BACLOFEN ATTENTUATES NICOTINE DEPENDENCE AND ABSTINENCE SYNDROME IN THE RAT

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Thesis

Presented to the Faculty of

The University of Houston Clear Lake

In Partial Fulfillment

Of the Requirements

For the Degree of

MASTER OF ARTS

THE UNIVERSITY OF HOUSTON CLEAR LAKE

December, 2004

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ABSTRACT

BACLOFEN ATTENUATES NICOTINE AND ABSTINENCE WITHDRAWAL SYNDROME IN THE RAT

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Baclofen is a $GABA_B$ agonist compound that has previously been shown to interfere with the self-administration of several addictive drugs, including nicotine. It was hypothesized that it might reverse some of the behavioral abstinence signs displayed by nicotine-dependent rats during nicotine withdrawal. Previous research has demonstrated that baclofen dose-dependently alleviated ongoing spontaneous nicotine abstinence syndrome in the rat. The present study determined that baclofen could dose-dependently reduce the state of nicotine dependence. This was indicated by a significantly reduced number of nicotine abstinence signs subsequently precipitated by the nicotinic antagonist mecamylamine, at a dose (1 mg/kg s.c.) that has been shown to precipitate a vigorous abstinence syndrome in nicotine dependent but not in non-dependent rats.

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CHAPTER I: INTRODUCTION

Tobacco Associated Problems

The general public has been aware of the hazards of smoking for decades. Ad campaigns and volumes of research have long warned of the dangers associated with sustained tobacco use. There are more smoking related deaths in the U.S. than car accidents, AIDS, alcohol, homicides and illegal drugs combined each year. Smokers currently number 1.2 billion people, worldwide. Of these, tobacco-related disease kills approximately 4 million of them, annually. By 2030, annual smoking related deaths could reach 10 million (Hays & Ebbert, 2003). Despite these facts, smoking remains the number one cause of preventable death in the U.S. (Arraya, & Laranjeira, 1991).

Cigarette smoke has been linked to a wide range of debilitating and sometimes fatal diseases. Smoking increased the risk of lung disease, multiple forms of cancer, heart disease and even blindness in populations of chronic smokers compared to non-smokers (DeBlack, 2003). Researhers found that smoking contributed to half of all tuberculosis deaths in rural and metropolitan India (Gajalakshmi, Peto, Kanaka, & Jha, 2003).

Tobacco-related cancers account for approximately ten percent of all deaths among smokers (Gajalakshmi, Peto, Kanaka, & Jha, 2003). Data show that smokers have

an increased risk of lung cancer (Rise, Strype, & Sutton, 2002), mouth cancer, cancer of the esophagus and cervical cancer (Marteu, Rana, Kubba, 2002) due to smoking compared to non-smokers. Lung cancer and cancer of the upper digestive tract are the most prevalent forms of tobacco related cancer (Gajalakshmi, Peto, Kanaka, & Jha, 2003). Marteu, Rana, and Kubba (2002) found that smoking more than doubled the risk of cervical abnormalities in women.

Smoking is also known to be one of the leading factors for two of the most common causes of blindness, cataracts and macular degeneration. Cigarette smoke is thought to cause damage to the eye by smoke passing directly into the eye or by the heat from the cigarette increasing the temperature of the lens. Research did find that those who successfully quit smoking and remained so had a lower chance of needing cataract surgery compared to those who continued to smoke (DeBlack, 2003). Tobacco smoke affects not only the smoker, but also those nearby. Passive or second hand smoke can be harmful as well. Research found individuals exposed to second hand smoke had an increased risk of smoking-related illness compared to non-smokers (Wall, Johnson, Jacob, & Benowitz, 1988). Smoking adversely affected unborn children in the womb of mothers that smoked during pregnancy (Maughan, Taylor & Taylor, 2001; Niaura, Bock, Lloyd, Brown, Lipsitt, & Buka, 2001). Higher rates of coronary heart disease as well as chronic pulmonary disease occur in smokers compared to non-smokers (Wagena, Zeegers, van Schayck, & Wouters, 2003).

From an economic standpoint, smoking-related costs continue to climb. Direct medical expenditures for illnesses attributed to smoking averaged $50 billion per year

over the past decade. Smoking accounted for 8% to 10% of personal health expenditures in 2002 and public funds paid for roughly 40% of this expense (DeBlack, 2003). Childhood medical expenditures exceed 6 billion annually, with a loss-of-life cost of more than $8.2 billion. Those who quit and maintained cessation experienced lower health care costs compared to those who continued to smoke (Lancet, 1997).

NICOTINE DEPENDENCE

Research has estimated that roughly 80% of all regular smokers have a desire to quit (Schuckit, Helzer, Cottler, Crowley, Nathan, & Woody, 1994). However, evidence shows that only approximately 4% of unaided quit attempts succeed, with the average relapse occurring in the first 8 days (Hughes, Keely & Naud, 2003). Smoking cessation is a challenge for those trying to quit smoking because nicotine, the main psychoactive ingredient in cigarettes, is highly addictive. Nicotine addiction has several components that make smoking cessation difficult.

The initial development of the tobacco habit is most likely due to nicotine's ability to act as a positive reinforcer (Corrigal, 1999). Nicotine produces stimulant effects in the reward pathway of the brain causing reinforcement and addiction without the subjective euphoria associated with other drugs like opiates or cocaine. This reinforcing pathway exists in the mesolimbic dopamine system, including the ventral tegmental area (VTA) and the nucleus accumbens (NAc). Nicotine's activation or depolariztion of ligand gated cation channels on nicotinic acetylcholine receptors (nACH) is responsible for nicotine's initial effects. When these channels are activated in the VTA, this causes the release of dopamine (DA) into the NAc stimulating the brain's reward pathway and thereby reinforcing the effects of nicotine. Self-administration of

nicotine, which is included in the definition of nicotine addiction, can be blocked by the opioid receptor antogonist, naltrexone, suggesting a link between the endogenous opioid system and nicotine reinforcement effects (Nestler, Hyman, & Malenka, 2001).

Additionally, chronic nicotine administration or tobacco use causes a state of physical dependence. Physical dependence is observed when an organism is in a state such that termination of drug administration initiates an abstinence syndrome (Malin, 2001). Signs and symptoms of this syndrome may include physiological changes as well as behavioral abnormalities and psychological distress. Abstinence syndromes are generally considered aversive. Symptoms can include irritability, sleep disturbances and weight gain. Smoking behaves as a negative reinforcer by relieving the aversive features of the abstinence syndrome (Levin, Morgan, Galvez, & Ellison, 1987). Positive reinforcement may play an important role in acquisition and maintenance of smoking, but the abstinence syndrome and the ability of nicotine to act as a negative reinforcer combine to make sustained smoking cessation very difficult.

Human Studies

Chronic nicotine exposure induces physical dependence in humans. This condition is recognized by the expression of a series of physiological and behavioral changes that occur during nicotine withdrawal or after administration of a centrally acting nicotinic receptor antagonist such as mecamylamine. Physiological dependence includes both somatic and motivational changes that occur during withdrawal from nicotine (DiChiara, 2000).

In humans, a large amount of the information available on human nicotine withdrawal was derived from investigating chronic tobacco smokers. However, a small number of studies investigating pure nicotine involved participants experiencing withdrawal from nicotine gum (Hughes, Hatsukami, & Skoog, 1986; West & Russel, 1985). These studies helped describe nicotine withdrawal through a physical dependence paradigm in humans. Despite the difference in pharmacokinetics of nicotine delivered by gum instead of cigarette smoke, evidence comparing abstinence syndrome between the two show differences in the number of signs rather than the type of signs. Withdrawal from nicotine gum and smokeless tobacco generally exhibit milder signs compared to withdrawal from tobacco smoke (Hughes, 1992; Hughes, Higgins, & Hatsukami, 1990; Snyder, Davis, & Henningfield, 1989).

Human nicotine withdrawal symptoms can usually be observed beginning at 6-12 hours after nicotine cessation, peak in 1-3 days and last for 3-4 weeks (Hughes, 1992; Hughes, Higgins, & Hatsukami, 1990; Shiffmann & Jarvik, 1976). Withdrawal is marked by physiological changes including a decrease in heart rate and an increase in body weight. Behavioral changes consist of restlessness and nocturnal awakening; mood changes including anxiety, depressed mood and irritability; motivational changes including craving and hunger, with craving and hunger lasting the longest of the withdrawal signs (Hughes et al.). Craving can last almost indefinitely and is considered a factor for relapse (DiChiara, 2000). Hughes and Hatsukami (1986) found no relationship between more withdrawal discomfort and a lower rate of smoking cessation.

Animal Models of Physical Dependence

Early research provided clues that termination of chronic nicotine exposure in the rat resulted in altered behavioral and physiological states. Benwell and Balfour (1979) found that withdrawal from chronic subcutaneous nicotine injections resulted in an increase in serum corticosterone, suggesting a stress response. Withdrawal from subcutaneous nicotine injections also showed stimulus generalization to an anxiogenic pentylenetetrazol (PTZ) cue (Harris, Emmett-Oglesby, Robinson, & Lal, 1986). Withdrawal from intraperitoneal nicotine injections altered preferences for light/dark in mice (Costall, Kelly, Naylor, & Onaivi, 1989). Nicotine withdrawal disrupted ongoing patterns of appetitive operant responding in rats trained on operant schedules (Caroll, Lac, Asencio, & Keenan, 1989; Corrigall, Herling, & Coen, 1989; Ford, & Balster, 1976). Despite a growing number of studies, Emmett-Oglesby et al. (1990) stated there were few robust and well accepted designs for evaluating nicotine withdrawal and dependence in the rat. Around this same time period, a research group at the University of Houston-Clear Lake (UHCL) began an attempt to discover such a model.

UHCL Model of Physical Dependence

Rodent models of nicotine abstinence have the potential to identify the mechanism of nicotine dependence and to evaluate proposed intervention that may aid in smoking cessation (Malin, 1992). While the few rat models available involve changes in conditioned responses or changes in body weight or food consumption to measure

withdrawal effects, the UHCL model primarily relies on the frequency of spontaneous behavioral signs observed in nicotine-dependent rats during nicotine withdrawal. This model is similar to rat models used in opiate abstinence syndrome and analogous to quantitative measures of nicotine abstinence in humans (Gianutsos, Drawbaugh, Hynes, & Lal, 1975).

From pilot studies, it was determined nicotine abstinence signs in the rat, including teeth-chatters, chews, abdominal writhes, ptosis, gasps, tremors and wet shakes were similar to those observed in opiate abstinence (Malin et al., 1992). This led to the development of a standard checklist of abstinence signs to be used in quantifying the intensity of the nicotine abstinence syndrome.

To determine if the behavioral signs on the checklist were a valid reflection of nicotine withdrawal in nicotine-dependent rats and that they specifically reflect chronic over-stimulation of nicotine cholinergic receptors followed by reduced stimulation, a series of validation studies was carried out. It was hypothesized that nicotine-abstinent rats should have more signs a day after nicotine withdrawal than during the pre-nicotine baseline, the nicotine administration period and a subsequent recovery period. Second, it was hypothesized that rats previously infused with nicotine should have more abstinence signs compared to control rats previously infused with saline. Third, the number of abstinence signs should increase at higher nicotine infusion rates. Finally, a small dose of nicotine should attenuate abstinence signs. Locomotor activity and weight gain were also monitored for any changes during withdrawal to further confirm the presence of an abstinence syndrome (Malin et al., 1992).

Research found that there were significantly more abstinence signs following termination of drug infusion than before infusion, during infusion or after a subsequent recovery period. Nicotine infused rats had more signs than saline infused rats and rats with higher rates of infusion produced more signs (Malin et al., 1992). Nicotine induced withdrawal was prevented by co-infusion of the nicotinic receptor antagonist mecamylamine (Levin, Morgan, Galvez, & Ellison, 1987). Nicotine abstinence was also found to be promptly and potently reversed by injection of nicotine. Weight gain showed an abrupt increase on the first but not the second day of nicotine abstinence and locomotor activity decreased dose-dependently in nicotine infused rats compared to the saline group (Malin et al.).

Precipitated abstinence is useful in drug dependence research designs due to the fact that a large number of abstinence signs are compressed in a short time frame (around 30 min). This continuous infusion model has many practical advantages. The method is fast and simple, with no major surgery, no complicated equipment or prolonged training of the subject. It is therefore well suited for repeated use in evaluating mechanisms of nicotine dependence or screening potential smoking cessation therapies (Malin, 2001). A number of independent research groups have confirmed the validity of this continuous infusion model (Carboni et al., 2000; Epping-Jordan et al., 1998; Hildebrand, Hartmann, Popp, & Bomhard, 1997; Watkins et al., 2000).

CURRENT TREATMENTS FOR NICOTINE DEPENDENCE

Pharmacological therapies for smoking cessation have gained in popularity among current treatments available (Shiffman, Dresler, & Rohay, 2004). Pharmacological therapies include non-nicotine treatments, such as clonidine or the antidepressants Bupropion or Nortriptyline and Nicotine Replacement Therapies (NRTs), including gum, transdermal patch, nasal spray, oral inhaler and sublingual tablet. Nicotine gum and the nicotine patch are the most common treatments possibly due to their mild side effects, over-the-counter availability, and successful marketing (Shiffman, Dresler, & Rohay).

Nicotine Replacement Therapy

Existing literature points out that compared to placebo, NRT is an efficacious aid to smoking cessation when used alone or in conjunction with behavioral support. A meta-analysis of current randomized controlled trials (RCTs) found global NRT abstinence rates were higher at a 6 month follow-up compared to placebo (Gold, Rubey, & Harvey, 2002; Martinez-Raga, Keaney, Sutherland, Perez-Galvez & Strang, 2003). Research (Gold, Rubey, & Harvey) showed that increased dosage increased efficacy and

a combination of two NRTs increased abstinence rates by an additional 5-10% over the control group. Mild side effects, including hiccups and sore mouth, throat and jaw, were most common for nicotine gum, while side effects for the transdermal patch resulted in mild skin irritation in a small number of the population evaluated (Foulds, 1993).

Bupropion

Although the specific mechanism of bupropion efficacy is not completely understood, bupropion is thought to be a weak inhibitor of the neuronal reuptake of noradrenaline (NA) and dopamine (DA) with a small affect on the reuptake of 5-HT (Martinez-Raga, Keaney, Sutherland, Perez-Galvez, & Strang, 2003). Blocking the reuptake of DA and NA, bupropion increased the amount of DA and NA in the reward center of the brain, replenishing the neurotransmitters depleted during nicotine withdrawal. Bupropion may also act as a nicotinic receptor antagonist, therefore blocking some of the reinforcing effects of nicotine when tobacco is used during treatment (Hays & Ebbert, 2003).

Several studies showed that Bupropion was dose dependently efficacious in the treatment of nicotine abstinence syndrome (Foulds, 1993; Hays & Ebbert, 2003 Martinez-Raga et al., 2003). Insomnia and dry mouth were the most commonly reported adverse effects and only a small percentage of patients discontinued therapy because of these effects (Hays & Ebbert, 2003).

The Need for New Approaches

Nicotine replacement therapy and bupropion have helped many smokers to quit. However, no treatment has resulted in long-term quit rates much higher that 30% (Gold, Rubey & Harvey, 2002). Medications affecting the transmitter GABA and its receptors have shown promise in combating addiction to several habit-forming drugs (Cousins, Roberts & de Wit, 2002). It was the purpose of this thesis to evaluate the potential of a GABAergic medication for combating nicotine dependence.

GABA AND ITS RECEPTORS

Gamma-aminobutyric acid (GABA) is an amino acid neurotransmitter present in a high proportion of inhibitory neurons of the central nervous system (CNS). Inhibitory neurons are neurons that, when activated, move the cell membrane potential away from action potential threshold, thus preventing electrical activation and neurons or interfering with transmitter release. GABA is found in highest concentration within the projection neurons and inter-neurons of the brain. Although once thought to be only inhibitory, GABA can produce an excitatory response under certain circumstances. A number of neuropsychiatric disorders are affected by altered GABA function. Epilepsy, Huntington disease, tardive dyskinesia, alcoholism, and sleep disorders are just a few of these disorders (Cooper, Floyd, & Roth, 2003). This suggests the crucial nature of GABA's functional roles.

Cellular GABA is manufactured from glucose and pyruvate in the neuron by the GABA shunt, a specific metabolic pathway. GABA is the only neurotransmitter in the brain that is synthesized solely by those neurons in the brain that specifically need it (Cooper, Floyd, & Roth, 2003). GABA, like most neurotransmitters, is packaged into seminal vesicles in the pre-synaptic terminal for eventual release into the synaptic cleft. After its release, several membrane GABA transporters remove GABA from the cleft. When an action potential is generated in a GABAergic neuron, neurotransmitter

When an action potential is generated in a GABAergic neuron, neurotransmitter filled vesicles travel to the pre-synaptic terminal where they fuse with the neuronal cell membrane. GABA is then released into the synaptic cleft. Gabapentin, an anticonvulsant drug, works by inhibition of this release process. The GABA molecules released into the cleft diffuse across the synapse, where they bind to and activate postsynaptic receptors, causing an inhibitory response in the neuron. GABA transporters and glial cells bind any free GABA remaining in the synaptic cleft and return it to the pre-synaptic terminal where the neurotransmitter is stored for future use (Cooper, Floyd, & Roth).

GABA receptors are divided into two distinct groups. The ionotropic $GABA_A$ receptor is a protein complex consisting of a GABA binding site coupled with an ion channel. $GABA_A$ receptors are identified by their ability to be positively modulated by the benzodiazepines and other anti-anxiety or tranquilizing drugs. $GABA_A$ receptors are blocked by the competitive antagonist bicuculline. Bicuculline is classified as a convulsant. Conversely, the $GABA_B$ receptor is a metabotropic, G protein-coupled structure. This means the ligand-binding domain of the $GABA_B$ receptor is not directly associated with an ion channel effector. $GABA_B$ receptors are resistant to bicuculline and are activated by baclofen, a competitive agonist, and inhibited by phaclofen, a competitive antagonist (Cooper, Floyd, & Roth, 2003).

The GABA receptor itself is a polyprotic or multiple protein structure consisting of several subunits that span the thickness of the neuronal membrane. $GABA_A$ receptors all have the same basic configuration. The structure includes a $GABA_A$ receptor attached to a pentameric protein ion channel. The five subunits conform together to make a water

filled pore within the neuronal membrane that allows ion flow into the cell. Specifically the structure consists of a long N-terminal extra-cellular domain, four trans-membrane spanning segments (M1-M4), an intracellular sequence between M3 and M4, and a short extracellular c-terminal loop. When a ligand binds to the $GABA_A$ site, a conformational change occurs, which opens the pore and Cl^- travels down its concentration gradient, hyperpolarizing the cell. $GABA_A$ receptor pharmacology originated with the discovery that the convulsant bicuculline antagonizes certain inhibitory actions of GABA.

By studying how various drugs interact with the $GABA_A$ receptors, researchers have gained a better understanding of how the $GABA_A$ receptor functions. $GABA_A$ receptors are recognized by their ability to bind benzodiazepines and other sedative drugs. This class of drug acts by increasing the receptor's affinity for GABA, which increases the frequency of channel openings. Barbiturates, ethanol and other related drugs increase the duration of channel openings, without affecting opening frequency. This mechanism maximizes the opportunity for Cl^- to travel down its concentration gradient. Barbiturates, benzodiazepines, and ethanol all have related actions on the same receptor substrate. Therefore, the combined use of these agents could lead to clinical complications. Overstimulating $GABA_A$ receptors could cause the central nervous system paralysis and consequent fatal respiratory depression. There is a real danger of overdose with $GABA_A$ agonists. Development of cross-dependence between different GABA agonist has been observed (Cooper, Floyd, & Roth, 2003).

$GABA_B$ receptors belong to the G-protein coupled receptor class of protein receptors. G-protein complexes operate by modulating the synthesis of effector molecules or second messengers, such as cAMP. The $GABA_B$ protein structure consists of seven transmembrane domains that make up the receptor complex. $GABA_B$ receptors were initially differentiated from $GABA_A$ receptors by their inability to bind bicuculline. Shortly after this discovery, the muscle relaxant baclofen was found to be an agonist at $GABA_B$ receptors. Unlike $GABA_A$, $GABA_B$ receptors are expressed on both presynaptic and postsynaptic membranes.

In contrast to $GABA_A$ receptors, postsynaptic $GABA_B$ receptors produce a slower and longer lasting inhibitory response. When activated, these receptors work by opening K^+ channels, decreasing Ca^{2+} conductance, and inhibiting adenyl cyclase. Because inhibitory postsynaptic potentials (IPSPs) mediated by $GABA_B$ receptors require a stronger stimulation of presynaptic neurons with longer duration and higher frequency, it is believed that some $GABA_B$ receptors may be extra-synaptic. This would explain the need for a stronger and more prolonged response in order for GABA released to travel to receptors outside the synaptic cleft (Cooper, Floyd, & Roth, 2003).

$GABA_B$ receptors can function as autoreceptors and inhibit further GABA release through inhibitory feedback. Other $GABA_B$ receptors are located on excitatory terminals. The activation of these terminals inhibits the release of the excitatory neurotransmitter glutamate. The release of GABA spills onto neighboring excitatory neurons, further preventing glutamate release. During repetitive stimulation, $GABA_B$

activation can also inhibit the release of the following neurotransmitters: norepinephrine, dopamine, serotonin, and substance P. The mechanism is believed to operate through inhibition of Ca^{2+} channels, which in turn decreases the amount Ca^{2+} influx into the terminal that triggers transmitter release. The muscle relaxant Baclofen is the only major drug in clinical use that interacts with $GABA_B$ receptors. New applications for $GABA_B$ receptor agonists are currently being investigated for the treatment of seizure disorders, anxiety, and depression. $GABA_B$ antagonists also show promise in cognition enhancement (Cooper, Floyd, & Roth).

BACLOFEN

An increasing amount of literature suggests that this $GABA_B$ receptor agonist can promote abstinence and reduce drug dependence and addiction (Cousins, Roberts, & de Wit, 2002). In multiple experimental animal models, baclofen appeared to reduce the reinforcing effects of abused drugs. A research team at Ciba-Geigy first described in a 1978 patent application baclofen's ability to attenuate naloxone-induced withdrawal in morphine dependent monkeys and reduce morphine self-administration in rats (Cousins, Roberts, & de Wit). Since then, a number of preclinical and clinical studies offer support to the idea that several GABAergic drugs, particularly baclofen, may increase abstinence from a variety of abused drugs. Recent studies have evaluated $GABA_B$ receptor agonists on reducing the reinforcing effects of cocaine, nicotine, heroin, and alcohol. Several researchers (Campbell, Lac & Carroll, 1999; Roberts, Andrews & Vickers, 1996; Shoaib, Swanner, Beyer, Goldberg & Schindler, 1998) evaluated the effects of baclofen and its role in reducing cocaine self-administration. Findings show that baclofen dose-dependently reduces self-administration of cocaine under fixed ratio reinforcement schedules compared to control group. However, the effects depend on the dose of cocaine self-administered. Baclofen (2.5, 5.0, 10.0 mg/kg, IP) reduced cocaine reinforced responding under fixed ratio schedules at low doses of cocaine (0.20 -0.66 mg/kg per injection) (Shoaib et al.; Campbell et al.; Roberts et al.). However, Roberts et

al. (1996) found that when using higher doses of cocaine (1.5 mg/kg per injection), baclofen (2.5 mg/kg) had no effect on cocaine self-administration. Further studies have found evidence that supports the findings that baclofen can reduce the reinforcing effects of cocaine, but this effect is surmountable with higher doses of cocaine (Brebner et al., 2000a).

One issue in interpreting drug self-administration reduction is the possibility that this reduction is due to non-specific factors such as sedation or motor impairment, rather than a specific reduction in reinforcing efficacy. Baclofen in the dose range of 2.5 – 5.0 mg/kg reduced locomotor activity and reduced response rate in certain operant tasks (Agmo & Giordano, 1985; Grech & Balster, 1993; Grech & Balster, 1997; McManus & Greenshaw, 1991; Paredes & Agmo, 1995; Zarrindast et al., 1989). Several studies have compared the effects of baclofen on cocaine and food-reinforced responding to address these concerns. In separate experiments, Roberts et al. (1996) and Brebner et al. (1999) provide evidence that the effects of Baclofen on food-reinforced responding were smaller than its effects on cocaine-reinforced responding. Additionally, Shoaib et al. (1998) conducted research that showed baclofen had a greater effect on cocaine self-administration than on food-reinforced responding.

The effects of baclofen on self-administration of ethanol and heroin have also been investigated. Daoust et al. (1987) provide evidence that low doses of baclofen selectively reduced ethanol intake. Colombo et al. (2000) found that over a range of doses

(2.5, 5.0, and 10.0 mg/kg, IP), baclofen dose-dependently reduced ethanol intake in rats. With regards to heroin, Xi and Stein (1999) investigated self-administration of heroin alone, baclofen alone, and heroin plus baclofen. Baclofen itself failed to maintain self-administration, but dose-dependently reduced heroin self-administration.

It is hypothesized that $GABA_B$ receptor agonists lessen the reinforcing effects of cocaine, nicotine, heroin and ethanol through dopamine transmission modulation. Drugs of abuse are believed to produce reinforcement by increasing the levels of DA in the nucleus accumbens, known as the brain's reward center. GABA neurons in the mesolimbic dopamine system are known to dampen dopamine neuron activity via inhibitory $GABA_B$ receptors (Cousins et al., 2002). Xi and Stein (1999) found that baclofen dose-dependently reduced nucleus accumbens DA release induced by heroin. These findings provide a plausible mechanism for the effects of baclofen on drug self-adminstration, which is a measure of psychological dependence. This raises the question does baclofen affect physical dependence in the form of nicotine withdrawal syndrome.

THE EFFECT OF BACLOFEN ON NICOTINE ABSTINENCE SYNDROME

Baclofen is a GABA-B agonist compound that has previously been shown to interfere with the self-administration of several addictive drugs, including nicotine (Corrigall, Coen, Adamson, Chow & Zhang, 2000; Fattore, Cossu, Martellotta, & Fratta, 2002). It was hypothesized that it might reverse some of the behavioral abstinence signs displayed by nicotine-dependent rats during nicotine withdrawal. Previous unpublished research in the UHCL laboratory has demonstrated that baclofen dose-dependently alleviated ongoing nicotine abstinence syndrome in the rat. The present study determined whether baclofen could dose-dependently reduce the state of nicotine dependence, as indicated by the number of nicotine abstinence signs subsequently precipitated by the nicotinic antagonist mecamylamine. The study employed a dose of mecamylamine (1 mg/kg s.c.) that has been shown to precipitate a vigorous abstinence syndrome in nicotine dependent but not in non-dependent rats (Malin et al., 1994).

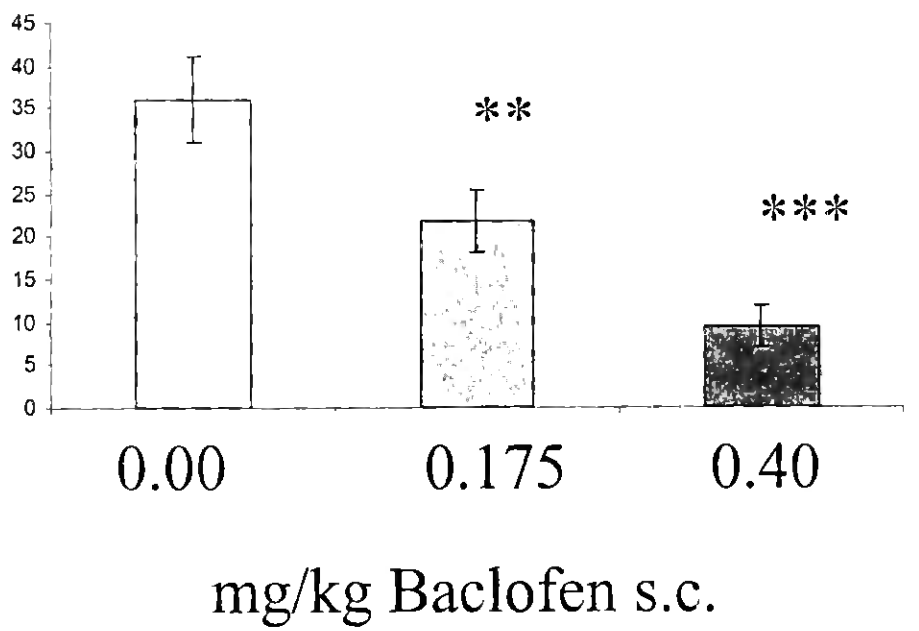
Methods

Under halothane anesthesia, 21 male 300-400g Sprague-Dawley rats were implanted sub-cutaneously in the scapular region with Alzet 2ML1 osmotic minipumps filled with a nicotine bitartrate dissolved in saline. They were rendered nicotine-dependent by seven days subcutaneous infusion of 9 mg/kg/day nicotine bitartrate (3.15mg/kg/day nicotine expressed as the base). Groups of seven rats were then injected respectively with either 0.175 or 0.400 mg/kg baclofen in saline or with saline alone. Solutions were coded to prevent observer bias. The higher dose was chosen because it reduced ongoing nicotine abstinence syndrome in the previous study without reducing locomotor activity. The lower dose was chosen on the basis of small pilot experiments that showed a reduction in nicotine abstinence syndrome. Ninety minutes after injection, each rat was injected with 1mg/kg mecamylamine HCl and was observed for thirty minutes under "blind" conditions for nicotine abstinence signs, utilizing a standard checklist (Malin et al., 1992; Malin et al., 2001). The rats were placed in a clear lexan container for viewing, with observers positioned at each corner to ensure all signs were counted. The categories of signs included gasps and writhes, teeth chattering and vacuous chewing, shakes and tremors, ptosis (drooping eyelid) and miscellaneous less-

frequent signs, such as hind-foot scratches, seminal ejaculations and backward locomotion. In the case of prolonged continuous teeth chattering, chewing or tremors, these signs were counted no more than once every fifteen seconds. Ptosis was counted no more than once a minute.

Results

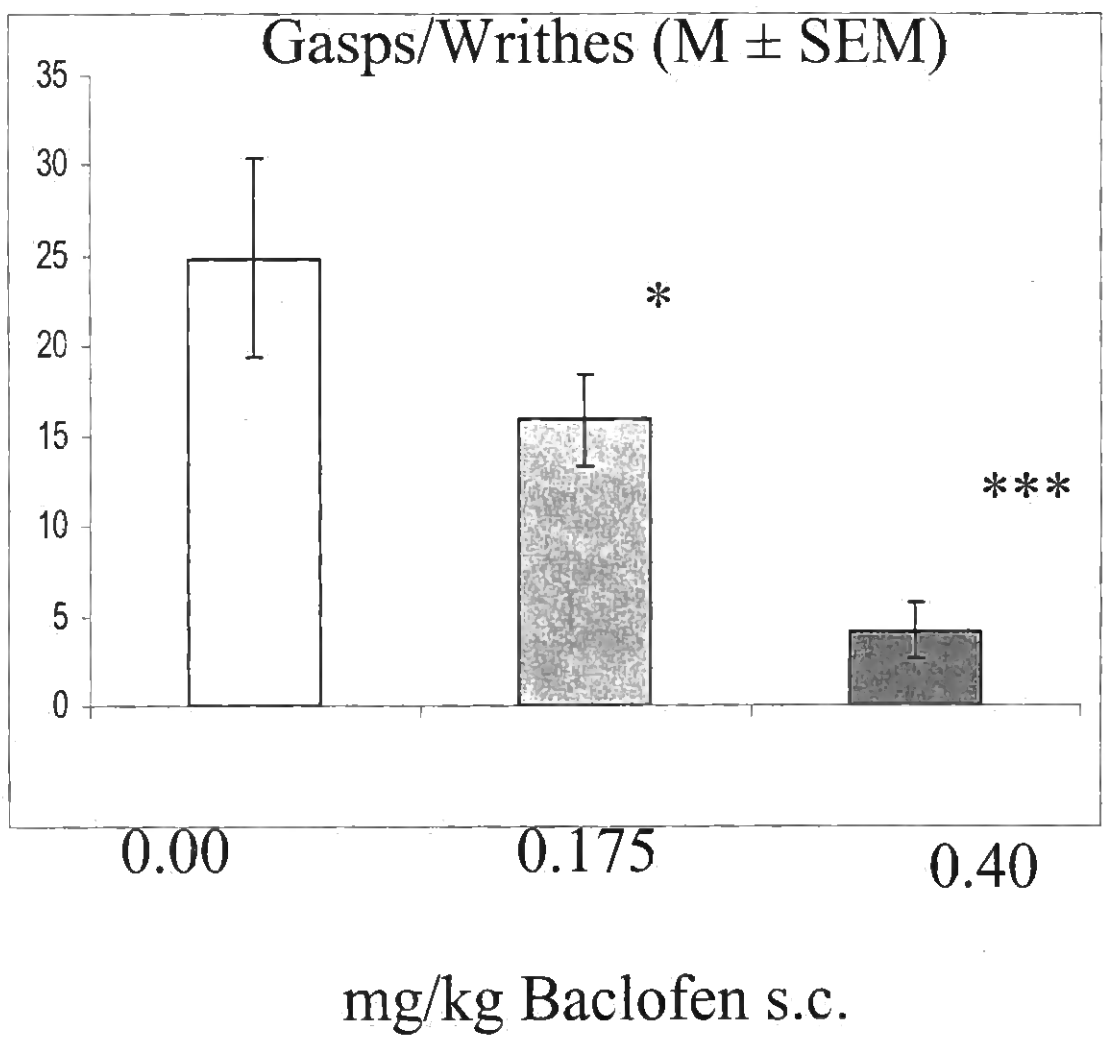
As shown in Figure 1, Baclofen injection resulted in a dose-dependent reduction of subsequent mecamylamine-precipitated nicotine overall abstinence signs (cumulated across all categories). One-way analysis of variance demonstrated a significant difference among treatment groups, $F(2,18) = 11.97$, $p < 0.001$. There was also a significant negative linear trend of overall signs as a function of baclofen dose, $F(1,18) = 23.58$, $p < 0.001$. Post-hoc pairwise comparisons (Fisher's LSD test) demonstrated that the 0.4 mg/kg dose group had significantly fewer overall abstinence signs than the saline control group, $p < 0.001$, and 0.175mg/kg group, $p = 0.018$. Also, the 0.175 mg/kg group differed significantly from the saline controls, $p = 0.008$.



** **P** = 0.009 vs 0.00 group. *** **p** < 0.001 vs. 0.00 group, **p** = 0.019 vs. 0.175 group

Fig. 1 Baclofen pretreatment dose-dependently reduced subsequent mecamylamine-Precipitated overall abstinence signs (cumulated across all categories) in nicotine dependent rats.
n = 7 in each dose group.

The predominant mecamylamine precipitated abstinence sign was gasps/writhes. There was a significant effect of baclofen on gasps/writhes (Fig. 2). ANOVA of gasps/writhes revealed a significant difference among the groups, $F(2,18) = 8.29$, $p = 0.003$. There was also a significant negative linear trend of gasps/writhes as a function of baclofen dose $F(1,18) = 16.57$, $p < 0.001$. Post-hoc analysis (Fisher's LSD test) revealed significant differences between the 0.4 mg/kg dose group and the saline group, $p = 0.001$, as well as the 0.175 mg/kg group, $p = 0.017$. The difference between the 0.0175 mg/kg group and the saline group was also significant, $p = 0.047$. ANOVA revealed no significant affect of dose on chews/teeth, $F(2,18) = 1.22$, $p = .318$; ptosis, $F(2,18) = .944$, $p = 0.408$; shakes/tremors, $F(2,18) = 2.40$, $p = 0.119$; and miscellaneous signs, $F(2,18) = 1.97$, $p = 0.169$.



* P = 0.047 vs 0.000 group. *** p < 0.001 vs.0.00 group, p = 0.017 vs. 0.175 group

Fig. 2 Baclofen pretreatment dose-dependently reduced subsequent mecamylamine precipitated gasps/writhes in nicotine dependent rats. n = 7 in each dose group

However, as shown in Fig. 3, the high dose group had fewer instances of each category of sign than the saline control group, except shakes/tremors.

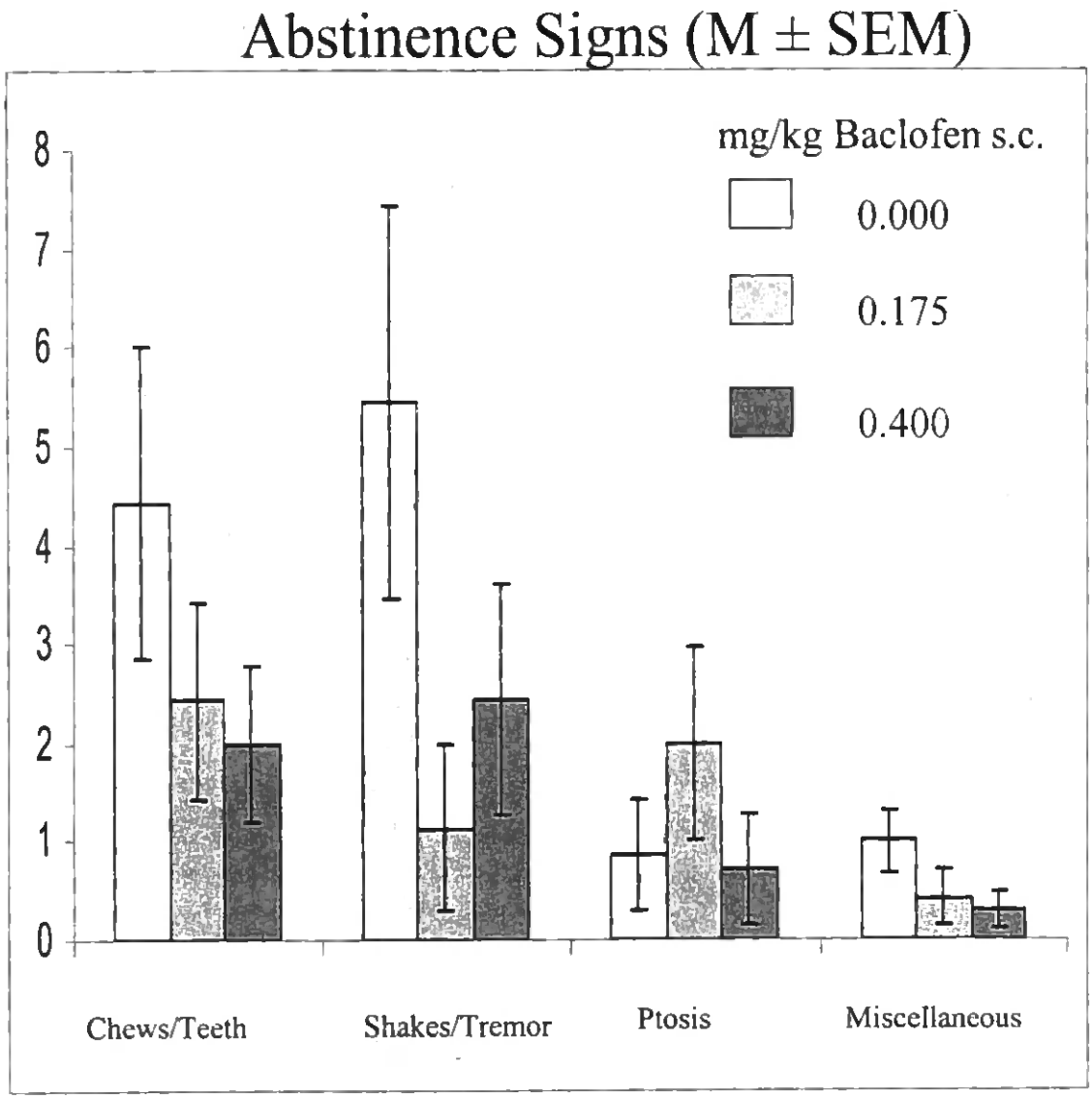


Fig. 3 **Occurrences of four less frequent nicotine abstinence signs in nicotine dependent rats injected with saline or baclofen (0.175 or 0.4 mg/kg s.c.) and challenged 90 minutes later with 1 mg/kg mecamylamine HCl. n = 7 in each dose group.**

Discussion

Baclofen dose-dependently attenuated nicotine dependence; it markedly reduced the ability of a subsequently administered nicotinic antagonist to precipitate a nicotine abstinence syndrome. The predominant mecamylamine-precipitated abstinence sign was gasps/writhes. There was a significant dose-related reduction of this category of sign. There was a non-significant trend in all other categories of abstinence sign for the higher dose group to have fewer signs than the saline-injected controls.

This result reinforced and extended the earlier unpublished finding in the UHCL lab, that subcutaneous baclofen injection dose-dependently reduced an abstinence syndrome that had been ongoing for 21.5 hours. It is not likely that either effect can be attributed to non-specific behavioral suppression by this GABA agonist drug. Data in the previous report clearly showed that the higher (0.4 mg/kg) dose does not reduce locomotor activity in nicotine-abstinent rats.

The results thus far may have some interesting implications for the potential use of GABA-B agonists in smoking cessation. They raise the possibility that administration of such compounds could be initiated before the target quit date to reduce nicotine dependence and prevent the full onset of nicotine withdrawal symptoms. Conversely, the previous data suggest that such medication could be continued after the target quit date to suppress withdrawal symptoms.

Drug dependence generally involves the reinforcing actions of drugs as well as physical dependence manifested by abstinence signs following drug withdrawal. Baclofen had already been shown to interfere with the reinforcing actions of nicotine (Corrigall, et al., 2000; Fattore, et al., 2002) and our results indicate that it also interferes both with nicotine dependence and subsequent nicotine abstinence syndrome. These triple-barreled actions might make $GABA_B$ compounds effective agents for smoking cessation during several different phases of the cessation process: pre-quitting, the withdrawal period and a longer term maintenance period.

Encouragingly, some $GABA_B$ compounds may have a fairly benign side-effect profile. $GABA_B$ compounds are less addictive and do not have the respiratory depression risk associated with $GABA_A$ compounds. The prototype compound, Baclofen, is an already-approved drug, as a muscle relaxant. An occasional side effect of oral baclofen prescribed for spasticity is a withdrawal syndrome upon abrupt discontinuation (Terrence & Fromm, 1981). This is more common with intrathecal administration, which is administered directly into the cerebrospinal fluid. It is important in this context that we have obtained the anti-nicotine dependence effects with only one-fifth to one-tenth the usual dose for muscle relaxant/anti-spasticity effects in the rat. This raises the possibility that analogously low doses might be useful for smoking cessation in human beings, thus reducing concerns about the need to carefully taper off the dosage of medication.

Universally, quit rates average between 15%-30% for smokers, with a combination of bupropion and cognitive behavioral therapy (CBT) being the most effective treatment (Gold, Rubey, & Harvey, 2002). The low success rate points to a need for new more effective treatments. Baclofen with its ability to block nicotine's rewarding effects and to diminish the adverse withdrawal signs associated with nicotine abstinence could be useful in maintaining sustained quit rates among smokers. Evidence shows that pharmacological treatments like baclofen are most efficacious when combined with cognitive behavioral therapy. Carroll (1997) found that CBT's like motivational enhancement, behavior modification, relapse prevention, and weight and nutritional counseling combined with pharmacotherapy increase quit rates by an additional 10%-15% over those not participating in therapy. This evidence points to the possibility that baclofen coupled with a CBT program could be efficacious in helping smokers quit. Future research could evaluate the effectiveness of combining baclofen with cognitive behavioral therapy.

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