on this deprivation schedule remain healthy and gain weight at approximately 1 gm/day, yet are highly motivated to respond for food. The testing apparatus and the PR schedule was identical to that used in self-administration experiments described above. Delivery of the first food pellet was contingent on a single lever response, thereafter the ratio of responses required to obtain the food pellet was incremented through the identical progression described for cocaine self-administration on a PR schedule. The session duration was 3 hours. Break point was defined by the number of completed increments in the schedule. CGP 44532 (0.063, 0.125, 0.25 or 0.5 mg/kg, IP) was administered 30 min prior to the food reinforced session. The testing order of the doses was counterbalanced according to a latinized design. At least three days of baseline training separated test days.

Discrete Trials Procedure

Additional groups of naive rats were tested using a discrete trials procedure. Rats were used that had displayed stable daily intake of cocaine (>30 injections/5 hrs) for at least

5 days on an FR1 schedule and had shown consistent post-infusion pauses. Parameters were chosen that produced a circadian pattern of responding (see Fitch & Roberts, 1993). Discrete trials were 10 min in duration and were initiated at 30 min intervals for the duration of the experiment. Each trial began with the introduction of a retractable lever into the cage. Completion of the FR requirement resulted in a cocaine injection (1.5 mg/kg per injection), illumination of a stimulus light for 20 sec, retraction of the response lever and termination of the trial. Failure to complete the FR within 10 min also terminated the trial.

Two discrete trials studies were conducted. In the first study, animals (N = 7) had *ad libitum* access to Purina® Food Chow and delivery of cocaine was controlled on an FR5 schedule of reinforcement. The FR value was set at 1 for the first few days of testing and was gradually increased to an FR5 by the tenth day of testing. The second study was a complete replication of the first in a separate group of animals (N = 6), except that cocaine was delivered on an FR1 schedule of reinforcement and the animals were given continuous access to a second response lever that controlled the delivery of 45 mg nutritionally balanced Noyes food pellets. These pellets were the only source of food for this group. Syringes were refilled, water bottles changed and waste pans cleaned during one of the inter-trial intervals (ITI) between 9 am and 10 am. After at least 14 days on the discrete trials schedule, the effect of pretreatment with various doses of the CGP 44532 was assessed. Rats received an injection of CGP 44532 (vehicle, 0.063, 0.125, 0.25 or 0.5 mg/kg, IP) 5-10 min prior to the 9 am trial. The order of testing was counterbalanced and at least three baseline days separated test days. The number of injections self-administered during the four hour period following treatment was used as the dependent measure. In the second study, the number of

responses on the food-reinforced lever responses during the same four hour period following treatment was recorded and analyzed separately.

Data Analysis

Separate one-way ANOVAs with repeated measures were used to examine the effect of Dose of CGP 44532 on cocaine reinforced responding on a PR schedule and on food reinforced responding. In the discrete trials experiments, the number of injections self-administered, or the number of food pellets delivered in the four hour time period following the injection of CGP 44532 was used as the dependent measure. The effect of Dose was examined in separate repeated measures ANOVAs. Analysis was restricted to animals that completed testing across all drug doses.

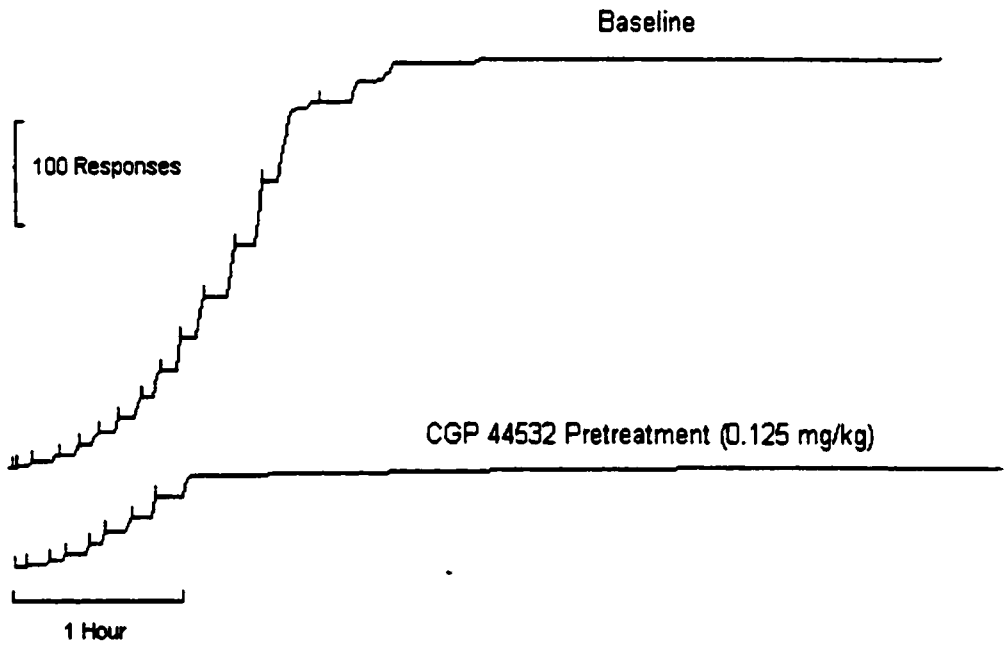
Drugs

CGP 44532 was synthesized and provided by Novartis Pharma AG. The compound was dissolved in sterile saline.

Results

Figure 10 (top) illustrates the pattern of responding on a PR schedule. Early in the session, cocaine injections were self-administered at regular intervals. Post-reinforcement pauses were followed by relatively consistent periods of lever responding which resulted in the delivery of an injection. Since the response requirements escalated following each injection, eventually a point was reached when responding ceased. Figure 10 (bottom) illustrates the effect of CGP 44532 (0.125 mg/kg) on responding on a PR schedule reinforced by cocaine. Responding ceased much earlier in the session, with the result that the animal

Figure 10: The effect of CGP 44532 on cocaine responding reinforced on a PR schedule. **Top:** The line represents a cumulative record from a baseline self-administration session. Responses are indicated by vertical increments. Injections of cocaine (0.75 mg/kg per injection) are represented by short tick marks. In this case, the animal responded for a total of fourteen injections which represents a final ratio of 77. **Bottom:** The line illustrates the response pattern for the same animal following pretreatment with CGP 44532 (0.125 mg/kg). See Results for description of pattern.


Baseline
100 Responses
CGP 44532 Pretreatment (0.125 mg/kg)
1 Hour

received 8 injections, with a final ratio of 20. While no appreciable change is evident during the early part of the session, responding ceased at a much earlier time point.

Figure 11 shows the group data for the effect of CGP 44532 on break points for responding for 0.75 mg/kg per injection of cocaine under a PR schedule. Analysis of the group data indicated a significant effect of Dose for CGP 44532 [$F(3,21) = 7.28$; $p < 0.01$]. The compound produced a dose-dependent decrease in breaking point. Also shown in Figure 11 is the effect of the $GABA_B$ agonist on responding on a PR schedule for food reinforcement. A significant effect of dose was observed for CGP 44532 [$F(3,21) = 5.43$; $p < 0.01$], although the reduction in breaking points was less pronounced for food reinforcement than for cocaine reinforcement. Cocaine and food supported similar baseline break points on the PR schedule (13.8 vs 14.6).

Figure 12 illustrates the dose-response curve for cocaine and the effect of pretreatment with CGP 44532. Higher unit doses of cocaine supported higher break points. Pretreatment with saline had no significant effect ($F < 1$), whereas pretreatment with the $GABA_B$ agonist was found to produce a significant main effect [$F(1,8) = 43.02$; $p < 0.001$]. No significant interaction was found between cocaine dose and the $GABA_B$ agonist ($F < 1$).

The discrete trials procedure engendered a pattern of cocaine self-administration consistent with previous reports (Roberts & Andrews, 1997; Fitch & Roberts, 1993). There were no statistically significant differences in the baseline levels and patterns of responding between the two replications. The data were therefore combined, resulting in a combined $N = 13$. Cocaine intake was observed to fluctuate across the light/dark cycle. The probability of animals self-administering cocaine during a trial in the light phase of the cycle was very

Figure 11: The effect of various doses of CGP 44532 on responding on a PR schedule reinforced by either cocaine (0.75 mg/kg per injection) or food (45 mg pellets). Points represent the mean (± S.E.M.) for separate groups of animals (N = 6-8). Data are presented as percent of baseline responding. Cocaine and food supported similar baseline break points on the PR schedule (13.8 vs 14.6).

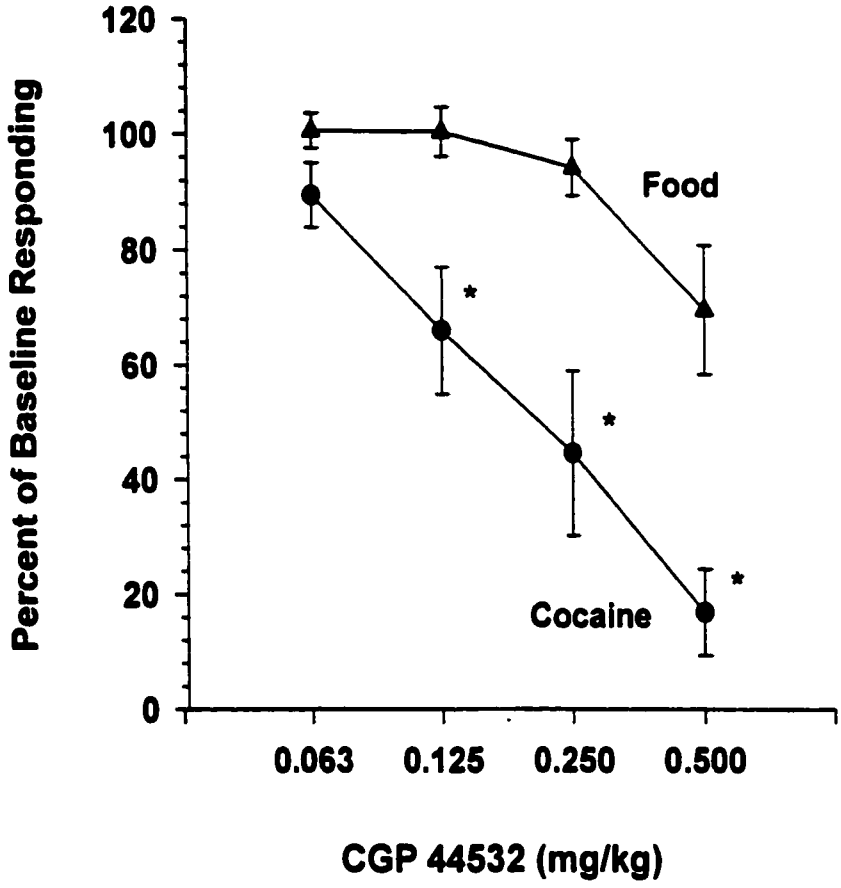
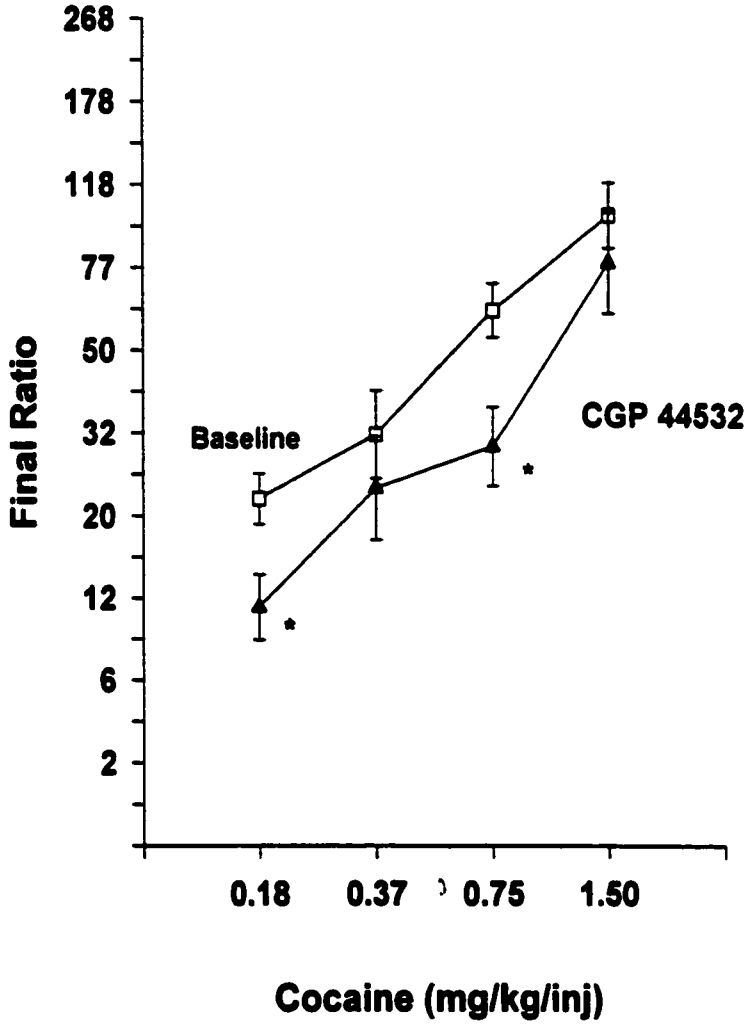

120
100
80
60
40
20
0
Percent of Baseline Responding
Food
Cocaine
*
*
*
0.063
0.125
0.250
0.500
CGP 44532 (mg/kg)

Figure 12: The effect of CGP 44532 on cocaine self-administration reinforced on a PR schedule. Points represent the average (± S.E.M.) break point for groups of rats pretreated with CGP 44532 (0.125 mg/kg). Rats were tested on various unit doses of cocaine in a Latinized design. Each point represents the mean (±S.E.M.) of at least 9 subjects.


268
178
118
77
50
32
20
12
6
2
Final Ratio
Baseline
CGP 44532
*
*
0.18
0.37
0.75
1.50
Cocaine (mg/kg/inj)

low, increased through the dark phase and reached a maximum immediately prior to the light change (See Figure 13). CGP 44532 produced a dose-dependently and statistically significant reduction in the number of cocaine injections self-administered during the four hour period immediately following treatment. Figure 14 (left) illustrates the effect of treatment with CGP 44532 on the total number of cocaine injections self-administered during the four hour post-treatment interval. ANOVA revealed a significant Dose effect [$F(4,20) = 28.7, p < 0.001$]on responding for cocaine. Newman Keuls multiple comparisons revealed the two highest doses of CGP 44532 significantly suppressed responding, with the highest dose of the compound causing a near total cessation of self-administration in most animals. The duration of the effect was dose-dependent. Figure 14 (right) shows the effect of CGP 44532 on the food-reinforced responding during the four hour post-treatment interval in the subset of animals that had concurrent access to a food lever. No significant alterations in responding for food were observed [$F(4,20) = 1.5$].

Discussion

The present results show that the $GABA_B$ agonist CGP 44532 produced a significant effect on cocaine self-administration reinforced in both the PR schedule and in the discrete trials procedure. The effect was found to be dose-dependent and low doses of the drug produced an apparent shift to the right of the cocaine dose-response curve. The data suggest that CGP 44532 attenuated the reinforcing effects of cocaine.

The effect of CGP 44532 was tested in two ways. First a cocaine dose from the middle of the ascending limb was used to examine the effect of various doses of the $GABA_B$

Figure 13: The effect of 0.5 mg/kg CGP 44532 on cocaine self-administration reinforced on a discrete trials procedure. Animals (N =13) were given the opportunity to self-administer a single injection of cocaine (1.5 mg/kg per injection) on an FR 5 (n=7) or an FR 1 (n=6) schedule during 10 min trials. Trials were initiated continually every 30 min. The proportion of injections self-administered by each animal during each trial was calculated across a three day baseline period. The mean (±S.E.M.) proportion was calculated for the group and shown on the graph as open squares. The observed number of injections self-administered on the CGP 44532 test day is shown as closed triangles. The time of the CGP 44532 injection (0.5 mg/kg) is shown by the arrow. CGP 44532 produced a marked reduction in cocaine self-administration for at least 4 hrs post-injection.

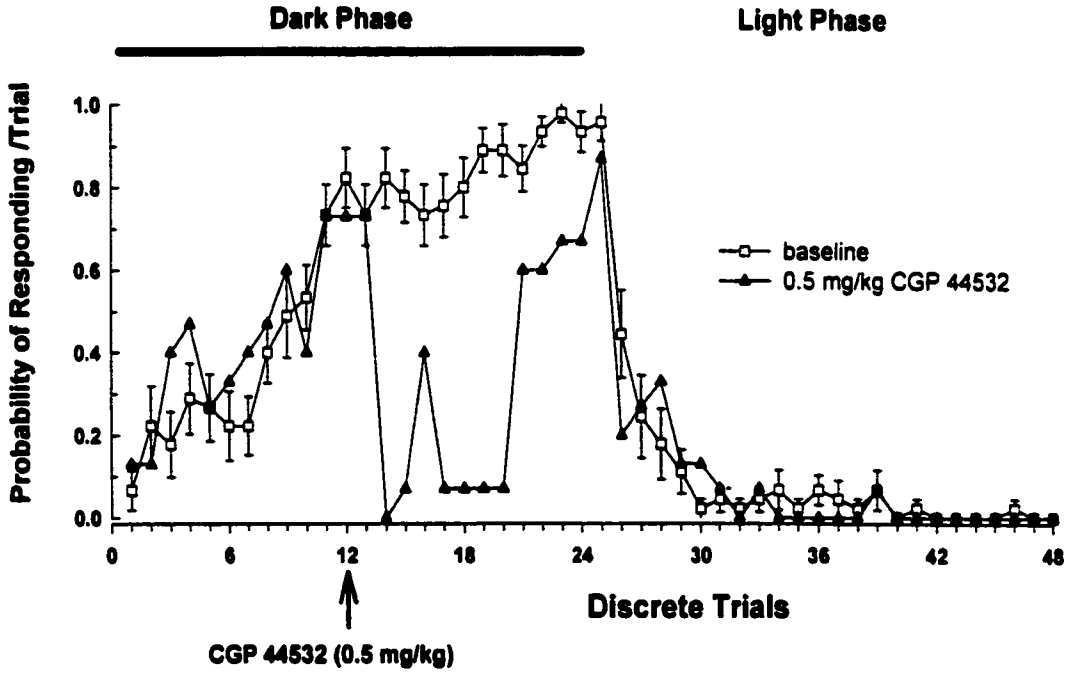
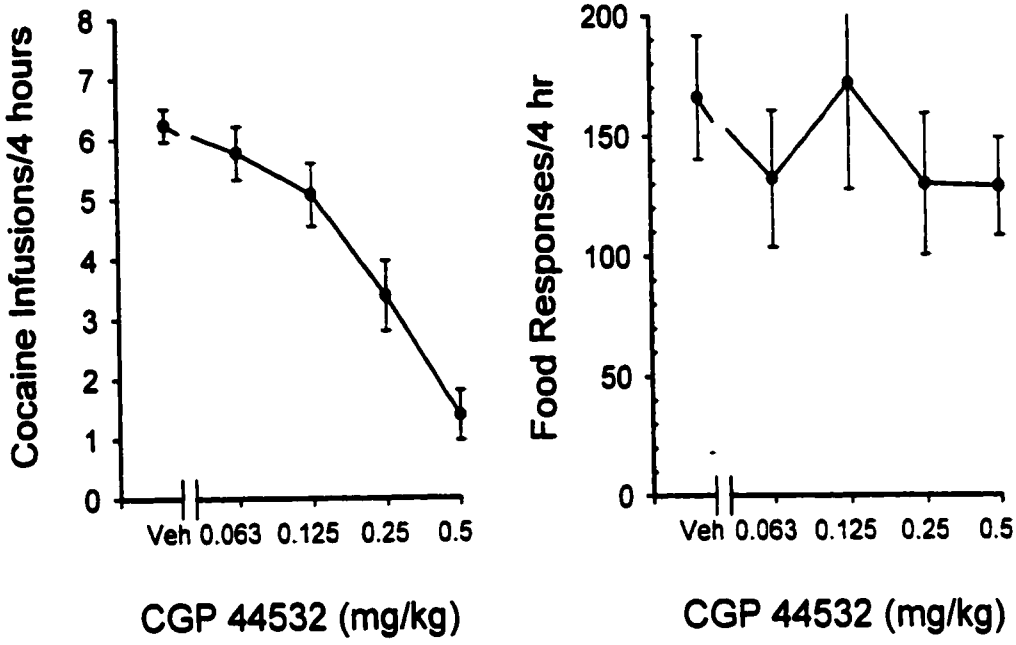

Dark Phase
Light Phase
Probability of Responding /Trial
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
30
36
42
48
baseline
0.5 mg/kg CGP 44532
Discrete Trials
CGP 44532 (0.5 mg/kg)

Figure 14: The effect of various doses of CGP 44532 on the probability of responding for cocaine (**left**) on the discrete trials procedure. Animals (N=13) were given the opportunity to self-administer a single injection of cocaine (1.5 mg/kg per injection) on an FR 5 (n=7) or an FR 1 (n=6) schedule during 10 min trials. Trials were initiated continually every 30 min. The total number of infusions of cocaine that were self-administered during the first four hours following CGP 44532 (0.063, 0.125, 0.25 and 0.5 mg/kg IP) administration was calculated for the group and compared to total number of infusions self-administered following treatment with saline. CGP 44532 decreased responding at all doses tested, with the two highest doses producing a significant reduction in cocaine self-administration. In contrast, CGP 44532 had no significant effect on the total number of lever presses on a second, continuously available, food reinforced lever (**right**) during the same four hour period (n=6).


Cocaine Infusions/4 hours
8
7
6
5
4
3
2
1
0
Veh 0.063 0.125 0.25 0.5
CGP 44532 (mg/kg)
Food Responses/4 hr
200
150
100
50
0
Veh 0.063 0.125 0.25 0.5
CGP 44532 (mg/kg)

agonist. Second, after an effective range had been established, a moderately low dose of CGP 44532 was selected to examine the effect on the cocaine dose-response curve.

CGP 44532 dose-dependently affected responding on a PR schedule reinforced by cocaine. Pretreatment with the $GABA_B$ agonist produced substantial decreases in breaking points at doses that had little or no effect on food reinforced responding. These data are consistent with the results from the experiments in Series 1 and 2 that demonstrated that baclofen, the prototypical $GABA_B$ agonist, decreased cocaine self-administration on a PR schedule at doses that had no effect on food reinforced responding.

Cocaine self-administration under an FR or PR schedule typically begins with a non-contingent infusion at the start of the session. It has been argued that this "priming" injection re-instates drug-seeking behaviour in an animal that may otherwise have remained in an abstinent state. Since rodents have been shown to titrate their cocaine intake so that the effects of one injection carry over from one administration to the next, such procedures may not be able to distinguish between factors that trigger relapse to drug use, and those that maintain it. The discrete trials procedure offers a different perspective on the motivation to seek cocaine. Manipulations of the inter-trial-interval (ITI) between discrete trials can engender very different patterns of cocaine self-administration behavior. When relatively short ITIs are used (15 min) a binge pattern emerges. Rats will self-administer cocaine on consecutive trials for many hours or even days (Fitch & Roberts, 1993). A very different pattern is observed when the ITI is lengthened. When trials are limited to two per hour, a regular circadian pattern of intake emerges. Rats maintain a regular daily pattern of cocaine self-administration yet also show normal eating and sleeping patterns. Figure 4 illustrates

that the probability of self-administering is very high (~85%) during the latter 6-8 hours of the dark cycle and very low during the light phase. We interpret these data to indicate that unlimited access to cocaine overwhelms normal behavior; however, if a 30 min ITI is imposed, then behavior becomes more susceptible to a number of physiological processes, including circadian rhythms. The fact that rats initiate drug taking at predictable times without 'priming' can be used to advantage to test the effects of potential therapeutic agents.

CGP 44532 dose-dependently inhibited cocaine self-administration on the discrete trials procedure. The length of the suppressive effect increased with the dose of CGP 44532, with the highest dose causing an almost complete cessation of drug seeking behaviour for four hours. It should be noted that cocaine was available on a low fixed-ratio schedule (FR5 or FR1) and therefore little effort was required in order to receive an injection. It is unlikely therefore that the results can be explained by a motor impairment. In one group, rats responded an average of 150 times for food pellets during the four hours after CGP 44532, yet declined to self-administer cocaine when it could be obtained with a single lever response. These data are consistent with our previous report that baclofen produced a dose dependent inhibition of cocaine intake on a discrete trials procedure (Roberts & Andrews, 1997), yet had no effect on food intake during the time when cocaine self-administration was suppressed.

The present data suggest that CGP 44532 can specifically attenuate the motivation to self-administer cocaine, and the results cannot be entirely accounted for by sedation. Continued screening of compounds in this series may resolve whether the muscle relaxant and anti-cocaine effects can be dissociated.

Series 4: To determine whether baclofen's anti-cocaine can be blocked by the $GABA_B$ antagonist CGP 56433A

The results of Series 1-3 suggest that GABAergic compounds can modulate cocaine reinforcement, an effect that is likely mediated through $GABA_B$ receptors located in the VTA. The $GABA_B$ agonist baclofen was shown to attenuate cocaine self-administration behaviour under a number of different schedules of reinforcement. These results add to several other studies that have demonstrated that $GABA_B$ receptor modulation has an impact on the reinforcing properties of cocaine (Roberts et al., 1996; Roberts & Andrews, 1997; Shoaib et al., 1998; Campbell et al., 1999).

Baclofen was developed in 1962, and until recently was the only agonist available for the study of $GABA_B$ effects. While it appears that baclofen has relatively high specificity to $GABA_B$ receptors (with respect to other known neurotransmitter receptors) the possibility remains that some of the effects of baclofen may occur via some means other than $GABA_B$ receptor stimulation (Fung et al., 1985). The experiments in Series 3 using the more specific agonist CGP 44532 do provide some evidence of the fact that $GABA_B$ receptor activation is responsible for attenuating cocaine self-administration. Another way to confirm that $GABA_B$ receptor activation is responsible for baclofen's anti-cocaine effect is to examine whether it can be blocked by a $GABA_B$ antagonist. The following Series of studies were conducted to determine whether the specific $GABA_B$ antagonist CGP 56433A ([3-1-(S)-[3-(cyclohexylmethyl)hydroxyphosphinyl]-2-(S)-hydroxypropyl]-amino]ethyl]benzoic acid) attenuated baclofen's effect on cocaine self-administration under FR1 and PR schedules of reinforcement.

Methods

Subjects

Male Sprague Dawley rats (Harlan, Indiana) weighing between 275 and 300 grams at the start of the experiment were housed under conditions identical to those described above.

Procedure

Surgical procedures and training of rats were as previously described.

CGP 56433A Dose-Response Curve under FR and PR schedules

The effect of the $GABA_B$ antagonist on cocaine-reinforced responding was assessed in two separate groups of rats, one of which was responding under an FR1 schedule (n = 4) and the other which was responding under a PR schedule of reinforcement (n = 5). The unit injection dose of cocaine for both groups of rats was 1.5 mg/kg per injection. Once stable rates of responding were established, all rats were pretreated with CGP 56433A (0.6, 1.0 or 1.8 mg/kg, IP) 30 min prior to the test sessions. The order of CGP 56433A injections was counterbalanced according to a Latin square design and test sessions were separated by at least three days.

Effect of Baclofen and CGP 56433A on cocaine self-administration

FR1 schedule:

In order to determine whether CGP 56433A blocked baclofen-induced decreases in cocaine self-administration, a group of rats (n = 8) was trained to respond for 1.5 mg/kg per injection of cocaine under an FR1 schedule of reinforcement as previously described. Once stable rates of responding were established under the FR1 schedule, rats were pretreated with

baclofen (5.6 mg/kg, IP) and CGP 56433A (vehicle, 0.6, 1.0 or 1.8 mg/kg, IP) administered in two successive injections 30 min prior to the start of the daily session. CGP 56433A injections were counterbalanced according to a Latin square design and test days were separated by at least 3 days of stable responding.

PR schedule:

Two additional groups of rats were trained to respond under a PR schedule of reinforcement (1.5 mg/kg per injection) as previously described. One group (n = 8) was pretreated with baclofen (1.8, 3.2 or 5.6 mg/kg, IP), while the second group (n = 8) was pretreated with CGP 56433A (1.8 mg/kg, IP) and baclofen (1.8, 3.2 or 5.6 mg/kg, IP) in two successive injections. All drug treatments were administered 30 min prior to the start of the daily session. Baclofen injections in both groups were counterbalanced according to a Latin square design and test sessions were separated by at least 3 days of responding over which break points varied by less than 4. Data were analyzed by a Two-way ANOVA with repeated measures on Dose of baclofen.

Drugs

CGP 56433A was synthesized and provided by Novartis Pharma AG. The compound was dissolved in sterile saline.

Results

CGP 56433A Dose-Response Curve under FR and PR schedules

The IP administration of the $GABA_B$ antagonist CGP 56433A had no effect on responding for cocaine (1.5 mg/kg per injection) under either an FR1 or PR schedule of

reinforcement. Figure 15 (Left) shows that under FR1 conditions there was no significant effect of CGP 56433A on the number of responses completed during the first 3 hours of the test session [F<1]. Figure 15 (Right) shows that there was no significant change in break points for responding for cocaine following any of the doses of CGP 56433A tested [F (3,12) = 1.99, NS].

Effect of Baclofen and CGP 56433A on cocaine self-administration

FR1 schedule:

Figure 16 illustrates the effect of combining a high dose of baclofen with various doses of CGP 56433A in rats responding for cocaine under an FR1 schedule of reinforcement. Baclofen (5.6 mg/kg, IP) caused a significant reduction in responding during the first 3 hours of the test session. ANOVA revealed that CGP 56433A dose-dependently blocked the effect of baclofen on responding [$F(4,28)=5.09$, $p<0.01$]. Newman Keuls multiple comparisons revealed that the effect of baclofen was significantly attenuated by the highest dose of CGP 56433A (1.8 mg/kg, IP).

PR schedule:

The effect of baclofen on cocaine-reinforced responding under a PR schedule is illustrated in Figure 17 (open squares). Baclofen produced a dose-dependent decrease in break points [$F(3,21)=21.72$ $p<0.01$]. Newman Keuls multiple comparisons revealed that the two highest doses of baclofen (3.2 and 5.6 mg/kg, IP) caused significant reductions in responding when compared to baseline (BL), with the highest dose causing an almost complete cessation of responding in the entire group of rats.

Figure 15: Effect of the $GABA_B$ antagonist CGP 56433A (0.6, 1.0, or 1.8 mg/kg, IP) on cocaine self-administration. **Left:** Rats (n = 4) were trained to respond for cocaine (1.5 mg/kg per injection) under an FR1 schedule of reinforcement. Points represent the mean (± S.E.M.) number of responses during baseline (BL) conditions, or following various doses of CGP 56433A. Animals were pretreated with CGP 56433A 30 min prior to the test session. There was no significant effect of treatment at any of the doses tested. **Right:** A separate group of rats (n = 5) responding for cocaine (1.5 mg/kg/ per injection) under a PR schedule of reinforcement also received CGP 56433A 30 min prior to the test session. Points represent the mean (±S.E.M.) break points under baseline (BL) conditions, or following various doses of CGP 56433A. The $GABA_B$ antagonist failed to have a statistically significant effect on cocaine-reinforced responding under the PR schedule.

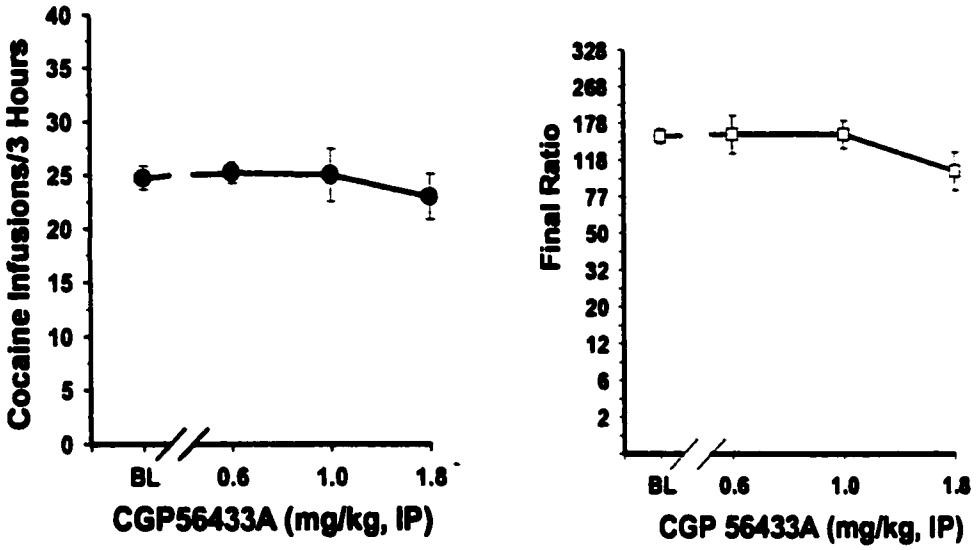

Cocaine Infusions/3 Hours
40
35
30
25
20
15
10
5
0
BL
0.6
1.0
1.8
CGP56433A (mg/kg, IP)
Final Ratio
328
268
178
118
77
50
32
20
12
6
2
BL
0.6
1.0
1.8
CGP 56433A (mg/kg, IP)

Figure 16: Effect of combining the $GABA_B$ agonist baclofen with various doses of CGP 56433A (0, 0.6, 1 or 1.8 mg/kg, IP) on responding for cocaine under an FR1 schedule of reinforcement. Points represent the mean (±S.E.M.) number of infusions of cocaine (1.5 mg/kg per injection) during the first 3-h of the session under baseline conditions (**open circles**) and over the test session (**closed circles**). Rats (n = 8) were pretreated with a high dose of baclofen (5.6 mg/kg, IP) and various doses of CGP 56433A. Drugs were administered in 2 consecutive injections 30 min prior to the test session. Baclofen produced a significant attenuation of responding when compared to baseline (BL). ANOVA indicated that CGP 56433A dose-dependently blocked the effect of baclofen on responding for cocaine. Newman Keuls revealed a significant difference between vehicle (0.0) and 1.8 mg/kg CGP 56433A (* $p<0.05$).

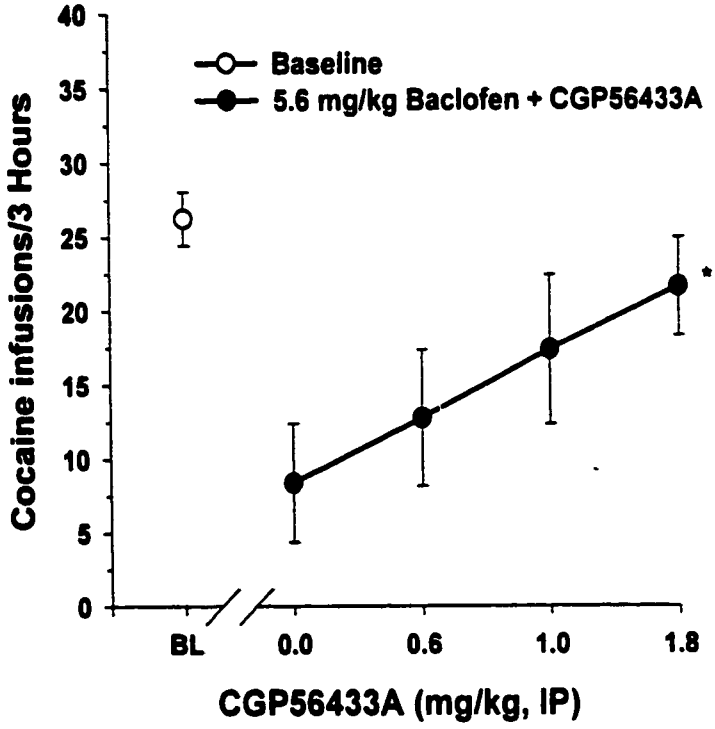
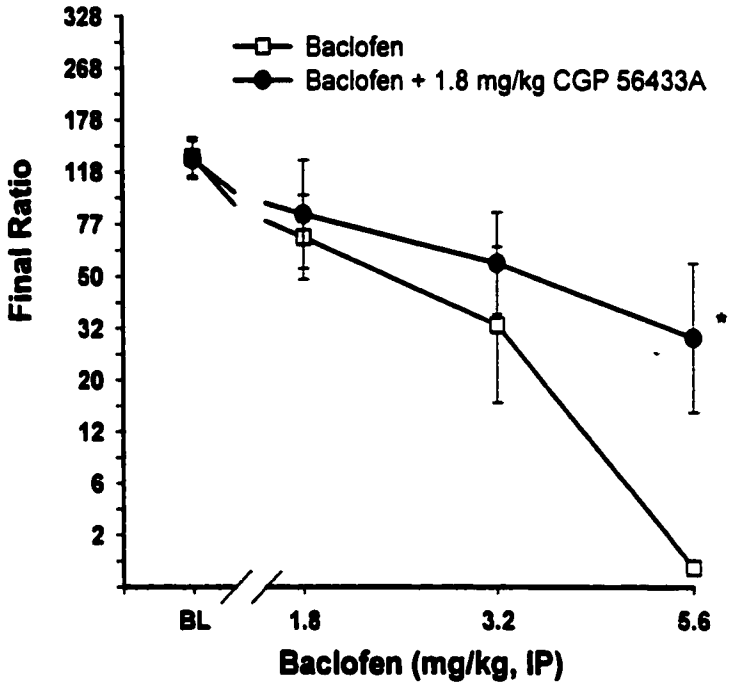

Baseline
5.6 mg/kg Baclofen + CGP56433A
Cocaine infusions/3 Hours
40
35
30
25
20
15
10
5
0
BL
0.0
0.6
1.0
1.8
*
CGP56433A (mg/kg, IP)

Figure 17: Effect of baclofen and the $GABA_B$ antagonist CGP 56433A on cocaine-reinforced break points. Separate groups of rats (n = 8 per group) were trained to respond for cocaine (1.5 mg/kg per injection) under a PR schedule of reinforcement. Points represent the mean (±S.E.M.) break points after various doses of baclofen (1.8, 3.6 or 5.62 mg/kg, IP), with (**squares**) or without 1.8 mg/kg CGP 56433A (**circles**). All drugs were administered 30 min prior to the test session. Break points were significantly reduced compared to baseline (BL) conditions after the intermediate and high doses of baclofen. CGP 56433A attenuated the effect of the highest dose of baclofen (*$p<0.05$).


Baclofen
Baclofen + 1.8 mg/kg CGP 56433A
Final Ratio
328
268
178
118
77
50
32
20
12
6
2
BL
1.8
3.2
5.6
*
Baclofen (mg/kg, IP)

Also illustrated in Figure 17 is the effect of CGP 56433A on baclofen-induced reductions in cocaine-reinforced responding under the PR schedule (closed circles). ANOVA showed a significant Drug X Dose interaction [$F(3,42) = 3.2$, $p < 0.05$]. Analysis of the simple main effects revealed a significant difference between the baclofen and CGP 56433A + baclofen groups indicating that the expected dose dependent decrease in responding produced by baclofen was attenuated by the co-administration of CGP 56433A.

Discussion

The results of the experiments in Series 4 provide further support for the hypothesis that $GABA_B$ receptor modulation attenuates cocaine reinforcement. While not all of baclofen's effects are necessarily mediated by $GABA_B$ receptors (Fung et al., 1985), the specific $GABA_B$ antagonist CGP 56433A attenuated the effect of baclofen on cocaine self-administration under both FR and PR schedules of reinforcement. These results are the first report that baclofen's anti-cocaine effect is attenuated by the co-administration of a $GABA_B$ antagonist.

Given that stimulation of $GABA_B$ receptors with baclofen causes an attenuation of cocaine self-administration, it might be predicted that blocking $GABA_B$ receptors could increase cocaine-reinforcement. No such increases were observed. It is possible that higher doses of CGP56433A may have affected cocaine self-administration, but seizures activity has been reported in some rats at doses higher than 6 mg/kg. The doses used in this study have been demonstrated to be physiologically active (Getova et al., 1997; 1998; Heese et al., 2000) The fact that CGP 56433A alone had no effect on cocaine self-administration would

seem to indicate that VTA DA levels are not under a significant $GABA_B$-mediated inhibitory tone under normal circumstances, or during exposure to cocaine.

Series 5: To characterize the effect of baclofen on heroin self-administration

Opioid drugs are characterized by their ability to bind to one of the 3 major classes (μ, δ, and κ) of opioid receptors. Diacetylmorphine, or heroin, is a lipid soluble μ opioid agonist that is derived from morphine. Upon entering the brain it is metabolized into 6-monoacetylmorphine and then into morphine, which is responsible for the pharmacological effects of heroin (Di Chiara & North, 1992).

It has been suggested that the mesolimbic DA system may be a common pathway for mediating the reinforcing properties of both psychostimulants and opiates (Zito et al., 1985; Koob, 1992; Di Chiara & North, 1992; Wise, 1996a). Cocaine self-administration is mediated by DA-dependent mechanisms, while heroin reinforcement appears to primarily involve DA-independent, μ and δ opiate receptors (Koob & Bloom, 1988; DiChiara & North, 1992; Negus et al., 1993; Hemby et al., 1995; Hemby et al., 1999). However, there is a large body of evidence that indicates that μ and δ opioid receptors located proximal to DA cell bodies in both the NAC and VTA have the potential to activate the VTA dopamine system and thereby affect heroin self-administration (Gysling & Wang, 1983; Latimer et al., 1987; Mansour et al., 1987; Leone et al., 1991; Negus et al., 1993; Shippenberg et al., 1992; Devine & Wise, 1994; Svingos et al., 1999).

There is some controversy over the importance of the mesolimbic DA system in the

reinforcing effects of opiates. On the one hand, morphine injections into the VTA produce conditioned place preference in rats. This effect has been linked to opiate-induced increases in DA release, as it can be blocked by haloperidol (Phillips et al., 1983) or dopaminergic lesions of the VTA (Phillips & LePaine, 1980; Phillips et al., 1983) or NAC (Spyraki et al., 1983). Moreover, rats will self-administer opiates or opiate agonists into the NAC and VTA (Bozarth & Wise, 1981; Goeders et al., 1984). However, while in some studies heroin self-administration is attenuated after DA antagonists or neurotoxic lesions of the NAC and VTA (Vaccarino et al., 1985; Zito et al., 1985; Bozarth & Wise, 1986; Van Ree & Ramsay, 1987; Corrigal & Vaccarino, 1988), in others this is not the case (Ettenberg et al., 1982; Pettit et al., 1984; Gerrits et al., 1994). Similarly, while many studies report increases in NAC DA levels following heroin self-administration (Petit & Justice, 1989; 1991; Wise et al., 1995), several others report only non-significant DA elevations (Hemby et al., 1995; Hemby et al., 1999; Gerasimov & Dewey, 1999).

The importance of VTA dopaminergic projections to the NAC in opiate self-administration is still unclear. However, the demonstration that opiates increase DA cell firing in the VTA and increase DA levels in the NAC supports the hypothesis that mesolimbic DA mechanisms are involved in the reinforcing effects of both cocaine and heroin. Opiate drugs such as heroin are thought to excite DA neurons in the VTA indirectly, by binding to μ opioid receptors and hyperpolarizing inhibitory, GABA-containing interneurons (Johnson & North, 1992). Support for this hypothesis comes from studies demonstrating that stimulation of μ opioid receptors inhibits GABA synaptic transmission in a number of brain regions (Renno et al., 1992; Lupica, 1995; Vaughan et al., 1997), while

systemic or iontophoretic morphine increases the firing rate of dopaminergic VTA neurons and decreases the firing of GABAergic interneurons (Kelley et al., 1980; Matthews & German, 1984; Johnson & North, 1992; Gysling & Wang, 1993).

Two recent studies have examined the effect of GABAergic drugs on heroin self-administration (Gerasimov et al. 1999; Xi & Stein, 1999). In these studies, systemic GVG, and intra-VTA baclofen blocked heroin self-administration, and the heroin-induced increase in NAC DA release. The effect of baclofen on the NAC DA release was blocked by intra-VTA microinjection of the $GABA_B$ antagonist 6-hydroxy-saclofen. These data provide preliminary evidence that heroin reinforcement might be modulated through $GABA_B$ receptors. The experiments in this Series examined the effect of several doses of baclofen on heroin self-administration under both an FR and PR schedule of reinforcement, in order to determine whether the effect of baclofen is specific to psychostimulant drugs. In addition, separate groups of rats were pretreated with the $GABA_B$ antagonist CGP 56433A alone, and in combination with baclofen, in order to determine whether the antagonist could block baclofen-induced decreases in heroin-reinforced responding.

Method

Subjects and Procedure

Subjects, surgical procedures and data analysis were identical to those described in Series 4, expect as noted below

Baclofen Dose-Response Curve under an FR1 schedule

Rats were trained to self-administer 50 μg/kg per injection of heroin under an FR1

schedule of reinforcement, according to the parameters previously described for cocaine. After responding stabilized (defined as completion of 40 injections within 5 hours), rats were randomly assigned to one of two groups, one that continued to respond for a 50 μg/kg per injection dose of heroin, while the other was switched to a unit injection dose of 25 μg/kg of heroin (n = 7 for both groups). Following three days of stable responding, rats received various doses of baclofen (vehicle, 1.8, 3.2 or 5.6 mg/kg, IP) 30 min prior to the test session. The order of baclofen injections was counterbalanced according to a Latin square design and test sessions were separated by at least three days.

CGP 56433A Dose-Response Curve under an FR1 schedule

The effect of the $GABA_B$ antagonist CGP 56433A on heroin-reinforced responding was assessed in a separate groups of rats trained to respond for a unit injection dose of 25 μg/kg of heroin under an FR1 schedule (n = 5) as previously described. Once stable rates of responding were established, rats were pretreated with various doses of CGP 56433A (0.6, 1.0 or 1.8 mg/kg, IP) 30 min prior to the test sessions. The order of CGP 56433A injections was counterbalanced according to a Latin square design and test sessions were separated by at least three days.

Baclofen Dose-Response Curve under a PR schedule

The effect of baclofen on heroin-reinforced responding under a PR schedule was assessed in a separate group of rats (n = 7). Rats were trained to self-administer 50 μg/kg per injection of heroin under an FR1 schedule as previously described. Once responding under FR1 conditions stabilized rats were switched to a 25 μg/kg per injection dose of heroin under a PR schedule. Daily sessions were 8 hr in length to ensure rats experienced at least 16 hr

heroin withdrawal prior to the beginning of daily sessions. When responding on the PR schedule stabilized (defined as 3 consecutive days over which break points varied by less than 3), rats received various doses of baclofen (1.8, 3.2 or 5.6 mg/kg, IP, counterbalanced according to a Latin square design) 30 minutes prior to the beginning of the session.

Effect of Baclofen and CGP 56433A on heroin self-administration

In order to determine whether CGP 56433A blocked baclofen-induced decreases in heroin self-administration two separate groups of rats were trained to respond for 25 μg/kg per injection of heroin under an FR1 (n = 7) or PR (n = 6) schedule of reinforcement as previously described. Once stable rates of responding were established under an FR1 schedule, rats were pretreated with various doses of baclofen (1.8, 3.2 or 5.6 mg/kg, IP) in combination with CGP 56433A (1.8 mg/kg, IP) 30 min prior to the start of the daily session. Baclofen injections were counterbalanced according to a Latin square design and test days were separated by at least 3 days of stable responding.

Rats responding for heroin under a PR schedule of reinforcement were pretreated with CGP 56433A (1.78 mg/kg, IP) in combination with various doses of baclofen (1.8, 3.2 or 5.6 mg/kg, IP) administered 30 min prior to the start of the daily session. Baclofen injections were counterbalanced according to a Latin square design and test sessions were separated by at least 3 days of responding over which break points varied by less than 3.

Heroin Dose-response Curve

A separate group of rats (n = 7) was used in order to establish a dose-response curve for heroin reinforced break points. Rats were trained to respond for 50 μg/kg per injection of heroin under FR1 conditions as previously described. Once responding stabilized rats

were switched to a PR schedule. Daily self-administration sessions were restricted to 8 hr in length and the unit injection dose (25, 50 or 100 μg/kg) of heroin were varied according to a Latin square design. Rats were allowed to respond for each dose of heroin for at least 4 days, or until break points varied by less than 3 for 3 consecutive days.

Drugs

Heroin was supplied by the National Institute on Drug Abuse (Research Triangle, NC). All drugs were dissolved in sterile saline.

Results

Baclofen Dose-Response Curve under an FR1 schedule

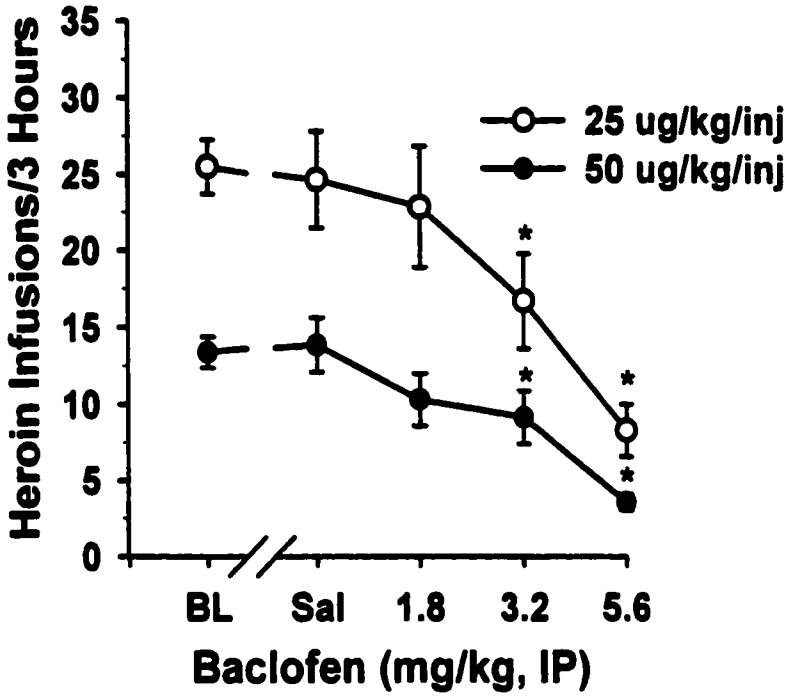
Baclofen dose-dependently attenuated the self-administration of both 25 and 50 μg/kg per injection of heroin under an FR1 schedule of reinforcement. Figure 18a shows that the two higher doses of baclofen (3.2 and 5.6 mg/kg, IP) caused a significant decrease in the number of injections of heroin that were self-administered during the first 3 hours of the session in both groups of animals [$F(4,24)=12.29, p<0.05$ for the group responding for 25 μg/kg per injection; $F(4,24)=12.64$, $p<0.05$ for the group responding for 50 μg/kg per injection]. Examination of the cumulative records (see Figure 18b) shows that the decrease in heroin self-administration was represented by periods of non-responding at the beginning of the session.

CGP 56433A Dose-Response Curve under an FR1 schedule

CGP 56433A pretreatment failed to produce a significant effect on heroin-reinforced responding under an FR1 schedule [$F<1$]. Figure 19 shows that the antagonist did not alter

Figure 18A: Effect of baclofen on heroin self-administration on a FR1 schedule. Points represent the mean (±S.E.M.) number of injections self-administered during a 3-h baseline (BL) or test session. Separate groups of rats (n = 7 per group) were trained to respond for either 25 or 50 μg/kg per injection of heroin and were pretreated with baclofen (vehicle, 1.8, 3.6 or 5.6 mg/kg, IP) 30 min prior to the daily session. The intermediate and high doses of baclofen produced a significant reduction in responding for both unit injection doses of cocaine (* $p<0.05$). **B:** A representative record of an animal responding for heroin (25 μg/kg per injection) showed that reductions in intake were accounted for by periods of non-responding at the beginning of the test session, which resulted in a decrease in the total number of injections.

A


Heroin Infusions/3 Hours
35
30
25
20
15
10
5
0
25 ug/kg/inj
50 ug/kg/inj
*
*
*
*
BL
Sal
1.8
3.2
5.6
Baclofen (mg/kg, IP)

B

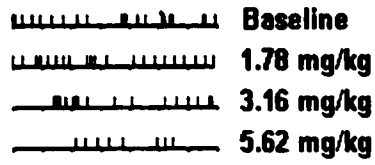
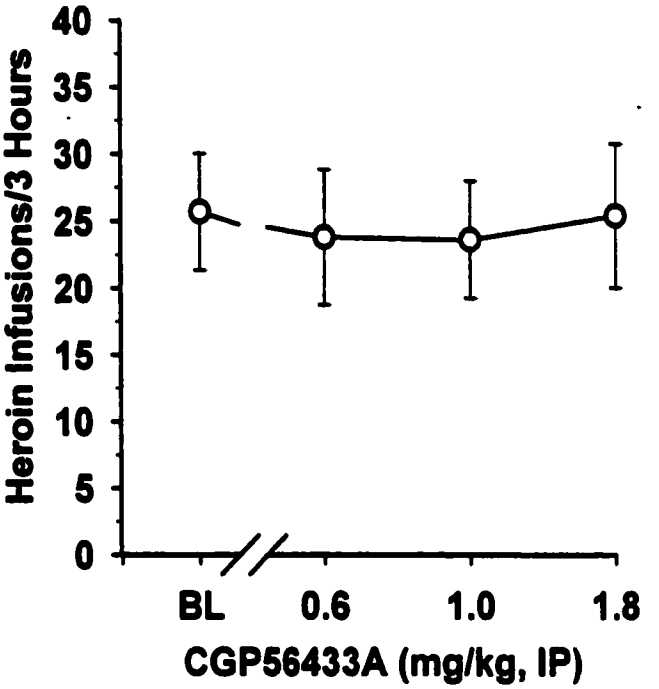

Baseline
1.78 mg/kg
3.16 mg/kg
5.62 mg/kg

Figure 19: Effect of the $GABA_B$ antagonist CGP 56433A (0.6, 1.0, or 1.8 mg/kg, IP) on heroin self-administration. A group of rats (n = 5) were trained to respond for heroin (25 µg/kg per injection) under an FR1 schedule of reinforcement. Animals were pretreated with CGP 56433A 30 min prior to the test session. Points represent the mean (± S.E.M.) number of injections during baseline (BL) conditions, or following various doses of CGP 56433A. There was no statistically significant effect of CGP 56433A treatment at any of the doses tested.


Heroin Infusions/3 Hours
40
35
30
25
20
15
10
5
0
BL
0.6
1.0
1.8
CGP56433A (mg/kg, IP)

rates of responding for 25 μg/kg per injection of heroin during the first 3 h of the test session when the effect of treatment would have been the strongest.

Baclofen Dose-Response Curve under a PR schedule

The effect of baclofen on heroin-reinforced responding (25 μg/kg per injection) under a PR schedule is illustrated in Figure 20. ANOVA revealed a significant effect of Dose when baclofen (1.8, 3.2 or 5.6 mg/kg) was administered 30 min prior to the beginning of the test session [$F(3,18) = 10.49$, $p<0.01$]. Newman Keuls multiple comparisons indicated that heroin-reinforced break points were significantly reduced following all three doses of baclofen ($p<0.01$).

Effect of Baclofen and CGP 56433A on heroin self-administration under an FR1 schedule

The effect of the $GABA_B$ antagonist CGP 56433A on the baclofen dose-response curve is illustrated in Figure 21 (open circles). ANOVA revealed a significant effect of Dose, but no effect of Group or Dose X Group interaction effect was seen. Although the two higher doses of baclofen significantly reduced responding during the first 3 hours of the test session CGP 56433A (1.8 mg/kg, IP) failed to block the effect (closed circles).

Effect of Baclofen and CGP 56433A on heroin self-administration under a PR schedule

Figure 22 illustrates that the $GABA_B$ antagonist CGP 56433A did not block baclofen's effect on heroin-reinforced break points. ANOVA revealed that CGP 5643A had no statistically significant effect on the baclofen dose-response curve [$F<1$].

Dose-Response curve for heroin under a PR schedule

It was not possible to establish a dose-response curve for heroin using the PR schedule (See Figure 23). Altering the unit injection dose of heroin failed to produce a

Figure 20: Effect of various doses of baclofen on responding on a PR schedule reinforced by heroin (25 μg/kg per injection). Points represent the mean break points (± S.E.M.) for a group of rats (n = 7) pretreated with baclofen (1.8, 3.2 or 5.6 mg/kg, IP) 30 min prior to the test session. Baclofen dose-dependently reduced heroin-reinforced break points, with the high dose causing an almost complete cessation of responding in all animals (*$p<0.05$).

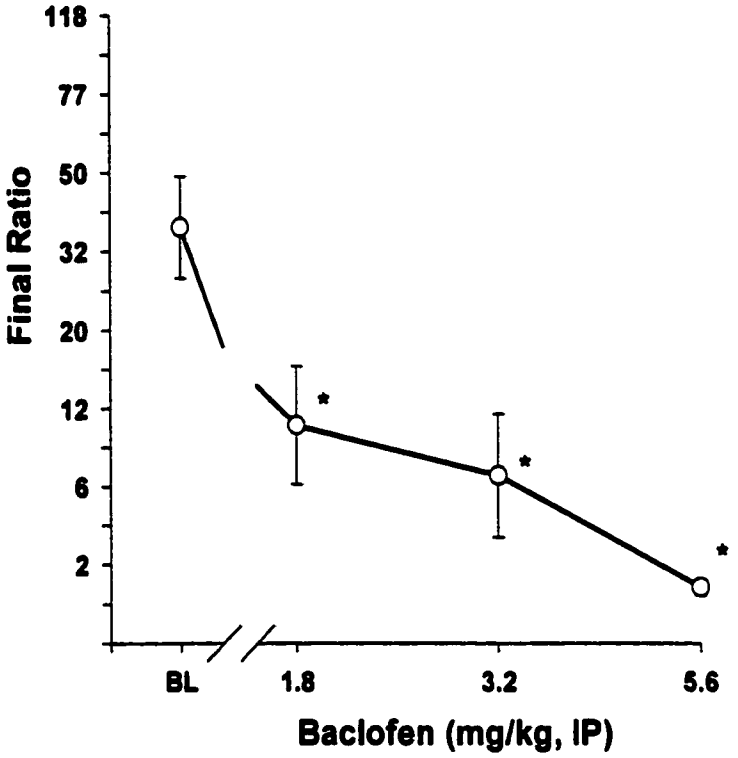

118
77
50
32
20
12
6
2
Final Ratio
*
*
*
BL
1.8
3.2
5.6
Baclofen (mg/kg, IP)

Figure 21: Effect of baclofen in combination with CGP 56433A on heroin self-administration under an FR1 schedule. Points represent the mean (±S.E.M.) number of infusions of heroin (25 μg/kg per injection) during the first 3-h of the session under baseline conditions (BL) and over the various test sessions. Two groups of rats (n = 7 per group) were pretreated with various doses of baclofen (1.8, 3.2 or 5.6 mg/kg, IP) alone (**closed circles**) or in combination with a high dose (1.8 mg/kg, IP) of CGP 56433A (**open circles**). All drugs were administered 30 min prior to the test session. The intermediate and high doses of baclofen produced a significant attenuation of responding when compared to baseline ($p<0.05$). The $GABA_B$ antagonist CGP 56433A failed to produce a statistically significant on the baclofen dose-response curve.

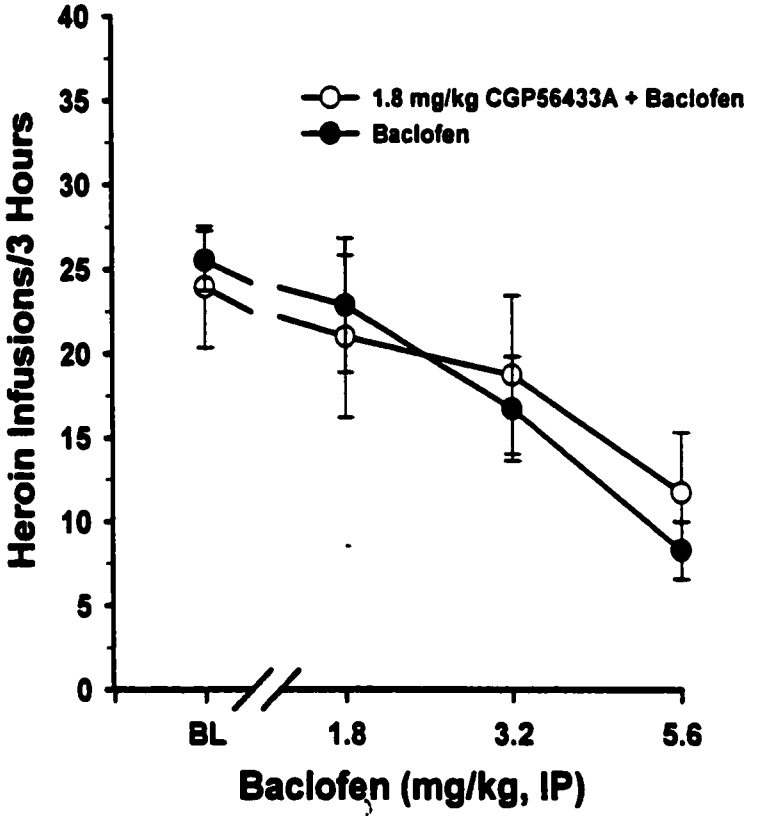

40
35
30
25
20
15
10
5
0
Heroin Infusions/3 Hours
1.8 mg/kg CGP56433A + Baclofen
Baclofen
BL
1.8
3.2
5.6
Baclofen (mg/kg, IP)

Figure 22: Effect of baclofen and CGP 56433A on heroin-reinforced break points. Two groups of rats were trained to respond for heroin (25 μg/kg per injection) under a PR schedule of reinforcement. *Points* represent the mean (±S.E.M.) break points after various doses of baclofen (1.8, 3.6 or 5.62 mg/kg, IP) alone (**open circles**, n = 7) or in combination with CGP 56433A (**closed circles**, n = 6). All drugs were administered 30 min prior to the test session. Break points were significantly reduced compared to baseline (BL) conditions after all three doses of baclofen ($p<0.01$). CGP 56433A failed to produce a statistically significant effect on the baclofen dose-response curve [$F<1$].

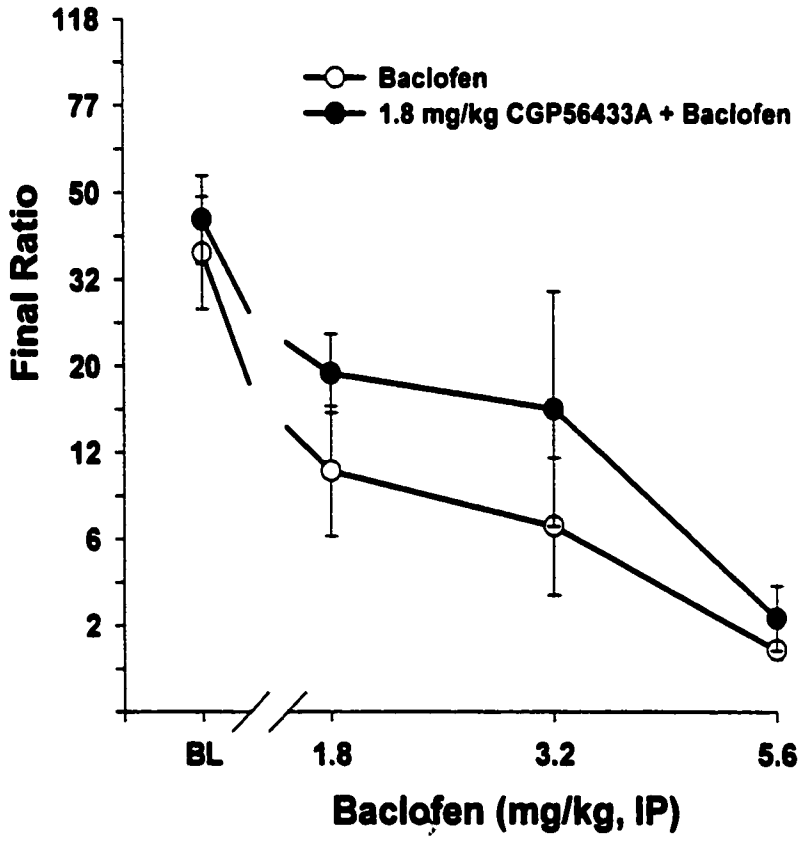
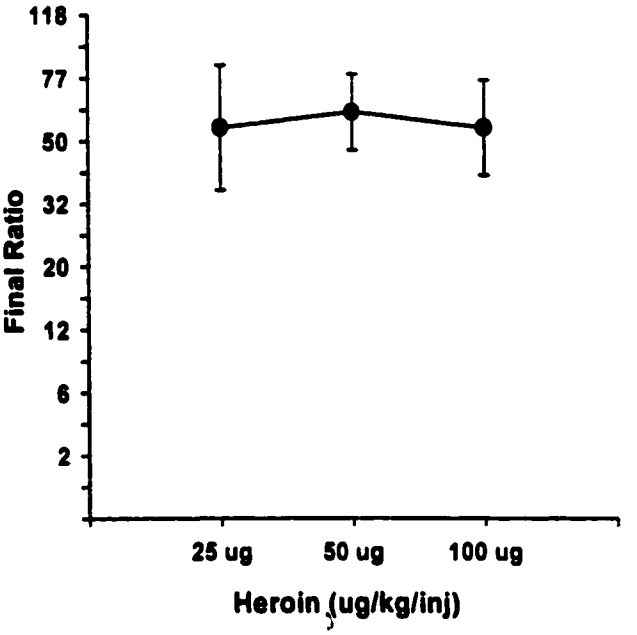

118
77
50
32
20
12
6
2
Final Ratio
Baclofen
1.8 mg/kg CGP56433A + Baclofen
BL
1.8
3.2
5.6
Baclofen (mg/kg, IP)

Figure 23: Dose-response curve for heroin self-administration under a PR schedule of reinforcement. Points represent mean (± S.E.M.) break points for each dose of heroin. A group of rats (n = 7) was trained to respond for 50 μg/kg per injection of heroin under FR1 conditions until responding stabilized. They were then switched to a PR schedule of reinforcement for daily 8-h sessions. Under the PR schedule, the unit injection dose of heroin (25, 50 or 100 μg/kg) was varied so that each rat was allowed to self-administer each dose for at least 4 days, or until they established stable break points (defined as 3 consecutive days over which break points varied by less than 3). The starting dose for the rats was varied according to a Latin square design. Altering the unit dose of heroin failed to have a statistically significant effect on break points, and no dose-dependent effect on responding was observed.


118
77
50
32
20
12
6
2
Final Ratio
25 ug
50 ug
100 ug
Heroin (ug/kg/inj)

statistically significant difference in break points across dose [F<1].

Discussion

The results of this Series of experiments demonstrate that the $GABA_B$ agonist baclofen reduced heroin intake. Baclofen produced a dose-dependent decrease in heroin self-administration under both an FR1 and PR schedule of reinforcement. While these results were robust, and in some respects parallel the effects observed for cocaine self-administration, they must, nonetheless, be interpreted with caution.

Interpreting changes in FR1 responding are always problematic. The decreases in intake in the present studies would seem to indicate that the reinforcing effects of heroin are diminished by baclofen pretreatment. However, it will be recalled that treatments that decrease reinforcing efficacy typically increase drug intake under FR schedules. For example rats increase their responding for cocaine and heroin after pretreatment with haloperidol or naloxone, respectively. Obviously there are problems with any theory that interprets both increases and decreases in response rate to mean the same thing. This interpretational difficulty is illustrated in the data of Xi & Stein (1999) who reported increases in heroin-reinforced responding after intra-VTA baclofen, and decreases in responding after systemic baclofen. They concluded that both changes indicated a decrease in reinforcing efficacy. Furthermore, it has been argued that rate of drug self-administration may be insensitive to changes in reinforcing efficacy (Arnold & Roberts, 1997). Some treatments have been documented to produce enormous changes in animals' motivation to self-administer drugs as shown by other schedules without producing a change in the rate of drug intake on an FR1

schedule. In the present studies, baclofen produced a decrease in heroin intake under an FR1 schedule. Since firm conclusions regarding reinforcing efficacy cannot be drawn from FR1 data, our working hypothesis is that baclofen attenuates heroin-reinforcement.

The PR schedule was used in this Series in an attempt to clarify the FR1 Examination of the effect of baclofen on PR responding indicated that rats self-administered fewer heroin injections after baclofen pretreatment. While baclofen reduced heroin-reinforced break points in a dose dependent manner, there are some unsettling features of these data that cause some concern. One of the problems associated with the PR schedule is that pretreatments that have significant motoric or sedative effects will interfere with an animal's ability to complete the operant response and result in a decrease in break point. In the present experiments 5.6 mg/kg of baclofen caused an almost complete cessation in responding under both the FR and PR schedules, which was not blocked by the antagonist CGP 56433A. Without evidence of the animals' capacity to perform we cannot rule out the possibility that the combination of baclofen and heroin produced motor or debilitating effects. Even more problematic was the failure to establish a dose-response curve for heroin under the PR schedule. Changes in the unit-injection dose (25, 50 or 100 μg/kg) of heroin failed to affect break points, suggesting that they were not sensitive to changes in reinforcing efficacy.

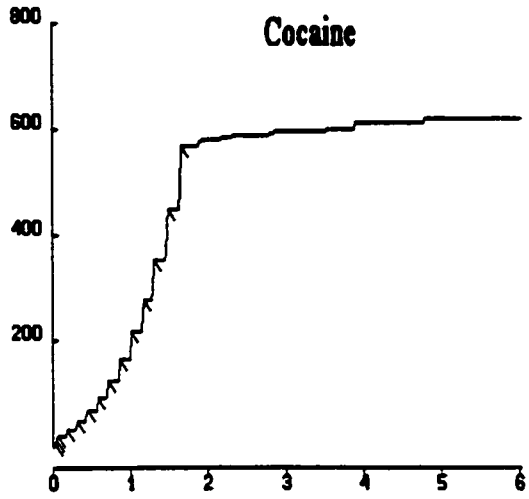
The PR schedule was developed specifically to examine psychostimulant reinforcement, and based on the results of the present experiments, it would appear that there are problems associated with applying it to the examination of heroin self-administration. Cocaine and heroin engender different patterns of responding, which suggests that they are quite different behaviourally. Examination of the cumulative record

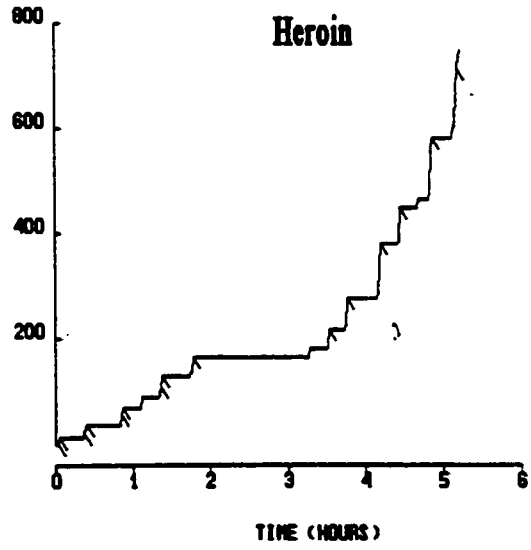
of a rat responding for cocaine under the PR schedule (see Figure 24, Top panel) shows that they take several injections at the start of the session before settling into a characteristic "step like" pattern. Under baseline conditions rats will typically reach their break point within 2-3 hours, and seldom respond after break point is reached. Break points are reliably dose-dependent and are exquisitely sensitive to pharmacological manipulation (see Arnold & Roberts, 1997 for review). By contrast, heroin-reinforced rats tend to respond in bursts, with two or more injections in a short period followed by prolonged periods of non-responding (See Figure 24, Bottom panel). Furthermore, they will intermittently resume responding at modest rates throughout the session, and break points tend to be quite modest.

Modified PR schedules have been used in an attempt to address these problems. Roberts & Bennett (1993) used a schedule in which the ratio requirement for the first injection of each session was adjusted according to the performance of the animal during the previous session. Using this schedule they showed that heroin animals seem most motivated to obtain the first injection of the session, but are less motivated for each subsequent injection; cocaine animals, on the other hand, appear most motivated to obtain the 'next' injection. While Roberts & Bennett (1993) were able to establish dose-dependent break-points using this schedule, it does not lend itself to experiments involving acute drug pretreatments, as it takes 3 or more days to establish break points.

It is clear that the development of more appropriate ways of measuring changes in rats' motivation to self-administer heroin is necessary before it will be possible to fully characterize the effect of $GABA_B$ drugs on heroin reinforcement. This analysis will be further complicated by the issue of the physical dependence that develops after exposure to

Figure 24: Cumulative records of baseline self-administration sessions in rats responding for cocaine- or heroin-reinforcement under a PR schedule. **Top:** Under a PR schedule, rats typically take several injections of cocaine (1.5 mg/kg per injection) at the start of the session (represented by short downward strokes) before settling into a characteristic "step like" pattern. Break points are often reached within 2-3 hours, and responding (represented by vertical increments) seldom occurs once break points are reached. **Bottom**: The pattern of responding in a rat self-administering heroin (25 μg/kg per injection) is quite different from cocaine. Rats tend to respond in bursts, taking two or more injections in a short time, followed by periods of non-responding. Intermittent responding can be observed over the 6 hours of the session.


Cocaine
800
600
400
200
0
1
2
3
4
5
6


Heroin
800
600
400
200
0
1
2
3
4
5
6
TIME (HOURS)

high doses of opiates. Positive reinforcement sustains responding for low doses of heroin, but as physical dependence develops, negative reinforcement associated with alleviating withdrawal symptoms also maintains responding. The doses used in the present investigation were deliberately chosen so as to minimize the possibility of physical dependence, and did not produce signs of withdrawal. The circuitry that is potentially involved in physical dependence may encompass a far greater constellation of neurochemical systems than are involved in mediating the reinforcing effects of heroin. It has been suggested that physical dependence involves not only the neural systems that mediate the affective components of opiate withdrawal (e.g. depression, anxiety, anhedonia), but also includes those controlling vegetative function (e.g. nausea and gastrointestinal disturbances) (Bozarth & Wise, 1984; Koob et al., 1992; Maldonado et al., 1992; Carrera et al., 1999; Koob, 2000). Although $GABA_B$ agonists appear to be somewhat effective at reducing heroin self-administration, possibly by interfering with its positive reinforcing effects, it is not yet clear whether they are also able to alleviate the negative reinforcing effects associated with withdrawal. There are no published reports addressing this possibility, although it is interesting to note that in the original patent application baclofen was said to depress the symptoms of withdrawal from addicting agents, particularly from morphine.

General Discussion

Baclofen clearly has an impact on cocaine self-administration under several different schedules of reinforcement, suggesting its potential utility as a medication for treatment of cocaine addiction. There are several issues raised by the present data that have implications

for its clinical possibilities. The experiments contained in this thesis demonstrate that the effect of baclofen on cocaine self-administration (1) is not the result of a generalized disruption in responding caused by sedation or locomotor depression, (2) is not due to a general suppression of other appetitive behaviours such as food-reinforced responding, and (3) is not accompanied by a compensatory increase in cocaine intake.

The muscle relaxant and sedation that can be associated with high doses of baclofen raise the possibility that the anti-cocaine effects in these studies were a result of a generalized disruption in operant responding, rather than a specific effect on cocaine-reinforcement. Indeed, two recent studies have reported that baclofen reduced consumption of liquid food in rats, and also affected food maintained responding in rhesus monkeys (Caine et al., 2000a; Stafford & Glowa, 2000). The results of Series 1 and 2, however, suggest that baclofen's effects on cocaine-reinforcement are somewhat specific. Baclofen did not suppress responding for food-reinforcement under a concurrent access schedule at even the highest doses tested, indicating it did it not interfere with the animals' ability to complete the operant response to obtain an infusion of cocaine. Rather, it appeared to redirect the animals' responding from the cocaine-reinforced lever toward the food-reinforced lever.

Close examination of the pattern of cocaine self-administration under an FR1 schedule suggests that baclofen suppresses cocaine self-administration in a manner that is qualitatively different from dopaminergic compounds. Typically, DA agonists and antagonists produce uniform increases or decreases in the rate of cocaine self-administration under FR schedules. In Series 1 the DA antagonist haloperidol increased self-administration

of cocaine on an FR1 schedule, in essence shifting the dose-response curve to the right. In contrast, baclofen shifted the cocaine dose-response curve downward on the FR1 schedule, and at no point was there any indication of an increase in cocaine intake. Baclofen-induced reductions in cocaine self-administration resulted from pauses in responding rather than a change in inter-injection intervals; when self-administration did occur, the timing of injections was not affected.

It seems paradoxical that a treatment that causes an attenuation of drug reinforcement would not have an effect on rate of drug intake. However, a variety of treatments have now been shown to produce dramatic changes in the motivation to self-administer cocaine, as demonstrated by the PR schedule, without causing corresponding changes in the rate of drug intake on an FR1 schedule (Roberts et al., 1989a; Loh & Roberts, 1990; McGregor & Roberts, 1993). These data suggest that there are some mechanisms that regulate drug intake that are distinct from those that mediate the reinforcing effects. It has been suggested that rate of drug intake is regulated not only by the reinforcing effects but also limited by toxic or aversive reactions (Roberts & Zito, 1987). Manipulations that influence both of these effects equally, such as dilution of the unit dose or blockade at the primary site of action, could produce a compensatory increase in intake without affecting this putative balance. Since animals do not show a compensatory increase in cocaine intake following baclofen pretreatment, we infer that the limiting factors that control drug intake are unchanged while the primary reinforcing properties of cocaine have been altered. A recent report has shown that baclofen has no effect on the discriminative stimulus properties of cocaine in rats (Munzar et al., 2000) To the extent that discriminative stimulus properties

may control FR1 responding, this would have been predicted by the present results.

The experiments in this thesis also demonstrate that the effect of baclofen on cocaine self-administration is dependent on the unit injection dose of cocaine, and on the response requirements of the schedule. Baclofen did not appear to alter intake of high unit-injection doses of cocaine on an FR schedule. Although this would seem to imply that the baclofen effect is surmountable, it would be a mistake to conclude that baclofen has no effect on high-dose cocaine. Baclofen is effective at decreasing intake of high unit injection doses of cocaine on a PR schedule, when the 'price' of obtaining an infusion is pushed.

In summary, it would appear that baclofen has a dramatic and specific effect on cocaine self-administration that cannot be ascribed to a generalized disruption in behaviour. The fact that baclofen causes a suppression of cocaine intake, rather than producing a compensatory increase, like that associated with DA antagonists would appear to have some clinical appeal.

Pharmacotherapies for cocaine addiction

The following brief review summarizes the primary strategies that have been used to develop a pharmacotherapy for cocaine addiction, and the problems associated with each. In many respects research into developing a medication to treat cocaine abuse is still in its infancy. Based on what is known about the role that DA plays in the reinforcing properties of cocaine, the majority of the pharmacotherapeutic interventions that have been developed are aimed at interfering with cocaine's effect on DA levels (see Roberts & Ranaldi, 1995; McCance, 1997 for review). Unfortunately, past efforts to treat cocaine abuse have translated poorly into the clinical setting (see Dackis et al., 1987; Gawin et al., 1989; Meyer, 1992;

Kleber, 1995; Carroll et al., 1999 for review).

One approach to developing a medication to treat cocaine dependence is to synthesize compounds with similar properties to cocaine, but with a longer duration of action (Bennett et al., 1995). These medications are being developed in the hopes of finding a non-addictive substitute for cocaine that can be used to reduce physiological cravings produced by withdrawal, much the same way methadone has been used to treat heroin addicts. Dopamine uptake inhibitors such as GBR12909 or cocaine analogues such as 2β-propanoyl-3β-(4-tolyl)-tropane (PTT), WF-23, and WF55 have high affinity for the DA transporter and have been evaluated as possible substitutes for cocaine (Roberts, 1993; Nader et al., 1997; Birmingham et al., 1998; Roberts et al., 1999). It is not yet clear whether the substitution approach will be successful with psychostimulants. While some preclinical studies indicate that these compounds can attenuate cocaine self-administration in rats and monkeys (Nader et al., 1997), others have reported that stimulant drugs do not satisfy cocaine-craving, but in fact may induce relapse (DeWit & Stewart, 1981).

A second major approach has been to use drugs such as haloperidol to antagonize the acute reinforcing effects of cocaine. Unfortunately, it appears that the benefits of using DA antagonists to treat cocaine addiction may be limited. Several studies have indicated that the receptor blockade produced by these antagonists is surmountable, and behaviourally active doses of many of these compounds have significant side effects. The resulting problems with patient compliance make their efficacy as therapies for cocaine addiction questionable (Caine & Koob, 1994; see Carroll et al., 1999 for review).

Serotonergic drugs have been shown to inhibit cocaine reinforcement and have also

been considered as potential candidates for treating cocaine dependence. In human trials, some studies have shown that fluoxetine decreased the subjective effects of cocaine (Walsh et al., 1994), while others reported that it was ineffective in reducing cocaine use or craving (Grabowski et al., 1995). Although subjects in these studies did appear to reduce cocaine intake as measured by plasma and urine cocaine concentrations, and remain in treatment for significantly longer than placebo-treated subjects, most subjects did not achieve abstinence.

The focus of medication development is now beginning to shift away from DA-based compounds. Very recent strategies include immunological-based therapies which seek to develop a vaccine (Kantak et al., 2000). These therapies have been used in rats to demonstrate that cocaine antibodies can bind to cocaine in the circulation and interfere with its ability to cross the blood brain barrier, without interfering with the normal rate of cocaine metabolism. An alternate strategy has been to develop a catalytic antibody that metabolizes circulating cocaine by converting it into the inactive ecgonine methyl ester before it can enter the brain (Landry et al., 1993; Mets et al., 1998). While these treatments do appear to attenuate cocaine self-administration, they also have drawbacks that potentially limit their usefulness. In particular, with the cocaine vaccine it can take days or sometimes weeks to generate a physiologically relevant antibody titre after immunization. While the catalytic antibody would be effective within minutes after immunization, efforts to develop an antibody that persists in the system and remain sufficiently active to neutralize typical street doses of cocaine have so far failed (De Prada et al., 2000). Unfortunately the effect of both the vaccine and the catalyic antibody can be overcome by increasing doses of cocaine, or increasing the rate at which cocaine is delivered and saturating the available antibody

(O'Brien, 1997; Sparenborg et al., 1997).

Baclofen's potential as a pharmacotherapy for cocaine addiction

While there is still no substantially effective treatment for cocaine addiction, the experiments described in this thesis and other promising preliminary data suggest more systematic studies of the effects of $GABA_B$ receptor agonists should be considered. Baclofen has shown some promise in the two clinical trials that have been conducted thus far. The preliminary evidence from these studies is somewhat inconclusive, but nevertheless suggests that baclofen has the potential to reduce cravings for cocaine, and therefore might be an effective pharmacotherapy for cocaine addiction. Ling et al. (1998) conducted the first study, which was an open-trial, that baclofen (20 mg, three times a day) reduced the frequency of cocaine craving and cocaine use. However, the second, larger study, which was a double-blind trial, indicated that while subjects were more likely to abstain from cocaine use during weeks 3-8 of the trial, there were no significant effects of baclofen over placebo on measures of treatment effectiveness (Shoptaw, 2000).

The more recent clinical trial appears to be somewhat discouraging; however, there is reason to remain hopeful. In both studies cocaine use was reduced for at least part of the clinical trial, and reports of reductions in cocaine-craving, which is a cardinal feature of addictive disorders, are encouraging. Childress et al. (1999; 2000a; 2000b) have used drug-related videos to evoke cocaine craving in cocaine patients. Using neuroimaging techniques, they have demonstrated that cue-induced craving is accompanied by limbic activation and basal ganglia deactivation. Pilot studies conducted in their laboratory have suggested that baclofen is effective in reducing limbic activation as measured by PET during

cue-induced craving in these patients.

Both the preclinical (present studies) and preliminary clinical data suggest that further investigation into $GABA_B$ agonists as potential pharmacotherapies for cocaine addiction is warranted. In our animal models, the magnitude of baclofen's effect depends on the dose of cocaine and the schedule of reinforcement. These results predict that in a clinical setting any potential therapeutic effect will interact with the cost and availability of cocaine. Therefore, although baclofen may not be effective in treating a cocaine addict in a situation involving unrestricted access to high doses of cocaine, it does appear that it can reduce cocaine-craving in some patients. Our data predict that baclofen may be most beneficial in a situation where access to cocaine is somewhat limited, and may prevent relapse in patients who are motivated to remain drug-free. Other advantages of baclofen are that it has a low abuse liability, does not promote relapse, and is well tolerated by most people. Although the sedative effects that can be associated with higher doses of baclofen could represent a drawback for some people, the development of non-sedative $GABA_B$ agonists may help resolve this issue. Finally, the demonstration that baclofen does not interfere with all appetitive behaviours (i.e. food reinforced responding) is encouraging, as a major obstacle to treatment compliance with medications such as haloperidol is complaints of reduced sensitivity to reinforcement from non-drug related activities (e.g. reduced libido).

In conclusion, $GABA_B$ receptor agonists appear to attenuate cocaine self-administration in rats, which suggests that they may also have potential therapeutic utility in the treatment of cocaine addiction. While the data from these studies are very robust, it

is not yet clear how they will translate into a clinical setting. Although preliminary studies in humans suggest that baclofen may be useful in the treatment of cocaine addiction, several questions remain to be answered, including what effect, if any, $GABA_B$ agonists might have on other aspects of the addictive process. For example, it is not yet clear whether $GABA_B$ agonists decrease drug use by reducing craving, or by dampening the euphorigenic effects of cocaine. Such research may also improve our understanding of the neurochemical mechanisms underlying the drug dependence process, and provide a strong rationale for conducting more systematic studies of the effects of $GABA_B$ receptor agonists in relation to drugs of abuse.

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