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

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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.

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 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 (Mohler et al., 2001). GABA$_B$ receptors can serve as pre-synaptic autoreceptors or heteroceptors 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.

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

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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; Lehmann 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-[^{14}C]-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., 2009; Liang et al., 2006; Paredes and Agmo, 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 a12-h light–dark cycle (lights on at 07:00). Food and water were available ad libitum.

Surgical procedures for the 2-[^{14}C]-DG studies followed those described by Torres-Reveron et al. (2006) and were carried out 24 h 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 h (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 intraperotineal (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 h (Wuis et al., 1989).

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

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 traveled. 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-[^{14}C]-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-[^{14}C] 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