Published in final edited form as:

Pharmacol Biochem Behav. 2013 May ; 106: 27–32. doi:10.1016/j.pbb.2013.03.001.

Differential development of tolerance to the functional and behavioral effects of repeated baclofen treatment in rats

T.J.R. Beveridge^{a,*}, H.R. Smith^a, and L.J. Porrino^a

^aDepartment of Physiology and Pharmacology, Wake Forest School of Medicine, Winston Salem, NC 27157, United States

Abstract

Baclofen, a gamma-aminobutyric acid (GABA)_B receptor agonist, has been used clinically to treat muscle spasticity, rigidity and pain. More recently, interest in the use of baclofen as an addiction medicine has grown, with promising preclinical cocaine and amphetamine data and demonstrated clinical benefit from alcohol and nicotine studies. Few preclinical investigations, however, have utilized chronic dosing of baclofen, which is important given that tolerance can occur to many of its effects. Thus the question of whether chronic treatment of baclofen maintains the efficacy of acute doses is imperative. The neural substrates that underlie the effects of baclofen, particularly those after chronic treatment, are also not known. In the present study, therefore, rats were treated with either a) vehicle, b) acute baclofen (5 mg/kg) or c) chronic baclofen (5 mg/kg, t.i.d. for 5 days). The effects of acute and chronic baclofen administration, compared to vehicle, were assessed using locomotor activity and changes in brain glucose metabolism (a measure of functional brain activity). Acute baclofen significantly reduced locomotor activity (horizontal and total distance traveled), while chronic baclofen failed to affect locomotor activity. Acute baclofen resulted in significantly lower rates of local cerebral glucose utilization throughout many areas of the brain, including the prefrontal cortex, caudate putamen, septum and hippocampus. The majority of these functional effects, with the exception of the caudate putamen and septum, were absent in animals chronically treated with baclofen. Despite the tolerance to the locomotor and functional effects of baclofen following repeated treatment, these persistent effects on functional activity in the caudate putamen and septum may provide insights into the way in which baclofen alters the reinforcing effects of abused substances such as cocaine, alcohol, and methamphetamine both in humans and animal models.

Keywords

2-[¹⁴C]-deoxyglucose; functional activity; locomotor activity; chronic baclofen; tolerance

1. Introduction

γ-aminobutyric acid (GABA), the predominant inhibitory neurotransmitter in the brain, achieves its effects through two main receptor subtypes, the ionotropic GABA_A and

© 2013 Elsevier Inc. All rights reserved.

*Corresponding author at Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston Salem, NC 27157, United States. Tel + 1 336 716 8669; fax + 1 336 716 8501., tbeverid@wakehealth.edu.
hsmith@wakehealth.edu; lporrino@wakehealth.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

metabotropic GABA_B. While the GABA_A receptor is a pentameric chloride ion channel, the GABA_B receptor is an inhibitory G-protein coupled receptor (Gi/Go) comprised of the GABA_B R1A/R1B and GABA_B R2 subunits (Möhler et al., 2001). GABA_B receptors can serve as pre-synaptic autoreceptors or heteroreceptors controlling neurotransmitter release, or as post-synaptic receptors, dampening the excitability of the post-synaptic neuron (Bowery and Enna, 2000). This overall inhibitory effect of GABA_B receptor activation results from the opening of inwardly rectifying potassium channels and the inactivation of voltage-gated sodium channels (Bowery and Enna, 2000). GABA_B receptors are widely distributed throughout the brain, with dense populations in the amygdala, hippocampus, thalamus and cortex (Bischoff et al., 1999). Impaired GABAergic function has been implicated in a range of conditions such as muscle spasticity, epilepsy, anxiety-disorder and Huntington's Disease (Wong et al., 2003).

Baclofen, a selective agonist at the GABA_B receptor, has been used clinically to treat muscle spasticity and rigidity (Montane et al., 2004) and has shown the potential to treat pain both in animal models (Brusberg et al., 2009; Hama et al., 2012) and clinically (Slonimski et al., 2004). In addition, there has been recent interest recently in the use of baclofen as an addiction medicine. In humans, baclofen has been shown to reduce the number of cigarettes smoked per day (Franklin et al., 2009) and reduce daily alcohol intake in alcohol-dependent individuals (Addolorato et al., 2011). Preclinical data also indicate that baclofen may be useful for treating psychostimulant addiction (Backes and Hemby, 2008; Brebner et al., 2000, 2005; Oleson et al., 2011). Studies have shown that baclofen reduces dopaminergic activity in the nucleus accumbens (Brebner et al., 2005; Fadda et al., 2003; Fu et al., 2012), an important brain region involved in the reinforcing effects of drugs, and it has been shown to reduce drug-motivated behaviors preclinically and clinically. Additionally, acute baclofen dose-dependently reduced responding for d-amphetamine (Brebner et al., 2005) and cocaine (Backes and Hemby, 2008; Brebner et al., 2005) on both fixed- and progressive-ratio schedules.

One of the common limitations in these preclinical studies, however, is the use of a single acute dose of baclofen rather than repeated treatment. This is problematic since baclofen is usually administered chronically in the clinical setting. Thus the question of whether chronic treatment maintains the efficacy of an acute dose in reducing drug-reinforced behavior is important. Indeed, it has been well recognized that tolerance to the effects of baclofen occurs. Multiple clinical and preclinical studies have reported reductions in the effects of baclofen following chronic treatment and the requirement of increasing doses to maintain its therapeutic effect, i.e. the development of tolerance (Dones and Broggi, 2010; Heetla et al., 2009; Lehman et al., 2003; Soni et al., 2003). A separate question is what are the underlying neural substrates of the effects of baclofen? Can we identify the brain regions involved in the functional effects of baclofen, and does activity change following repeated treatment? The answers to these questions are important since they will help us better understand how baclofen acts in the brain and how useful it and similar compounds may be as substance abuse medications. Additionally, given the widespread distribution of GABA_B receptors and the variety of behavioral effects of baclofen, whole brain neuroimaging methods may be particularly well-suited for identifying the neural substrates of its actions. The present study was designed, therefore, to determine the influence of the acute administration of baclofen on functional activity within the brain, using the 2-[¹⁴C]-deoxyglucose method, and assess whether tolerance to these effects occurred with repeated baclofen treatment. In order to have an additional measure by which to determine the degree of any behavioral tolerance that developed, we also measured locomotor activity in the same animals. Acute treatment with GABA_B agonists is well known to produce decreases in locomotor activity (Frankowska et al., 1999; Liang et al., 2006; Paredes and Almo, 1989) and thus behavioral measures can provide an index of the development of tolerance.

2. Methods

2.1. Animals

All animal procedures were performed in accordance with protocols approved by Wake Forest University School of Medicine Animal Care and Use Committee and were consistent with the NIH *Guide for the Care and Use of Laboratory Animals*. Male Sprague-Dawley rats (280-300 g) were obtained from Harlan Industries (Indianapolis, IN). All animals were maintained in a temperature-and humidity-controlled vivarium with a 12-h light-dark cycle (lights on at 07:00). Food and water were available *ad libitum*.

Surgical procedures for the 2-[¹⁴C]-DG studies followed those described by Torres-Reveron et al. (2006) and were carried out 24 hours before the 2DG procedure to allow for anesthetic clearance and recovery. Briefly, rats were lightly anesthetized with a mixture of isoflurane and nitrous oxide and catheters were implanted in the jugular vein. Catheters were filled with heparinized saline and run subcutaneously to exit at the nape of the neck. Surgery lasted no longer than 45 min, following which animals were returned to their home cages for recovery. Animals were food-deprived for 8 hours (overnight) before the initiation of the 2DG procedure.

2.2. Drug Treatment

Baclofen (Sigma Aldrich, St. Louis, MO) was dissolved in saline and injected via the intraperitoneal (i.p.) route in a 1 ml/kg volume. Animals were administered either baclofen or vehicle 3 times daily (*ter in die* (t.i.d.), 5 mg/kg) for 5 consecutive days, followed by an additional injection on the morning of day 6. The dose of baclofen was chosen based on data from previous studies demonstrating the ability of this dose to block the development of cocaine sensitization (Frankowska et al., 2009) and reduce responding for amphetamine on a fixed-ratio and progressive ratio schedule (Brebner et al., 2005). We chose to administer baclofen t.i.d. due to its relatively short half-life, which has been estimated to range from 4.58 (Anderson et al., 1984) to 6.8 hours (Wuis et al., 1989).

2.3. Locomotor Activity

Prior to any drug treatment, all animals (n = 24) were habituated to locomotor chambers for two daily 2 h sessions (Day 1 and 2; Table 1) prior to baseline locomotor testing (Day 3). Locomotor activity was measured in open-field Plexiglas® test chambers (42 × 42 × 30 cm) by electronic counters that detected interruptions of 8 independent infrared photocell beams (Omnitech, Columbus, OH). Photocell counts were recorded for 15 min and the following measures were calculated: horizontal activity and total distance travelled. After measurement of baseline locomotor activity, animals were assigned to one of 3 groups, matched for levels of baseline locomotor activity, 1) control (n = 8), 2) acute baclofen (n = 8) and 3) chronic baclofen (n = 8). On the final test day (Day 9), animals were administered either saline or baclofen (5 mg/kg, i.p.) 15 min prior to being placed in the locomotor chamber. Locomotor activity was recorded for 15 min, then the 2-[¹⁴C]-deoxyglucose method was initiated (see below). Given the relatively short half-life and duration of action of baclofen, locomotor activity was recorded for 15 min in order to capture the maximum effect without interference of blood sampling necessary for the 2DG procedure. Previous data have shown baclofen to be behaviorally active after a similar pretreatment time (Paredes and Agmo, 1989). This allowed both procedures to occur within the timeframe of maximal drug effect without interference with the measurement of either.

2.4. 2-[¹⁴C] Deoxyglucose method

Local cerebral glucose utilization was measured according to the method of Sokoloff et al. (1977), as adapted for use in freely moving animals (Crane and Porrino, 1989; Torres-

Reveron et al. 2006). Jugular catheters were utilized in order to prevent any reduced mobility that might result from surgery of the femoral artery and vein thus interfering with the measurement of spontaneous locomotor activity. This modification of the 2DG procedure using the jugular to collect timed blood samples has been verified to provide similar results as those obtained with arterial sampling (Torres-Reveron et al. 2006). Surgeries took place on the morning of Day 8 (5th day of drug treatment) and took no longer than 45 min to complete (including induction and recovery from anesthesia), ensuring that the next two injections of baclofen or vehicle could take place that day. 24 h after the surgery, on the final test day (Day 9 or immediately after the final drug dose), rats were given an intravenous pulse of 2-[¹⁴C]deoxyglucose (75 μ Ci/kg; specific activity 50–55 mCi/mmol; Perkin Elmer, Waltham, MA) and the catheter was flushed with 0.2 ml saline. Timed venous blood samples were then drawn over the next 45 min by applying slight negative pressure on a 1 ml syringe. The catheter was flushed with saline after each blood sample was taken. Samples were centrifuged and plasma concentrations were measured for 2[¹⁴C]DG and glucose. At the end of the 45-min sampling procedure, animals were sacrificed using sodium pentobarbital (100 mg/kg, i.v.). Brains were rapidly removed and frozen in isopentane at -45°C , then stored at -80°C until sectioning.

Coronal sections (20 μm) were cut in a cryostat maintained at -22°C . Sections were picked up on glass coverslips, dried on a hotplate (60°C), and then exposed to film (Kodak MRM film, Kodak, Rochester, New York) along with calibrated [¹⁴C] standards (Amersham, Arlington Heights, IL) for 12–15 days. Autoradiograms were then analyzed by quantitative densitometry with a computerized image processing system (MCID Imaging Research, Cambridge, England). Optical density measurements for each brain structure were made in a minimum of five sections. Local cerebral glucose utilization was calculated using the operational equation defined by Sokoloff et al. (1977). Rates of glucose utilization were determined in 35 brain structures selected on the basis of previous reports (Whitlow et al., 2002); according to the rat brain atlas (Paxinos and Watson, 1997).

2.5. Statistical Analysis

Measures of locomotor activity were analyzed by means of one way analyses of variance (ANOVA) followed by planned Bonferroni's test for multiple comparisons. Global rates of glucose utilization were calculated as the weighted average of rates of glucose metabolism across analyzed brain regions. Global rates of glucose utilization were then compared using one way ANOVA. Rates of local cerebral glucose utilization were analyzed in specific functional groups of brain regions (cortex, basal ganglia, limbic, thalamus, midbrain) using two way ANOVA, brain region group $\times$ treatment (vehicle vs. acute baclofen vs. chronic baclofen) with brain region group as a repeated measure. These were followed by planned Bonferroni's tests for multiple comparisons. Statistical significance was considered as $p < 0.05$.

3. Results

3.1. Locomotor Activity

The effects of acute and chronic baclofen treatment on locomotor activity are shown in Figure 1. When compared to vehicle treatment, the acute administration of baclofen (5 mg/kg) significantly reduced spontaneous locomotor activity. Measures of both total distance traveled and horizontal movement of the baclofen treated animals were significantly lower than those of vehicle treated rats. In contrast, chronic baclofen administration (5 mg/kg, t.i.d. for 5 days) did not significantly alter locomotor activity as compared to controls (see Figure 1).

3.2. Cerebral Glucose Utilization

Local rates of cerebral glucose utilization of vehicle-treated, acute baclofen- and chronic baclofen-treated animals are shown in Table 2 and Figure 2. Global rates of glucose utilization were significantly lower in animals treated acutely with baclofen, compared to vehicle treated controls (mean $\pm$ S.E.M., $86.6 \pm 3.8 \mu\text{mol}/100 \text{ g/min}$, controls vs. $68.3 \pm 2.9 \mu\text{mol}/100 \text{ g/min}$, acute baclofen, $p < 0.001$). In contrast, global rates of glucose utilization in animals chronically treated with baclofen were not significantly different from controls ($86.6 \pm 3.8 \mu\text{mol}/100 \text{ g/min}$, controls vs. $82.7 \pm 3.5 \mu\text{mol}/100 \text{ g/min}$, chronic baclofen).

3.3. Acute Baclofen Administration

Two way ANOVAs were used to compare rates of glucose utilization (drug condition $\times$ brain region group). In the cortex, there were significant main effects of drug condition ($F_{(2, 13)} = 10.62$, $p = 0.002$) and brain region ($F_{(6, 78)} = 63.29$, $p < 0.001$), as well as a significant interaction ($F_{(12, 78)} = 3.33$, $p = 0.001$). Multiple comparisons showed that acute baclofen administration resulted in significantly lower rates of glucose utilization throughout many portions of the cortex, compared to vehicle treated controls. These regions included prelimbic (–23%), infralimbic (–20%), agranula insula (–15%), anterior cingulate (–20%), posterior cingulate (–26%) and motor (–13%) cortex.

Within the basal ganglia, there were main effects of drug condition ($F_{(2, 12)} = 20.93$, $p < 0.001$) and brain region ($F_{(7, 84)} = 160.62$, $p < 0.001$), as well as a significant interaction ($F_{(14, 84)} = 3.38$, $p < 0.001$). Multiple comparisons showed that acute baclofen treatment resulted in significantly lower functional activity in the dorsomedial caudate (–26%), dorsolateral caudate (–22%), putamen (–21%), rostral nucleus accumbens (–21%), nucleus accumbens core (–25%), substantia nigra pars compacta (–35%), substantia nigra pars reticulata (–29%) and subthalamic nucleus (–30%), compared to controls.

Within limbic regions, there were significant main effects of drug condition ($F_{(2, 13)} = 3.94$, $p < 0.05$) and brain region ($F_{(10, 130)} = 83.94$, $p < 0.001$), as well as a significant interaction ($F_{(20, 130)} = 4.21$, $p < 0.001$). Multiple comparisons showed that rates of glucose utilization were significantly lower following acute baclofen treatment, compared to controls, in the lateral septum (–21%), medial septum (–36%) and hippocampal CA3 region (–23%).

Within the thalamus, there were significant main effects of drug condition ($F_{(2, 13)} = 7.78$, $p < 0.01$) and brain region ($F_{(3, 39)} = 8.91$, $p < 0.001$), but no significant interaction. Multiple comparisons showed that significantly lower levels of functional activity were present in the paraventricular (–26%), lateral (–20%) and ventral (–21%) nuclei. Within the midbrain and brainstem, there were significant main effects of drug condition ($F_{(2, 13)} = 11.20$, $p < 0.001$) and brain region ($F_{(18, 234)} = 50.673$, $p < 0.001$), but no significant interaction. Multiple comparisons showed that rates of glucose utilization in the lateral geniculate (–23%), superior colliculus (–25%), dorsal periaqueductal grey (–31%) and medial raphe (–32%) were significantly lower after acute baclofen administration, compared to controls.

3.4. Chronic Baclofen Treatment

In contrast, multiple comparisons revealed that rates of glucose utilization of rats receiving chronic administration of baclofen did not significantly differ from those of vehicle-treated rats in the majority of brain regions examined. Chronic baclofen treatment did, however, significantly reduce rates of glucose utilization in dorsomedial caudate (–8%), dorsolateral caudate (–14%) and lateral septum (–20%), as compared to vehicle-treated controls.

4. Discussion

The data from the present study demonstrate that acute administration of baclofen reduced rates of glucose metabolism throughout a diffuse network of brain areas, compared to controls, consistent with the decreased levels of spontaneous locomotor activity seen in the same animals. In contrast to these widespread reductions in metabolic activity, chronic baclofen administration resulted in lower rates of glucose utilization in a very restricted pattern of brain areas, as compared to those of saline-treated controls. This is consistent with the tolerance that developed to the effects of baclofen on locomotor activity seen here, as well as reports of tolerance to the other behavioral effects that accompany baclofen treatment. There was, however, a subset of structures, mainly within the nigrostriatal and mesolimbic systems, in which baclofen significantly altered functional activity even after repeated baclofen treatment.

The finding that acute baclofen treatment reduced locomotor activity agrees with a substantial body of literature demonstrating a motor impairing effect of baclofen (Lobina et al., 2005; Paredes and Agmo, 1989; Van Nieuwenhuijzen et al., 2009) and is in line with its clinical usage as a muscle relaxant (Richard and Menei, 2007). For example, 5 mg/kg baclofen (the dose used in the current study) has been shown to acutely inhibit locomotor activity by approximately 30 – 60% (Frankowska et al., 2009; Paredes and Agmo, 1989). GABA has been shown to directly inhibit dopamine-containing neurons in the substantia nigra via the striatonigral and pallidonigral pathways (Bunney and Aghajannian, 1976; Kubota et al., 1987). Moreover, since GABA_B receptors have been shown to be located in the substantia nigra (Bowery et al., 1987; Boyes and Bolam, 2003), it is possible that elevated levels of GABAergic activity resulted in less dopaminergic activity via inhibition of nigral dopamine cells, thereby suppressing levels of locomotor activity (Beninger, 1983).

Our findings of lower levels of functional brain activity following acute baclofen treatment are also in line with previous studies utilizing a different GABA_B agonist, γ -hydroxybutyric acid (GHB; Crosby et al., 1983; Kuschinsky et al., 1985). Kuschinsky and colleagues reported lower rates of glucose utilization following acute GHB administration (Kuschinsky et al., 1985), in a similarly distributed network to that reported here following acute baclofen. For example, functional activity in the frontal cortex, caudate, substantia nigra, thalamus and hippocampus was lower in GHB-treated animals, compared to controls. Though GHB is generally regarded as a GABA_B agonist, it has been suggested that GHB may also interact with its own distinct receptor (Andriamampandry et al., 2003, 2007). Regardless, the overlap of brain areas affected by GHB and baclofen, and the selectivity of baclofen for the GABA_B receptor would suggest that many of the effects of GHB may be mediated by GABA_B receptors.

The brain regions in which significant changes in functional activity following acute baclofen were measured also correlate well with the known distribution of the GABA_B receptor (Bischoff et al., 1999; Bowery et al., 1983). There are three splice variants of the GABA_B receptor – the R1A and R1B variants (Kaupmann et al., 1997, 1998a) and R2 variant (Kaupmann et al., 1998b). GABA_B receptors are heteromers formed from the GABA_B R1A or R1B and R2 variants in a stoichiometry of 1:1 (Kaupmann et al., 1998). Bischoff et al. (1999) investigated the relative distribution and density of the GABA_B R1A and R1B variants throughout the rat brain. While the highest densities of the R1A variant were located in the substantia nigra, ventral tegmental area, periaqueductal grey, hippocampus and cortex, the R1B variant was more restricted in its distribution with higher concentrations in the thalamus, habenula and cerebellum (Bischoff et al., 1999). As expected, since the GABA_B R2 variant is required for a functional GABA_B receptor, the distribution of the R2 variant largely parallels that of the R1A and R1B (Möhler et al.,

2001). Interestingly, many of the brain areas in the current study that exhibited the greatest degree of tolerance to the functional effects of baclofen (for example substantia nigra, hippocampus, thalamus and cerebellum) also contain the highest densities of GABA_B receptors (Bischoff et al., 1999), while regions with more moderate levels of GABA_B receptors, such as the caudate putamen, exhibited a lesser degree of tolerance. Finally, it is tempting to speculate on some of the functional outcomes of baclofen-induced decreases in rates of glucose metabolism in specific brain areas. For example, perhaps the ability of baclofen to relax muscles stems from its effects within brain regions such as the caudate putamen, globus pallidus and cerebellum, while its pain relieving effects may result from activity in regions such as the dorsal periaqueductal grey.

In contrast to the acute effects of baclofen on locomotor activity and functional activity, chronic treatment with baclofen failed to significantly alter either ambulatory behavior or rates of glucose utilization in the majority of brain areas measured. These data suggest that tolerance developed over the 5 days of repeated injections. Previously, evidence of the development of tolerance to the effects of baclofen has come from both clinical and preclinical reports (Akman et al., 1993; Gianutsos and Moore, 1978; Hefferan et al., 2006; Soni et al., 2003) and we have previously shown the 2DG method to be sensitive to drug-induced tolerance to the metabolic effects of drugs (Whitlow et al., 2003). However, studies have shown that tolerance does not develop to all the central and physiological effects of baclofen. For example, the antinociceptive effect of acute baclofen remains following chronic treatment, but not the acute locomotor effects (Levy and Proudfit, 1977).

Though the underlying molecular mechanism involved in the development and expression of this tolerance is not certain, it is possible that repeated baclofen treatment may alter the density, or result in desensitization, of GABA_B receptors (Kohout and Lefkowitz, 2003). Indeed, previous studies have shown that the density and functional response of GABA_B receptors can decrease following prolonged treatment with selective agonists, such as baclofen (Malcangio et al., 1993) or, conversely, increase following chronic treatment with specific antagonists (Malcangio et al., 1993; Pirbri et al., 2005). However, there is evidence both for, and against, the involvement of GABA_B receptor alterations in the development of tolerance to baclofen. For example, Sands et al. (2003) reported tolerance to the sedative effects of baclofen over 7 days of treatment, which was accompanied by GABA_B receptor desensitization, as measured by [³⁵S] GTPγS binding. In contrast, Lehmann et al. (2003) demonstrated that tolerance developed to the hypothermic effects of baclofen following repeated treatment, but this was not associated with significant changes in either GABA_B receptor density or mRNA expression. Either way, it is apparent from Fairfax et al. (2004) that the mechanism of baclofen-induced GABA_B receptor degradation is quite different from other classic G-protein coupled receptors and tolerance may occur to some effects of baclofen and not to others.

Although tolerance developed to the depressant effects of baclofen administration in a wide range of brain regions, persistent significant decreases in functional activity were observed in the striatum and septum, brain areas that have been shown to play an important role in the regulation of reward, reinforcement and habit formation (Balleine, 2005; Dalley and Everitt, 2009; Taber et al., 2012). In addition, there were trends towards continued decreases in the medial forebrain bundle and ventral tegmental area. That functional activity remains depressed in these portions of the mesolimbic system suggests that the effects of baclofen in these areas persist even after prolonged administration; and may, therefore, be the substrates of its ability to reduce the reinforcing effects of a variety of abused drugs, such as alcohol, cocaine, amphetamine and nicotine (Addolorato et al., 2011; Brebner et al., 2000, 2005; Fattore et al., 2009;). These effects are also consistent with those reported in a recent study by Franklin et al. (2011). These authors measured resting brain cerebral blood flow in

humans before and after 21 days of baclofen treatment and found that baclofen resulted in significant decreases in blood flow in the ventral striatum, medial orbitofrontal cortex and insula. The persistent functional effects of baclofen in these limbic areas, even after chronic dosing, suggests that baclofen may have potential as an effective addiction medicine, and that it may remain efficacious even with long term treatment.

In summary, the present report describes the effects of the administration of baclofen on rates of local cerebral glucose utilization. Acute baclofen administration at a dose that depressed spontaneous locomotor activity produced widespread decreases in functional activity throughout the central nervous system. In contrast, after chronic treatment, baclofen administration no longer depressed locomotor activity and, in the majority of brain regions, rates of glucose utilization were no longer different from control rates. However, rates of glucose utilization in the striatum and septum remained decreased at levels similar to those following acute baclofen administration. These persistent effects on functional activity may provide insights into the way in which baclofen alters the reinforcing effects of abused substances such as cocaine, alcohol, and methamphetamine both in humans and animal models.

Acknowledgments

The authors wish to thank Mack Miller for his technical help. This research was funded by NIDA grant P50 DA06634.

Abbreviations

ANOVA	analysis of variance
GABA	γ -aminobutyric acid
2DG	2-[^{14}C]-deoxyglucose
GHB	γ -hydroxybutyric acid

References

- Addolorato G, Leggio L, Ferrulli A, Cardone S, Bedogni G, Caputo F, et al. Dose-response effect of baclofen in reducing daily alcohol intake in alcohol dependence: secondary analysis of a randomized, double-blind, placebo-controlled trial. *Alcohol Alcohol*. 2011; 46(3):312–317. [PubMed: 21414953]
- Akman MN, Loubser PG, Donovan WH, O'Neill ME, Rossi CD. Intrathecal baclofen: does tolerance occur? *Paraplegia*. 1993; 31(8):516–520. [PubMed: 8414636]
- Anderson P, Noher H, Swahn CG. Pharmacokinetics in baclofen overdose. *Journal of toxicology Clinical toxicology*. 1984; 22(1):11–20. [PubMed: 6492227]
- Andriamampandry C, Taleb O, Kemmel V, Humbert JP, Aunis D, Maitre M. Cloning and functional characterization of a gamma-hydroxybutyrate receptor identified in the human brain. *Faseb J*. 2007; 21(3):885–895. [PubMed: 17197387]
- Andriamampandry C, Taleb O, Viry S, Muller C, Humbert JP, Gobaille S, et al. Cloning and characterization of a rat brain receptor that binds the endogenous neuromodulator gamma-hydroxybutyrate (GHB). *Faseb J*. 2003; 17(12):1691–1693. [PubMed: 12958178]
- Backes EN, Hemby SE. Contribution of ventral tegmental GABA receptors to cocaine self-administration in rats. *Neurochem Res*. 2008; 33(3):459–467. [PubMed: 17943439]
- Balleine BW. Neural bases of food-seeking: affect, arousal and reward in corticostriatolimbic circuits. *Physiology & behavior*. 2005; 86(5):717–730. [PubMed: 16257019]
- Beninger RJ. The role of dopamine in locomotor activity and learning. *Brain Res*. 1983; 287(2):173–196. [PubMed: 6357357]

- Bischoff S, Leonhard S, Reymann N, Schuler V, Shigemoto R, Kaupmann K, et al. Spatial distribution of GABA(B)R1 receptor mRNA and binding sites in the rat brain. *J Comp Neurol*. 1999; 412(1):1–16. [PubMed: 10440706]
- Bowery NG, Enna SJ. gamma-aminobutyric acid(B) receptors: first of the functional metabotropic heterodimers. *The Journal of pharmacology and experimental therapeutics*. 2000; 292(1):2–7. [PubMed: 10604925]
- Bowery NG, Hill DR, Hudson AL. Characteristics of GABAB receptor binding sites on rat whole brain synaptic membranes. *Br J Pharmacol*. 1983; 78(1):191–206. [PubMed: 6297646]
- Bowery NG, Hudson AL, Price GW. GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience*. 1987; 20(2):365–383. [PubMed: 3035421]
- Boyes J, Bolam JP. The subcellular localization of GABA(B) receptor subunits in the rat substantia nigra. *The European journal of neuroscience*. 2003; 18(12):3279–3293. [PubMed: 14686901]
- Brebner K, Ahn S, Phillips AG. Attenuation of d-amphetamine self-administration by baclofen in the rat: behavioral and neurochemical correlates. *Psychopharmacology (Berl)*. 2005; 177(4):409–417. [PubMed: 15630589]
- Brebner K, Phelan R, Roberts DC. Intra-VTA baclofen attenuates cocaine self-administration on a progressive ratio schedule of reinforcement. *Pharmacol Biochem Behav*. 2000; 66(4):857–862. [PubMed: 10973526]
- Brusberg M, Ravnefjord A, Martinsson R, Larsson H, Martinez V, Lindstrom E. The GABA(B) receptor agonist, baclofen, and the positive allosteric modulator, CGP7930, inhibit visceral pain-related responses to colorectal distension in rats. *Neuropharmacology*. 2009; 56(2):362–367. [PubMed: 18824012]
- Bunney BS, Aghajanian GK. Dopaminergic influence in the basal ganglia: evidence for striatonigral feedback regulation. *Research publications - Association for Research in Nervous and Mental Disease*. 1976; 55:249–267. [PubMed: 826996]
- Crane AM, Porrino LJ. Adaptation of the quantitative 2-[14C]deoxyglucose method for use in freely moving rats. *Brain Res*. 1989; 499(1):87–92. [PubMed: 2804673]
- Crosby G, Ito M, Kaufman E, Nelson T, Sokoloff L. Naloxone pretreatment alters the local cerebral metabolic effect of gamma-hydroxybutyrate in rats. *Brain Res*. 1983; 275(1):194–197. [PubMed: 6626978]
- Dalley JW, Everitt BJ. Dopamine receptors in the learning, memory and drug reward circuitry. *Seminars in cell & developmental biology*. 2009; 20(4):403–410. [PubMed: 19560045]
- Dones I, Broggi G. A case of very long-term appearing drug tolerance to intrathecal baclofen. *Journal of neurosurgical sciences*. 2010; 54(2):77–78. [PubMed: 21313959]
- Fadda P, Scherma M, Fresu A, Collu M, Fratta W. Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse*. 2003; 50(1):1–6. [PubMed: 12872287]
- Fairfax BP, Pitcher JA, Scott MG, Calver AR, Pangalos MN, Moss SJ, et al. Phosphorylation and chronic agonist treatment atypically modulate GABAB receptor cell surface stability. *The Journal of biological chemistry*. 2004; 279(13):12565–12573. [PubMed: 14707142]
- Fattore L, Spano MS, Cossu G, Scherma M, Fratta W, Fadda P. Baclofen prevents drug-induced reinstatement of extinguished nicotine-seeking behaviour and nicotine place preference in rodents. *Eur Neuropsychopharmacol*. 2009; 19(7):487–498. [PubMed: 19250803]
- Franklin TR, Harper D, Kampman K, Kildea-McCrea S, Jens W, Lynch KG, et al. The GABA B agonist baclofen reduces cigarette consumption in a preliminary double-blind placebo-controlled smoking reduction study. *Drug Alcohol Depend*. 2009; 103(1-2):30–36. [PubMed: 19398283]
- Franklin TR, Wang Z, Sciortino N, Harper D, Li Y, Hakun J, et al. Modulation of resting brain cerebral blood flow by the GABA B agonist, baclofen: a longitudinal perfusion fMRI study. *Drug Alcohol Depend*. 2011; 117(2-3):176–183. [PubMed: 21333466]
- Frankowska M, Nowak E, Filip M. Effects of GABAB receptor agonists on cocaine hyperlocomotor and sensitizing effects in rats. *Pharmacol Rep*. 2009; 61(6):1042–1049. [PubMed: 20081239]
- Fu Z, Yang H, Xiao Y, Zhao G, Huang H. The gamma-aminobutyric acid type B (GABAB) receptor agonist baclofen inhibits morphine sensitization by decreasing the dopamine level in rat nucleus accumbens. *Behavioral and brain functions : BBF*. 2012; 8

- Gianutsos G, Moore KE. Tolerance to the effects of baclofen and gamma-butyrolactone on locomotor activity and dopaminergic neurons in the mouse. *The Journal of pharmacology and experimental therapeutics*. 1978; 207(3):859–869. [PubMed: 731436]
- Hama AT, Broadhead A, Lorrain DS, Sagen J. The antinociceptive effect of the asthma drug ibudilast in rat models of peripheral and central neuropathic pain. *Journal of neurotrauma*. 2012; 29(3):600–610. [PubMed: 21806469]
- Heetla HW, Staal MJ, Kliphuis C, van Laar T. The incidence and management of tolerance in intrathecal baclofen therapy. *Spinal cord*. 2009; 47(10):751–756. [PubMed: 19333246]
- Hefferan MP, Fuchigami T, Marsala M. Development of baclofen tolerance in a rat model of chronic spasticity and rigidity. *Neurosci Lett*. 2006; 403(1-2):195–200. [PubMed: 16716507]
- Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, et al. Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature*. 1997; 386(6622):239–246. [PubMed: 9069281]
- Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, et al. GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature*. 1998a; 396(6712):683–687. [PubMed: 9872317]
- Kaupmann K, Schuler V, Mosbacher J, Bischoff S, Bittiger H, Heid J, et al. Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K⁺ channels. *Proceedings of the National Academy of Sciences of the United States of America*. 1998b; 95(25):14991–14996. [PubMed: 9844003]
- Kohout TA, Lefkowitz RJ. Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization. *Molecular pharmacology*. 2003; 63(1):9–18. [PubMed: 12488531]
- Kubota Y, Inagaki S, Kito S, Wu JY. Dopaminergic axons directly make synapses with GABAergic neurons in the rat neostriatum. *Brain Res*. 1987; 406(1-2):147–156. [PubMed: 2882818]
- Kuschinsky W, Suda S, Sokoloff L. Influence of gamma-hydroxybutyrate on the relationship between local cerebral glucose utilization and local cerebral blood flow in the rat brain. *J Cereb Blood Flow Metab*. 1985; 5(1):58–64. [PubMed: 3972924]
- Lehmann A, Mattsson JP, Edlund A, Johansson T, Ekstrand AJ. Effects of repeated administration of baclofen to rats on GABAB receptor binding sites and subunit expression in the brain. *Neurochem Res*. 2003; 28(2):387–393. [PubMed: 12608713]
- Levy RA, Proudfit HK. The analgesic action of baclofen [beta-(4-chlorophenyl)-gamma-aminobutyric acid]. *The Journal of pharmacology and experimental therapeutics*. 1977; 202(2):437–445. [PubMed: 886473]
- Liang JH, Chen F, Krstew E, Cowen MS, Carroll FY, Crawford D, et al. The GABA(B) receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. *Neuropharmacology*. 2006; 50(5):632–639. [PubMed: 16406445]
- Lobina C, Colombo G, Gessa GL, Carai MA. Different sensitivity to the motor incoordinating effects of gamma-hydroxybutyric acid (GHB) and baclofen in GHB-sensitive and GHB-resistant rats. *Brain Res*. 2005; 1033(1):109–112. [PubMed: 15680346]
- Malcangio M, Da Silva H, Bowery NG. Plasticity of GABAB receptor in rat spinal cord detected by autoradiography. *Eur J Pharmacol*. 1993; 250(1):153–156. [PubMed: 8119313]
- Mohler H, Benke D, Fritschy JM. GABA(B)-receptor isoforms molecular architecture and distribution. *Life sciences*. 2001; 68(19-20):2297–2300. [PubMed: 11358339]
- Montane E, Vallano A, Laporte JR. Oral antispastic drugs in nonprogressive neurologic diseases: a systematic review. *Neurology*. 2004; 63(8):1357–1363. [PubMed: 15505149]
- Oleson EB, Richardson JM, Roberts DC. A novel IV cocaine self-administration procedure in rats: differential effects of dopamine, serotonin, and GABA drug pre-treatments on cocaine consumption and maximal price paid. *Psychopharmacology (Berl)*. 2011; 214(2):567–577. [PubMed: 21110008]
- Paredes R, Agmo A. Stereospecific actions of baclofen on sociosexual behavior, locomotor activity and motor execution. *Psychopharmacology (Berl)*. 1989; 97(3):358–364. [PubMed: 2497485]
- Paxinos, G.; Huang, XF.; Toga, AW. *The rhesus monkey brain in stereotaxic coordinates*. Academic Press; San Diego, CA: 2000.

- Pibiri F, Carboni G, Carai MA, Gessa GL, Castelli MP. Up-regulation of GABA(B) receptors by chronic administration of the GABA(B) receptor antagonist SCH 50,911. *Eur J Pharmacol.* 2005; 515(1-3):94–98. [PubMed: 15893746]
- Richard I, Menei P. Intrathecal baclofen in the treatment of spasticity, dystonia and vegetative disorders. *Acta neurochirurgica Supplement.* 2007; 97(Pt 1):213–218. [PubMed: 17691379]
- Sands SA, McCarson KE, Enna SJ. Relationship between the antinociceptive response to desipramine and changes in GABAB receptor function and subunit expression in the dorsal horn of the rat spinal cord. *Biochem Pharmacol.* 2004; 67(4):743–749. [PubMed: 14757174]
- Slonimski M, Abram SE, Zuniga RE. Intrathecal baclofen in pain management. *Regional anesthesia and pain medicine.* 2004; 29(3):269–276. [PubMed: 15138913]
- Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, et al. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *Journal of neurochemistry.* 1977; 28(5):897–916. [PubMed: 864466]
- Soni BM, Mani RM, Oo T, Vaidyanathan S. Treatment of spasticity in a spinal cord-injured patient with intrathecal morphine due to intrathecal baclofen tolerance--a case report and review of literature. *Spinal cord.* 2003; 41(10):586–589. [PubMed: 14504619]
- Taber KH, Black DN, Porrino LJ, Hurley RA. Neuroanatomy of dopamine: reward and addiction. *The Journal of neuropsychiatry and clinical neurosciences.* 2012; 24(1):1–4. [PubMed: 22450608]
- Torres-Reveron A, Melnick SM, Stephenson SI, Dow-Edwards DL. Standardization of a novel blood-sampling method through the jugular vein for use in the quantified [14C] 2-deoxyglucose method. *Journal of neuroscience methods.* 2006; 150(2):143–149. [PubMed: 16430967]
- van Nieuwenhuijzen PS, McGregor IS, Hunt GE. The distribution of gamma-hydroxybutyrate-induced Fos expression in rat brain: comparison with baclofen. *Neuroscience.* 2009; 158(2):441–455. [PubMed: 18996447]
- Whitlow CT, Freedland CS, Porrino LJ. Functional consequences of the repeated administration of Delta9-tetrahydrocannabinol in the rat. *Drug Alcohol Depend.* 2003; 71(2):169–177. [PubMed: 12927655]
- Wong CG, Bottiglieri T, Snead OC 3rd. GABA, gamma-hydroxybutyric acid, and neurological disease. *Annals of neurology.* 2003; 54(Suppl 6):S3–12. [PubMed: 12891648]
- Wuis EW, Dirks MJ, Termond EF, Vree TB, Van der Kleijn E. Plasma and urinary excretion kinetics of oral baclofen in healthy subjects. *European journal of clinical pharmacology.* 1989; 37(2):181–184. [PubMed: 2792173]

Highlights

- Tolerance has been reported to occur to many of baclofen's effects in humans
- In rats, acute baclofen resulted in reduced locomotor activity and brain metabolism
- Tolerance developed to the locomotor effects of chronic baclofen
- Tolerance developed to the metabolic effects except in striatum and septum
- Lack of tolerance in these areas suggests addiction treatment potential

Figure 1. Effects of acute (5 mg/kg) and chronic baclofen (5 mg/kg t.i.d. for 5 days) treatment on spontaneous locomotor activity. Panel A displays total distance traveled (total cm), Panel B displays horizontal distance traveled (total cm). ** significantly different ($P < 0.01$) from control.

Figure 2.
Pseudocolor enhanced autoradiograms of local rates of cerebral glucose utilization following vehicle (Panels A, D), acute (Panels B, E) or chronic (Panels C, F) baclofen treatment at the frontal cortical (Panels A, B, C) and hippocampal level (Panels D, E, F).

Table 1
Experimental Timeline

Day	Procedure
1	Locomotor Habituation
2	Locomotor Habituation
3	Baseline Locomotor
4 – 8	Vehicle or Baclofen Injections t.i.d.
8	Surgery for 2-DG
9	Test Day: Vehicle or Baclofen Injection + Locomotor + 2DG

Table 2

Effect of acute and chronic baclofen on rates of local cerebral glucose metabolism

Brain Region	Vehicle Treated (N=7)	Acute Baclofen (N=6)	Chronic Baclofen (N=7)
<i>Cortex</i>			
Prelimbic	119 ± 6.1	92 ± 5.1	120 ± 5.1
Infralimbic	97 ± 4.7	77 ± 5.4	102 ± 3.6
Agranular insula	86 ± 1.7	73 ± 3.6	81 ± 3.3
Anterior cingulate	125 ±	99 ± 3.1	123 ± * 6.8
Motor	110 ± 4.5	96 ± 2.8	106 ± 2.2
Posterior cingulate	145 ± 3.4	107 ± 7.3	133 ± 7.4
<i>Basal ganglia</i>			
Dorsomedial caudate	122 ± 4.5	91 ± 2.2	112 ± 3.6
Dorsolateral caudate	128 ± 4.3	100 ± 3.7	112 ± 4.3
Putamen	110 ± 3.1	87 ± 1.1	94 ± 4.8
Nucleus accumbens core	84 ± 2.3	67 ± 4.3	78 ± 5.4
Nucleus accumbens shell	78 ± 4.7	70 ± 4.8	81 ± 6.2
Substantia nigra, pars compacta	77 ± 3.3	50 ± 3.3 *	69 ± 5.9
Substantia nigra, pars reticulata	56 ± 5.3	40 ± 3.4 *	52 ± 2.4
Ventral tegmental area	59 ± 5.9	48 ± 4.4	46 ± 3.8
Subthalamic nucleus	93 ± 6.0	65 ± 6.4 *	84 ± 3.6
<i>Limbic</i>			
Lateral septum	56 ± 2.1	44 ± 3.3	45 ± 2.3
Medial septum	95 ± 6.2	61 ± 4.9	87 ± 3.1
Bed nucleus of stria terminalis	42 ± 6.0	41 ± 3.2	39 ± 2.0
Medial forebrain bundle	53 ± 4.8	45 ± 4.1	44 ± 6.0
Amygdala, central nucleus	81 ± 4.3	69 ± 4.2	78 ± 3.3
Amygdala, basolateral nucleus	56 ± 3.9	50 ± 3.6	59 ± 3.5
Ventromedial hypothalamus	41 ± 5.4	37 ± 3.8	36 ± 3.0

Brain Region	Vehicle Treated (N=7)	Acute Baclofen (N=6)	Chronic Baclofen (N=7)
Hippocampus, CA1	70 ± 6.0	54 ± 3.8	68 ± 3.5
Hippocampus, CA3	78 ± 4.8	60 ± 3.9 *	75 ± 1.9
Hippocampus, dentate gyrus	79 ± 5.3	64 ± 5.3	79 ± 3.5
<i>Thalamus</i>			
Medial thalamus	107 ± 4.9	77 ± 6.5	106 ± 13.0
Lateral thalamus	96 ± 4.2	76 ± 5.4	94 ± 2.3
Ventrolateral thalamus	105 ± 6.4	83 ± 4.4	89 ± 5.7
<i>Midbrain/Brainstem</i>			
Lateral geniculate	94 ± 3.6	73 ± 5.9	97 ± 2.8
Medial geniculate	131 ± 5.3	110 ± 7.7	130 ± 5.8
Superior colliculus	90 ± 5.9	67 ± 3.7	94 ± 5.5
Periaqueductal grey	64 ± 4.0	44 ± 4.3	66 ± 4.4
Dorsal raphe	85 ± 9.1	61 ± 4.5	80 ± 11.1
Medial raphe	106 ± 5.4	72 ± 3.5	96 ± 5.6
Dorsal tegmental area	94 ± 6.7	64 ± 3.6	93 ± 5.0

Data are expressed as mean ($\mu\text{mol}/100 \text{ g/ min}$) $\pm$ S.E.M.

* P<0.05 compared to vehicle treated controls