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Review

Abuse and dependence liability of benzodiazepine-type drugs: $GABA_A$ receptor modulation and beyond

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Abstract

Over the past several decades, benzodiazepines and the newer non-benzodiazepines have become the anxiolytic/hypnotics of choice over the more readily abused barbiturates. While all drugs from this class act at the $GABA_A$ receptor, benzodiazepine-type drugs offer the clear advantage of being safer and better tolerated. However, there is still potential for these drugs to be abused, and significant evidence exists to suggest that this is a growing problem. This review examines the behavioral determinants of the abuse and dependence liability of benzodiazepine-type drugs. Moreover, the pharmacological and putative biochemical basis of the abuse-related behavior is discussed.

Keywords: Benzodiazepines; $GABA_A$ receptor; Abuse potential; Reinforcing effects; Self-administration; Tolerance; Dependence

Contents

Benzodiazepine-type drugs (benzodiazepines and the newer non-benzodiazepines) are similar to older sedative/hypnotic drugs, such as the barbiturates, in that they act at the $GABA_A$ receptor (Ator and Griffiths, 1987; Tone, 2005). Unfortunately,

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benzodiazepine-type drugs also retain the liability for abuse and dependence associated with the earlier anxiolytics (Nutt, 2005; Woods and Winger, 1995). Action at $GABA_A$ receptors likely plays a key role in both the therapeutic as well as abuse-related effects of this important class of drugs. While the extent to which therapeutic efficacy and abuse potential can be dissociated is not yet understood fully, the biochemical processes underlying these behavioral effects are even less understood. A more comprehensive understanding of the etiology of benzodiazepine-type drug-induced abuse and dependence is likely to provide information that can inform drug development strategies to help design anxiolytics and hypnotics that have maximum clinical benefit with reduced abuse potential. Thus, this review will explore issues related to the abuse and dependence potential of benzodiazepine-type drugs and the role that $GABA_A$ receptors play in this phenomenon. Further, this review will discuss putative intracellular events that may occur as a result of the interaction between benzodiazepine-type drugs and $GABA_A$ receptors, and how those events may ultimately give rise to the abuse-related behaviors associated with these drugs.

1. $GABA_A$ receptor modulators

Sedative/hypnotic drugs include those that are typically considered to be tranquilizers such as the barbiturates, benzodiazepines, and newer non-benzodiazepines. Clinically, these drugs are prescribed as anxiolytics, sedatives, anticonvulsants, and muscle relaxants, and share in common an ability to interact with the $GABA_A$ receptor (Bateson, 2004; Saunders and Ho, 1990). Barbiturates and benzodiazepine-type drugs are positive allosteric modulators of the receptor complex. They each bind to a distinct site on the $GABA_A$ receptor and increase the affinity of the receptor by favoring an open state, thereby increasing chloride conductance (Campo-Soria et al., 2006, Tallman et al., 1978). Many studies over the past decades have revealed the existence of multiple subtypes of the $GABA_A$ receptor (e.g., McKernan and Whiting, 1996; Pritchett et al., 1989), and research with transgenic mice and subtype-selective ligands has postulated that the diverse behavioral effects of benzodiazepine-type drugs in particular may reflect action at different subtypes of $GABA_A$ receptors (Löw et al., 2000; McKernan et al., 2000; Platt et al., 2002; Rowlett et al., 2005; Rudolph et al., 2001).

The $GABA_A$ receptors in the central nervous system are pentamers composed of subunits from at least five different families of distinct proteins (for review, see Rudolph et al., 2001). While the majority of $GABA_A$ receptors consist of α, β, and γ subunits, classical benzodiazepines bind predominantly to a site on the native $GABA_A$ receptor that occurs at the interface between the $\gamma 2$ subunit and either an $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunit (McKernan et al, 1995; Pritchett and Seeburg, 1991; Stephenson et al., 1990; Wieland et al., 1992). In contrast, these drugs are inactive at corresponding $\alpha 4$- and $\alpha 6$-subunit containing receptors (Pritchett and Seeburg, 1990).

More than 90% of the $GABA_A$ receptors in the brain contain $\alpha 1$, $\alpha 2$, and $\alpha 3$ subunits (McMahon et al., 2001), and despite the existence of other subunits within the receptor, benzodiazepine action appears to be determined by the presence of particular α subunits (McKernan and Whiting, 1996; Pritchett et al., 1989; Rudolph et al., 2001). $GABA_A$ receptors containing $\alpha 1$ subunits ($\alpha 1GABA_A$ receptors) recently have been implicated in the sedative effects of benzodiazepine-type drugs (McKernan et al., 2000; Platt et al., 2002; Rudolph et al., 1999), whereas $GABA_A$ receptors containing $\alpha 2$ and $\alpha 3$ subunits ($\alpha 2GABA_A$ and $\alpha 3GABA_A$ receptors) have been implicated in the anxiolytic effects of benzodiazepine-type drugs (Löw et al., 2000; McKernan et al., 2000). Receptors containing $\alpha 5$ subunits ($\alpha 5GABA_A$ receptors), while being a relatively minor population of $GABA_A$ receptors, may play a role in memory processes, but likely not anxiolysis or motor effects (Collinson et al., 2002; Crestani et al., 2002).

To the extent that the different behavioral effects of benzodiazepines are attributable to different receptor subtypes, it is feasible that a subset of receptors is responsible for the abuse-related effects of these drugs. Consequently, the heterogeneity of $GABA_A$ receptors raises the possibility that compounds lacking abuse liability can be found. However, as will be discussed later, a complex picture is emerging with respect to abuse of benzodiazepine-type drugs and the role of different $GABA_A$ receptor subtypes.

2. Behavioral effects of benzodiazepine-type drugs

Benzodiazepines were developed in the 1960s in response to a need for safe and effective anxiolytics. Barbiturates had lost favor as anxiolytics and anticonvulsants due to their low therapeutic index and high abuse potential (Morgan, 1990). The successor to the barbiturates, meprobamate, met a similar demise as reports of overuse and illicit diversion gradually negated its clinical usefulness and popularity (Littrell et al., 1993). The introduction of meprobamate, however, was the beginning of modern psychopharmacology, and led to an intense interest in the development of novel anxiolytic drugs with reduced side effects. The interest in that endeavor continues to this day (Tone, 2005).

2.1. Therapeutic efficacy

Chlordiazepoxide (Librium) and diazepam (Valium) were among the earliest benzodiazepine anxiolytics to be developed. Diazepam in particular was extremely popular, and became the most widely prescribed drug in the United States and Europe between 1968 and 1987 (Speaker, 1997). Within the past decade diazepam has maintained its popularity and along with alprazolam (Xanax), clonazepam (Klonopin), and lorazepam (Ativan), has appeared on a list of the top 100 most commonly prescribed medications (American Druggist, 1996). Among the advantages of prescribing benzodiazepines as broad-spectrum anxiolytics and hypnotics is that in addition to how well-tolerated they are they exhibit rapid onset of action and variable, yet predictable, half-lives (Greenblatt et al., 1981).

While the hallmark of their therapeutic efficacy is their ability to reduce anxiety and seizure activity acutely as well as to induce sleep, benzodiazepines are useful for treating a variety of specific conditions (Hollister et al., 1993; Pollack, 1993). Most notably, with respect to anxiety disorders, this group of drugs has been demonstrated empirically to treat the somatic

symptoms associated with generalized anxiety disorder (e.g., Fontaine et al., 1986), panic disorder (e.g., Dunner et al., 1986), and obsessive–compulsive disorder (e.g., Hewlett et al., 1990, 1992). Status epilepticus, either as a result of neurological illness or as a precursor to epilepsy, also has been shown to benefit from treatment with benzodiazepines (Mitchell, 1996). Not only are benzodiazepines the traditional prescription for treating insomnia (Kales and Kales, 1983), but their amnestic properties make them invaluable when used during pre-surgical and dental sedation (Dundee et al., 1984). This broad range of clinical uses signifies that benzodiazepines are some of the most important psychoactive drugs developed over the past century.

The 1980s brought reports of the new "Z-drug" hypnotics. These drugs have rapid onset and short duration of action (Arbilla et al., 1985), thus making them attractive non-benzodiazepine alternatives for the short-term treatment of insomnia. Although they are structurally distinct from benzodiazepines, zolpidem, zaleplon, and zopiclone (and more recently, its active enantiomer eszopiclone), all act at the benzodiazepine recognition site on the $GABA_A$ receptor. However, zolpidem and zaleplon are selective for those receptors containing an α1 subunit (Benavides et al., 1988; Sanna et al., 2002), while zopiclone appears to be less specific (Concas et al., 1994; Doble, 1999). Interestingly, they are also structurally unrelated to one another; zolpidem is an imidazopyridine, zaleplon is a pyrazolopyrimidine, and both zopiclone and eszopiclone are cyclopyrrolones.

Of the three hypnotics, zolpidem (Ambien) is probably the most frequently prescribed non-benzodiazepine hypnotic in the United States (Morlock et al., 2006), and the most potent. Its potency has been demonstrated in vitro using oocytes expressing recombinant $\alpha 1GABA_A$ receptors. The potentiation of GABA-evoked chloride currents was measured, showing that zolpidem potentiated these currents with an $EC_{50}=78$ (Sanna et al., 2002), zopiclone had an $EC_{50}=107$ (Reynolds and Maitra, 1996), and zaleplon had an $EC_{50}=169$ (Sanna et al., 2002). In vivo, zolpidem can be distinguished from conventional benzodiazepines (e.g., Arbilla et al., 1985; Depoortere et al., 1986; Sanger and Zivkovic, 1986, 1987) such that its predominant behavioral effect is sedation despite its ability to engender anxiolytic-like, anticonvulsant, and myorelaxant effects in rodents. Moreover, sedation was observed at much lower doses than those required to engender the other effects (Depoortere et al., 1986; Sanger et al., 1987). Clinically, zolpidem demonstrated hypnotic efficacy in people with sleep disturbances comparable to the benzodiazepines, but without the disruption of sleep architecture (Besset et al., 1995; Blois et al., 1993; Lavoisy et al., 1992; Quera-Salva et al., 1994; Terzano and Parrino, 1994).

Zaleplon (Sonata) has been shown to have similar preclinical (Sanger et al., 1996) and clinical (see review by Dooley and Plosker, 2000) behavioral pharmacological profiles to zolpidem. However, at therapeutic doses the agonist effects of zolpidem are greater than those of zaleplon (Greenblatt et al., 1998). Eszopiclone (Lunesta) and zopiclone (Imovane) are similar to zolpidem and zaleplon such that they also induce anxiolytic, anticonvulsant, myorelaxant, and sedative effects in rodents (Carlson et al., 2001). Clinically, eszopiclone appears to be comparable to the other non-benzodiazepine hypnotics with respect to pharmacokinetics and ability to induce and maintain sleep (see review by Najib, 2006), but it is unique in that it retains its safety and efficacy for 6–12 months (Krystal et al., 2003; Roth et al., 2005). All together, the "Z-drugs" have become the first-line medication treatment for insomnia (Erman, 2005; Neubauer, 2006).

2.2. Abuse liability

Despite the usefulness of benzodiazepine-type drugs across many clinical indications, their myriad behavioral effects may sometimes be perceived as side effects, thus limiting their utility. Among those effects are daytime sedation, motor incoordination, and memory impairment (Fisch et al., 1990; Verster et al., 2002; Wesensten et al., 1995). In contrast, effects such as abuse and dependence serve no clinical purpose, and are always perceived as undesirable (Griffiths and Wolf, 1990; Korpi et al., 1997).

The abuse potential of benzodiazepines was recognized as early as 1967, as reports in the popular media were warning of their illicit and non-medical use particularly by youth and the counter-culture (Speaker, 1997; Tone, 2005). In fact, benzodiazepines had entered the popular culture. For example, the Rolling Stones' song "Mother' Little Helper" referred to a street name associated with the perceived widespread use of diazepam by middle-class housewives (although it is not entirely clear the extent to which this street name refers to benzodiazepines only). Another example is the prominent role played by Valium in Jacqueline Susann's 1966 novel "Valley of the Dolls". The story revolves around ambitious young women who medicate themselves with Valium in order to cope with the pressures they face in their personal lives and careers. During these years, doctors were generous with prescriptions prompting Valium to become a coping tool for everyone from overworked business executives to frazzled housewives. In 1975, the United States Drug Enforcement Agency (DEA) began regulating valium and several other benzodiazepines as Schedule IV drugs, and by 1979 the government used congressional hearings on the "Valium scare" (U.S. Committee on Labor and Human Resources, 1979) to urge more judicious prescribing practices.

While the notion that benzodiazepine-type drugs have the potential to be abused is not new, recent epidemiological findings suggest that their abuse may be on the rise. One prominent example comes from recent reports prepared by the Drug Abuse Warning Network (DAWN), in which yearly estimates of drug abuse-related emergency department visits from a large network of hospitals in the United States are compiled. According to the most recent data available, the number of emergency room visits associated with the use of sedative/hypnotics in 2005 was 34% of the total visits involving non-medical use of prescription drugs (Substance Abuse and Mental Health Services Administration, 2007; see Fig. 1). More strikingly, the number of benzodiazepine-related emergency department visits was not only comparable to those involving misuse of prescription opiates (approximately 29% of sedative/hypnotic visits), but they had increased 19% since 2004. These statistics are in agreement with current reports based on substance abuse treatment admissions. Based on

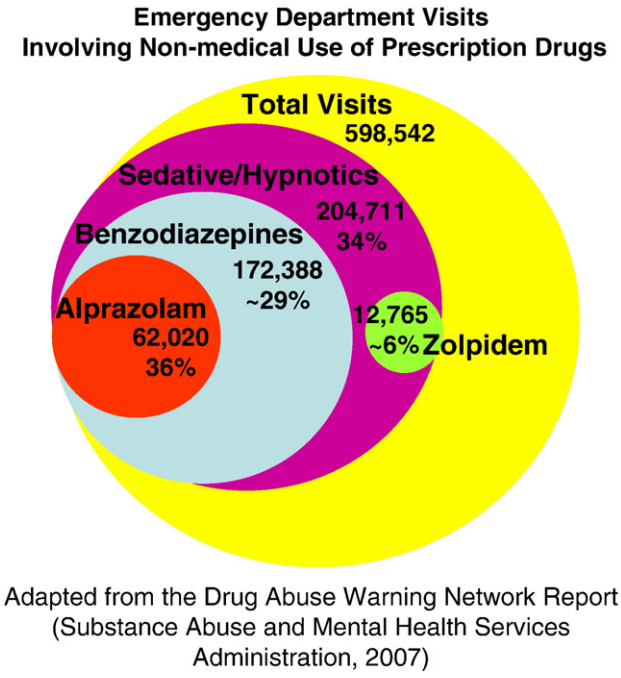

Fig. 1. Recent emergency department visits involving the non-medical use of prescription drugs, adapted from the Drug Abuse Warning Network report (Substance Abuse and Mental Health Services Administration, Office of Applied Studies, 2007). Percentages are approximate.

findings from the Treatment Episode Data Set (TEDS), an annual compilation of patient characteristics in substance abuse treatment facilities in the United States, admissions due to "primary tranquilizer" use (including, but not limited to, benzodiazepine-type drugs) increased 79% from 1992 to 2002 (The DASIS Report, 2005). Thus, the DAWN and TEDS data sets demonstrate clearly that the misuse of these sedative/hypnotics is on the rise, and cause for concern.

Within the general population there are certain sub-populations who are at greater risk for inappropriate benzodiazepine taking. These groups include polydrug abusers, patients with histories of alcohol abuse, and the elderly (Griffiths and Weerts, 1997; Woods et al., 1992). With respect to polydrug abuse, benzodiazepine-type drugs are often co-abused with opiates and alcohol (Crane and Nemanski, 2004). Upwards of one-third of opiate-addicted individuals have reported taking benzodiazepines in combination with opioid drugs, particularly with methadone (Darke et al., 1995, 1994; Du Pont, 1988; Forsyth et al., 1993; Iguchi et al., 1993; Metzger et al., 1991; Preston et al., 1984, Segura et al., 2001; Stitzer et al., 1981). Clinical and preclinical evidence suggests that benzodiazepines enhance the abuse-related effects of opiates or "boost" their high. In that respect, opiate users report enhanced subjective effects with the combination relative to either drug alone (Lintzeris et al., 2007; Preston et al., 1984), while otherwise ineffective doses of alprazolam and heroin engendered a significant place preference in rodents when tested in combination (Walker and Ettenberg, 2001, 2003, 2005). Similarly, people with a history of moderate-to-heavy alcohol use tend to have a higher degree of long-term benzodiazepine use (often without a prescription) and appear more sensitive to the effects of these drugs (Ciraulo et al., 1988; de Wit and Doty, 1993; Evans et al., 1996). And while the elderly likely do not engage in recreational abuse, prevalence of use is typically higher than in the general population (Griffiths and Weerts, 1997; Woods et al., 1992).

There also are other unique instances of susceptibility to the abuse of benzodiazepine-type drugs. For example, iatrogenic factors have been shown to contribute to dependence, particularly when benzodiazepine-type drugs are used in the comfort and care of the critically ill. Intensive care units utilize benzodiazepines in high volumes, and patients often undergo withdrawal upon discontinuation despite the use of standard tapering management protocols (see review by Taylor, 1999). This is a pathway to dependence that is often overlooked in both adults (Cammarano et al., 1998; Diehl et al., 2000) and children (Carnevale and Ducharme, 1997; Franck et al., 2004; van Engelen et al., 1993). Similarly, treatment of medication-induced insomnia also has the potential to lead to dependence on benzodiazepine-type drugs. This can be a problem particularly in the elderly for whom there is an increased likelihood of polypharmacy (Kamel and Gammack, 2006; Salzman, 1985), or in those individuals being treated with other medications such as antidepressants (Jindal and Thase, 2004; Londborg et al., 2000). Overall, it can be concluded that benzodiazepine-type drugs have serious abuse and dependence liability, even in seemingly innocuous medical situations.

3. Behavioral determinants of abuse and dependence liability

Drug seeking and drug taking behavior together is a complex phenomenon comprised of discrete behavioral components. The most likely property of a compound that predicts inappropriate use is the degree to which the compound has reinforcing effects. A drug is said to have reinforcing effects if its presentation increases the probability of subsequent responses to produce it. The study of the reinforcing effects of drugs has been an important emphasis of drug abuse research for decades, and the demonstration of a drug's reinforcing effects in the laboratory forms a key component of abuse liability assessment required by worldwide regulatory agencies (Ator, 2005; Ator and Griffiths, 2003; Griffiths et al., 2003).

Another major determinant of the extent to which a drug has abuse liability is the occurrence of physical dependence with repeated administration. Physical dependence is characterized by the emergence of a withdrawal syndrome upon cessation of chronic drug treatment. Tolerance to some or all of the effects of a drug often accompanies the development of physical dependence. It is important to note that abuse can occur in the absence of physical dependence—thus dependence is a predictor of abuse potential, but it is not a necessary condition. As with reinforcing effects, regulatory agencies also consider the extent to which a compound induces physical dependence following chronic treatment as part of scheduling decisions (Ator and Griffiths, 2003).

A final property often considered to be a key component of a drug's abuse liability is the subjective, or interoceptive effect produced by it. These effects often are assessed with drug discrimination procedures in which subjects typically are trained to distinguish the presence and absence of a drug, i.e.

a response is correct or incorrect based on whether drug or placebo is administered. In their most basic form, these procedures determine the extent to which one drug shares discriminative stimulus effects with another drug—if the latter is an abused drug of a particular class, then the likelihood that the compound of interest has subjective effects in common with the drug of abuse is high (Ator, 2005; Lelas et al., 2000).

Of the three properties of drugs that are considered for determination of abuse liability, the following sections will focus on the reinforcing effects and propensity to induce physical dependence of benzodiazepine-type drugs. The discriminative stimulus effects of benzodiazepine-type drugs have been reviewed extensively elsewhere (e.g., Ator, 2005; Lelas et al., 2000) and will not be discussed in detail further.

3.1. Self-administration of benzodiazepine-type drugs

A consistent finding in human laboratory studies is that benzodiazepine-type drugs have reinforcing effects primarily in subjects with histories of drug or alcohol abuse, in anxious subjects, and patients with sleep disorders (Griffiths and Weerts, 1997; Woods et al., 1992). However, unlike other abused drugs, benzodiazepine-type drugs do not function as reinforcers consistently if subjects lack these characteristics. While it is unclear why the reinforcing effects should depend on subject characteristics and/or histories, it can be hypothesized that individuals who suffer from some type of anxiety self-administer benzodiazepine-type drugs because of their therapeutic efficacy; i.e., in order to alleviate anxiety (Griffiths and Weerts, 1997; Helmus et al., 2005). In fact, it is completely plausible that highly anxious individuals find benzodiazepine-type drugs very reinforcing. Polydrug abusers and alcoholics likely self-administer these compounds due to some interaction that exists between the therapeutic effects of benzodiazepines and the reinforcing effects that are subsequent to prior exposure to abused substances. Although some reports have demonstrated evidence that this population may use benzodiazepine-type drugs to self-medicate "emotional disturbances" or insomnia (e.g., Gelkopf et al., 1999; Perera et al., 1987), and others have observed that benzodiazepines are co-administered with other substances primarily to boost a drug "high" (e.g., Darke et al., 1995; Iguchi et al., 1993), one study has found a combination of these effects. Among a population of patients maintained on methadone for treatment of opioid dependence, relatively large proportions of the subjects self-administered benzodiazepines for either recreational purposes or treating "emotional problems", however, approximately one-third reported taking benzodiazepines for both reasons (Gelkopf et al., 1999).

With respect to self-administration in laboratory animals, it is predicted that if benzodiazepine-type drugs have reinforcing effects, then these compounds should be effective in models of self-administration in controlled laboratory settings. Indeed, this prediction does hold, as benzodiazepine-type compounds show reinforcing effects under a variety of experimental conditions (e.g., Ator, 2005; Bergman and Johanson, 1985; Broadbear et al., 2005; Griffiths et al., 1991; Rowlett et al., 2005). These studies employed i.v. self-administration procedures, in which subjects are trained to press a lever in order to receive an i.v. drug injection via a chronic venous catheter. Reinforcing effects of the drug are affirmed if it maintains a higher degree of self-administration compared to that observed under conditions of vehicle availability.

Although benzodiazepines do produce self-administration behavior above levels maintained by vehicle, they might be relatively weak reinforcers in general (Weerts et al., 1998b), and especially compared to other drugs of abuse. For instance, the peak levels of self-administration maintained by diazepam were below the peak levels maintained by the training drug methohexital, a short-acting barbiturate (Winger et al., 1975). This observation could be explained by a difference in pharmacokinetics between these drugs. Shorter-acting compounds have a tendency to maintain higher levels of self-administration compared to compounds with a longer duration of action (e.g., Griffiths et al., 1991).

Alternatively, other drugs of abuse may indeed be more reinforcing compared to benzodiazepines. In recent years, we have evaluated self-administration of benzodiazepine-type drugs and other types of drugs of abuse using progressive-ratio schedules of intravenous drug injection in monkeys. In this procedure, the response requirement increases across a session until responding stops, permitting the determination of "break point", which is defined as the last response requirement completed in a session. In our studies, drugs of abuse such as cocaine and opioid receptor agonists are typically studied at higher response requirements than benzodiazepine-type drugs. For example, the initial response requirement (IRR) of a progressive-ratio sequence used to study cocaine's reinforcing effects is 100 (e.g., Rowlett et al., 2002), whereas IRRs of 40 were used to evaluate benzodiazepine-type drugs (e.g., Rowlett et al., 2005). Recently, we have evaluated self-administration of zolpidem and the short-acting benzodiazepine midazolam under a relatively wide range of IRRs (Rowlett and Lelas, 2007), allowing us to make comparisons of the relative reinforcing strength of these drugs with stimulants and opioids under similar experimental conditions. As shown in Fig. 2, break

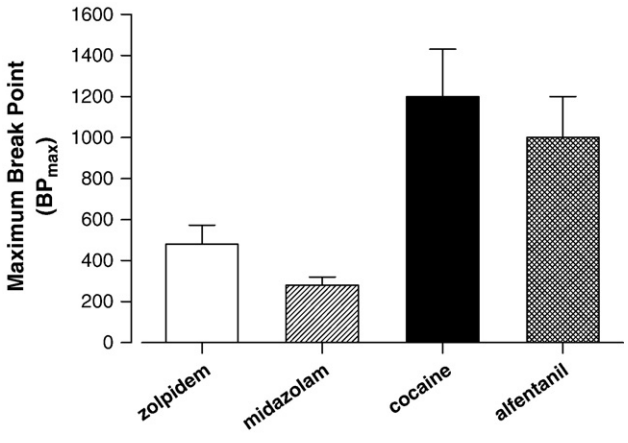

Fig. 2. Break points maintained by zolpidem, midazolam, cocaine, or alfentanil, in rhesus monkeys trained under a progressive-ratio schedule of intravenous drug delivery. Break point was defined as the maximum response requirement obtained in a session, and the data represent the maximum break points irrespective of dose tested (referred to as "BP_{max}"). Data are means ± SEM for $N = 4$ monkeys for each drug, and were obtained from Rowlett et al. (2005, 2002) and Rowlett and Lelas (2007).

points maintained by both cocaine and alfentanil, a selective mu opioid receptor agonist, were markedly higher than the break points maintained by zolpidem and midazolam. These data provide clear support for benzodiazepine-type drugs being weaker reinforcers than other drugs of abuse (cf. Woods et al., 1992).

3.2. Physical dependence following chronic treatment with benzodiazepine-type drugs

Prolonged use of benzodiazepine-type drugs can lead to physical dependence, which in turn may contribute to the abuse liability of these drugs (Ashton, 1991; Petursson, 1994). For example, abrupt cessation of benzodiazepine use after prolonged treatment at a therapeutic dose can result in a withdrawal syndrome (for review, see Griffiths and Weerts, 1997; Woods and Winger, 1995). Benzodiazepine withdrawal is characterized by many signs that are opposite to the therapeutic effects of benzodiazepines (e.g. anxiety, insomnia) and, in more severe cases, patients may experience seizures (Griffiths and Weerts, 1997; Mintzer and Griffiths, 2005; O'Brien, 2005). Comprehensive reviews discussing the evidence of physical dependence to benzodiazepines, as well as the factors that may influence the development of physical dependence to chronic benzodiazepine treatment can be found elsewhere (Griffiths and Weerts, 1997; Woods et al., 1992; Woods and Winger, 1995).

Physical dependence to a benzodiazepine-type drug is often measured in the laboratory as the emergence of characteristic withdrawal signs upon cessation of the drug that is reversed with subsequent drug administration (spontaneous withdrawal) or precipitated by administration of an antagonist, such as flumazenil (precipitated withdrawal; Woods et al., 1992). Controlled studies examining patients who use low therapeutic doses of benzodiazepines chronically have demonstrated that flumazenil can precipitate withdrawal symptoms (Bernik et al., 1991; Bernik et al. 1998; Harrison-Read et al., 1996; Mintzer et al., 1999). Likewise, precipitated withdrawal also has been observed in healthy human volunteers following daily exposure to a relatively high therapeutic dose of a benzodiazepine (Mintzer and Griffiths, 2005). In preclinical studies, the severity of withdrawal has been shown to be dose-dependent in non-human primates (Lukas and Griffiths, 1984) and dogs (Sloan et al., 1993).

Duration of treatment may also contribute to the severity of the withdrawal, although the empirical data are mixed. A study in healthy human volunteers demonstrated precipitated withdrawal as soon as 7 days after daily exposure to diazepam, but withdrawal severity did not increase with increased exposure (i.e., withdrawal symptoms were similar on days 7, 14, and 28; Mintzer and Griffiths, 2005). In contrast, a study undertaken in baboons concluded that the severity of withdrawal increased with the duration of treatment (Lukas and Griffiths, 1984).

Precipitated or spontaneous withdrawal from benzodiazepines in laboratory animals can be used to detect a negative affective or subjective state induced by withdrawal. For example, flumazenil administration conditioned a place aversion following chronic treatment with diazepam in rats (Allison et al., 2002). Similarly, spontaneous withdrawal from diazepam increased the amount of time spent in the drug-paired context of a conditioned place preference paradigm, and literally drove the animals away from the withdrawal-associated context (Souza-Pinto et al., 2007). It is not clear if these observations are manifestations of withdrawal-induced anhedonia or anxiety-like behavior, both of which have been implicated in the discontinuation of drug use (e.g., Lago and Kosten, 1994; Shippenberg et al., 2007), but one study has demonstrated the ability of antidepressants to reverse the escape deficit induced by diazepam withdrawal in a shock avoidance task (Lacerra et al., 1999).

The concerns about dependence following long-term treatment are becoming more prominent as the popularity of the newer benzodiazepine-type hypnotics is on the rise. Most of the newer hypnotic benzodiazepine-type drugs are relatively short-acting, raising concerns over the possibility of more severe withdrawal after chronic treatment (O'Brien, 2005; Woods and Winger, 1995). However, little evidence exists for a more severe withdrawal syndrome engendered by short-acting drugs. For example, short-acting benzodiazepines, such as midazolam, produced physical dependence similar in magnitude to longer-acting drugs such as chlordiazepoxide (Woods et al., 1992). Similarly, a review of hypnotic abuse liability led to the conclusion that the withdrawal observed after therapeutic doses of zolpidem (no information was available for zaleplon) was rated as intermediate, i.e. similar to conventional benzodiazepines (Griffiths and Johnson, 2005). Importantly though, clinical studies find consistently that not all patients develop physical dependence to benzodiazepine-type drugs (Woods et al., 1992).

3.3. Tolerance following chronic treatment with benzodiazepine-type drugs

In addition to the development of physical dependence, chronic benzodiazepine treatment can result in tolerance to some behavioral effects. It is important to note that the development of physical dependence does not require the development of tolerance, and that tolerance can occur in the absence of physical dependence (Woods et al., 1992). Moreover, the time course for the development of tolerance varies for different behavioral effects of benzodiazepine-type drugs. In humans, for example, tolerance develops rapidly to sedative effects and motor coordination deficits; whereas tolerance does not always develop to the anxiolytic or memory impairing effects of benzodiazepine-type drugs after long periods of use (Cowley et al., 1995; Griffiths and Weerts, 1997; Stoops and Rush, 2003). A clear gap in our knowledge about tolerance development is the extent to which the reinforcing effects of benzodiazepine-type drugs change over time, i.e. whether or not tolerance to the reinforcing effects of benzodiazepines develops after chronic exposure. Based on available information, tolerance to reinforcing effects of benzodiazepine-type drugs appears unlikely, since self-administration of midazolam or zolpidem was shown to be stable over relatively long durations of exposure (Weerts and Griffiths, 1998; Weerts et al., 1998b). Moreover, indirect evidence that tolerance to the reinforcing effects of benzodiazepines does not occur comes from the observation that long-term use by humans is not associated with

escalation in the ingested dose of drug across time (Griffiths and Weerts, 1997; Woods et al., 1992).

4. $GABA_A$ receptor contribution to abuse and dependence liability

Recently, selective pharmacological tools have been developed that allow investigators to probe the $GABA_A$ receptor mechanisms underlying behaviors engendered by benzodiazepine-type drugs (Dawson et al., 2005). For example, although the hypnotic benzodiazepine-type drugs zolpidem and zaleplon interact with the benzodiazepine-binding site on the $GABA_A$ receptor, they enhance GABA-mediated chloride currents in recombinant $GABA_A$ receptors containing α1 subunits more selectively than those containing α2 or α3 subunits (Hadingham et al., 1993; Sanna et al., 2002). These findings in addition to other accumulating behavioral data led investigators to formulate the hypothesis that α1$GABA_A$ receptors are critical mediators of the sedative effects of benzodiazepine-type drugs (McKernan et al., 2000; Platt et al., 2002; Rudolph et al., 1999). In contrast, ligands such as L-838,417 (McKernan et al., 2000) and TPA023 (Atack et al., 2006), lack intrinsic efficacy at α1$GABA_A$ receptors, and have been helpful in understanding the relationship of sedative vs. anxiolytic, myorelaxant, and anticonvulsant activity of these compounds (see Table 1). The following sections will review how pharmacological tools such as these have contributed to the current state of knowledge about the role of $GABA_A$ receptors in mediating behavior associated with the abuse liability of benzodiazepine-type drugs.

4.1. $GABA_A$ receptor subtypes and the reinforcing effects of benzodiazepine-type drugs

Although benzodiazepine-type drugs generally have relatively modest reinforcing effects, notable exceptions have been observed with the hypnotics zolpidem and zaleplon. In non-human primates, zolpidem self-administration was not only greater than conventional benzodiazepines, but it was comparable to behavior maintained by barbiturates (Griffiths et al., 1992; Rowlett et al., 2005; see also Fig. 2 for comparison of break points maintained by zolpidem vs. midazolam). Similarly, another study demonstrated that zaleplon was self-administered to the same extent as zolpidem (Ator, 2000). Both drugs display selectivity for the α1$GABA_A$ receptor, raising the possibility that this receptor subtype may be an important substrate for self-administration of benzodiazepine-type drugs (Ator, 2005; Griffiths et al., 1992; Rowlett et al., 2005).

Further support for a critical role for α1$GABA_A$ receptors in the reinforcing effects of benzodiazepine-type drugs was observed in studies involving benzodiazepine-type compounds with efficacy at specific $GABA_A$ receptor subtypes (Ator, 2005). TPA123 is a partial benzodiazepine-binding site agonist that exhibits low intrinsic efficacy in vitro at α1$GABA_A$ receptors, while TPA023 is similar in that it is also a partial agonist, but it lacks efficacy at α1$GABA_A$ receptors (i.e. it is essentially an antagonist in vitro at α1$GABA_A$ receptors) and exhibits very low efficacy at α2$GABA_A$ receptors (12% potentiation of GABA-mediated currents vs. 81% for diazepam; see Table 1 as well as Atack et al., 2006). In those studies, TPA123 functioned as a reinforcer in baboons trained to self-administer intravenous injections of cocaine, whereas TPA023 was ineffective (Ator, 2005). Together with the findings obtained with zolpidem and zaleplon, these results raise the possibility that a benzodiazepine-type compound's potential for abuse may be directly related to its efficacy in vitro at α1$GABA_A$ receptors.

Although the findings of Ator (2005) and our laboratory are seemingly contradictory, it may simply be the case that not enough data are available to make firm conclusions. Table 1 compares published in vitro receptor activity and self-administration results for TPA023 and L-838,417, zolpidem, and two non-selective benzodiazepines (diazepam and triazolam). In order to compare these results across studies using different methodologies and species, we developed a scale of zero, low, intermediate, and high degrees of self-administration based on previous work by Griffiths et al. (1991, 1992) and Ator (2005). As can be seen in Table 1, for these particular compounds, the available results are concordant across the two laboratories, and with only minor differences (e.g., Griffiths, Ator and colleagues showed greater reinforcing effectiveness of triazolam vs. diazepam, whereas we have not observed this difference consistently). Importantly,

Table 1
Non-selective and selective benzodiazepine-type drugs: relationship of receptor binding, intrinsic efficacy and relative reinforcing effectiveness

	Selective affinity [a]	Selective efficacy [a]				Baboon [b]	Rhesus monkey [c]
		α1	α2	α3	α5		
Diazepam	None	1.0	1.0	1.0	1.0	Low-intermediate	Intermediate
Triazolam	None	1.7	1.2	1.3	1.4	Intermediate	Intermediate
Zolpidem	α1>α2=α3>>α5	1.6	1.3	1.2	–	High	High
TPA023	None	0.01	0.15	0.38	0.11	0	NA [d]
L-838,417	None	0.02	0.53	0.48	0.68	NA [d]	Low

[a] Binding and efficacy data are from cloned human receptors (Atack et al., 2006; McKernan et al, 1995; Smith et al., 2001). Efficacy represents % potentiation of Cl− currents at an EC_{20} concentration of GABA, divided by the values obtained for diazepam (α1=71%, α2=81%, α3=88%, α5=57%).

[b] Relative reinforcing effectiveness, i.v. self-administration in baboons (Ator, 2005; Griffiths et al., 1991; Griffiths et al., 1992): 0 = not different from vehicle; low = below a mean of 4 injections/session; intermediate = 4–6 injections/session; high = 6–8 injections/session.

[c] Relative reinforcing effectiveness using a scale adapted from baboon studies (see Ator, 2005); i.v. self-administration in rhesus monkeys (Rowlett et al., 2005; except for triazolam, which is unpublished data from N=4 monkeys): 0 = mean 0–4 injections/session (not different from vehicle); low = 5–8 injections/session; intermediate = 9–12 injections/session; high = 13–20 injections/session.

[d] NA: not available.

critical information is missing, including tests of TPA023 self-administration in rhesus monkeys as well as tests of L-838,417 self-administration in baboons.

Regardless of our gaps in knowledge concerning self-administration of subtype-selective compounds, the comparisons in Table 1 provide information to draw preliminary conclusions and formulate hypotheses. First, no clear relationship between a compound's affinity or in vitro intrinsic efficacy at $\alpha5GABA_A$ receptors and its subsequent relative reinforcing effectiveness was observed. For example, while both zolpidem and TPA023 lack activity at the $\alpha5GABA_A$ receptor, zolpidem was self-administered robustly whereas TPA023 lacked reinforcing effects. Second, it appears that action at $\alpha1GABA_A$ receptors is not necessary for reinforcing effects. The primary evidence for this hypothesis is the results with L-838,417, which has no efficacy at $\alpha1GABA_A$ receptors and did function as a reinforcer. Because TPA023 also exhibits no efficacy at $\alpha1GABA_A$ receptors and was not self-administered, more conclusive studies need to be undertaken in which compounds with different degrees of activity at $\alpha1GABA_A$ receptors are evaluated. Finally, intrinsic efficacy may play a key role in the differences in reinforcing effectiveness among the compounds. This hypothesis is supported by the observation that low intrinsic efficacy in general appears to predict lower reinforcing efficacy, irrespective of action at the different receptor subtypes. Moreover, based on comparisons between the findings with TPA023 and L-838,417, it appears that a compound may require a degree of efficacy at $\alpha2GABA_A$ receptors greater than ~10%, and/or at least ~40% efficacy at $\alpha3GABA_A$ receptors, in order to have reinforcing effects. This latter idea assumes that action at $\alpha1GABA_A$ receptors is not necessary for reinforcing effects, as described above.

Although differences in binding selectivity and intrinsic efficacy at $GABA_A$ receptors provide intriguing hypotheses for the observed differences in self-administration compiled in Table 1, some methodological factors must also be considered. For example, the baboons in the Ator (2005) studies were trained to self-administer under a cocaine baseline, whereas the rhesus monkeys in Rowlett et al. (2005) were trained to self-administer intravenous injections of the short-acting barbiturate, methohexital. Moreover, self-administration by the baboons employed a fixed-ratio schedule of intravenous drug delivery, contrasting with the progressive-ratio used in the rhesus monkey report (see Griffiths et al., 1991; Rowlett et al., 2005 for comparisons of the procedures). As discussed above, the history of drug use by human subjects in a major determinant of the reinforcing effects of benzodiazepines. Some evidence exists for a similar phenomenon in the animal literature. In this regard, a previous study has shown that the number of rhesus monkeys that self-administered diazepam was significantly lower when self-administration was trained with cocaine compared to pentobarbital (Bergman and Johanson, 1985). The extent to which differences in baseline training conditions influenced the findings in Table 1 is unknown, and underscores the need for more research on not only pharmacological, but behavioral factors underlying benzodiazepine self-administration.

Another key factor that deserves consideration in explanations of the differences in reinforcing effectiveness among the compounds in Table 1 is pharmacokinetics. While little has been published regarding the pharmacokinetic parameters of TPA023 and L-838,417 following intravenous administration in monkeys, L-838,417 is purported to have a relatively short half-life similar to that of midazolam (Rowlett et al., 2005; J.R. Atack, personal communication). In contrast, TPA023's duration of receptor occupancy in rodents suggests that this compound may be relatively long-acting (Atack et al., 2006). These findings suggest that TPA023 might not maintain self-administration behavior due to its long duration of action. However, other compounds with a long duration of action (e.g. diazepam) clearly are self-administered under the procedures used by both Ator (2005) and Rowlett et al. (2005). In fact, onset of action may be the most important pharmacokinetic factor that determines the degree of reinforcing effects of abused drugs (Griffiths and Weerts, 1997), but empirical information regarding the onset of action for TPA023 and L-838,417 is not yet available.

4.2. $GABA_A$ receptor subtypes and physical dependence on benzodiazepine-type drugs

Withdrawal from benzodiazepine-type drugs has been characterized extensively in both humans and non-human animals, but the underlying mechanisms of benzodiazepine physical dependence have not been determined (Perrault et al., 1992; Wafford, 2005). A study using a drug discrimination model of withdrawal in rhesus monkeys has provided preliminary evidence that the acute effects and withdrawal-associated effects of benzodiazepines might be mediated via different mechanisms (McMahon et al., 2001). In this drug discrimination model of withdrawal, monkeys were treated chronically with diazepam and trained to discriminate flumazenil from vehicle injections (presumably a discrimination based on interoceptive cues associated with precipitated withdrawal). These authors demonstrated that the potencies of a series of benzodiazepines and related compounds to attenuate the withdrawal-inducing effects of flumazenil did not correlate with the potencies of these drugs to engender benzodiazepine-like discriminative stimulus effects in non-dependent monkeys. Thus, these findings suggest that distinct receptor mechanisms underlie physical dependence compared to benzodiazepine-related interoceptive effects in non-dependent subjects (McMahon et al., 2001).

As with reinforcing effects, the $\alpha1GABA_A$-selective agonist zolpidem provides a unique opportunity to probe the contribution of $\alpha1GABA_A$ receptors to the physical dependence on benzodiazepine-type drugs. However, it has been unclear the extent to which chronic treatment with this selective compound induces physical dependence. Studies examining chronic treatment with zolpidem in mice (Elliott and White, 2000; Perrault et al., 1992; VonVoigtlander and Lewis, 1991), as well as survey and epidemiological data of patients who had used zolpidem (Jaffe et al., 2004; Soyka et al., 2000), have suggested a reduced propensity to induce physical dependence compared with classical benzodiazepines. Empirical studies in non-human primates, however, have found that zolpidem can engender a withdrawal syndrome that is quite similar to that observed after chronic treatment with benzodiazepines (Griffiths et al., 1992; Weerts et al., 1998a; Weerts and

Griffiths, 1998). In fact, this finding is consistent with human case reports (see review by Hajak et al., 2003; Liappas et al., 2003; Quaglio et al., 2005), and suggests that α1GABA$_A$ receptors do indeed play a role in the development of physical dependence. Lending further support for this hypothesis, another α1GABA$_A$-selective agonist, zaleplon, engendered a withdrawal syndrome similar to zolpidem in baboons (Ator et al., 2000).

With respect to the α2GABA$_A$, α3GABA$_A$, and/or α5GABA$_A$ receptors, compounds with selective efficacy at these subtypes have provided the opportunity to evaluate their roles in physical dependence induced by benzodiazepine-type drugs. Using compounds that vary in both selectivity and efficacy at GABA$_A$ receptor subtypes, a recent study evaluated the degree to which chronic treatment engendered seizures in mice following administration of the inverse agonist FG-7142 (Mirza and Nielsen, 2006). Chronic treatment with zolpidem, as well as the selective compounds L-838,417 (partial agonist at α2GABA$_A$, α3GABA$_A$, and α5GABA$_A$ receptors) and SL651498 (full agonist at α2GABA$_A$ and α3GABA$_A$ receptors, partial agonist at α1GABA$_A$ and α5GABA$_A$ receptors), did not result in seizures following FG-7142 administration. Similarly, chronic treatment with TPA023 (partial agonist at α2GABA$_A$, α3GABA$_A$, and α5GABA$_A$ receptors) also did not result in FG-7142-induced seizures in mice (Atack et al., 2006). Together, these findings suggest that physical dependence does not occur with subtype-selective compounds. Rather, these data suggest that an interaction with all GABA$_A$ receptor subtypes is required for physical dependence to develop, at least as measured by inverse agonist-induced seizures. This is not an unlikely hypothesis, given that physical dependence is associated with a plethora of behavioral effects. Of note, chronic treatment with non-selective partial agonists did not result in FG-7142-induced seizures, suggesting that relatively high efficacy also might be a requirement for the development of physical dependence (Mirza and Nielsen, 2006).

5. Neuroadaptations following benzodiazepine administration: what is the biochemical basis of abuse-related effects?

Recent research efforts have been aimed at delineating the GABA$_A$ receptor mechanisms that underlie benzodiazepine-type drug-induced behavior, but relatively little is known about the downstream events that occur between allosteric modulation of the receptor by these drugs and the subsequent behavioral outcome. With respect to their abuse potential, the neurochemical, cellular, and molecular sequelae of events that occur following administration of benzodiazepine-type drugs are largely and surprisingly ignored in the vast literature aimed at understanding the neuroadaptations associated with addiction-like behavior. Instead, the preponderance of data surrounding the rewarding properties of drugs of abuse has focused on stimulants and opioids (e.g. for review, see Kalivas and Volkow, 2005). The following sections will discuss briefly the neuroadaptive changes that occur following the interaction between benzodiazepine-type drugs and GABA$_A$ receptors, and how those changes may be related to the observable behavior associated with their abuse potential, namely tolerance and dependence.

5.1. GABA$_A$ receptor regulation following benzodiazepine administration

Many studies have demonstrated GABA$_A$ receptor down regulation following chronic exposure to benzodiazepine agonists (e.g., see review by Klein and Harris, 1996). Although the number of receptors at the cell surface may not change (Shibla et al., 1981), their ability to bind benzodiazepines (Miller et al., 1989; Sher et al., 1983) and enhance GABA neurotransmission (Gallager et al., 1984; Hu and Ticku, 1994; Roca et al., 1990; Wu et al., 1994a) becomes compromised. For instance, a 40–80% decrease in allosteric binding site coupling has been demonstrated within days of drug exposure in neuronal cultures (Hu and Ticku, 1994; Roca et al., 1990), and over the course of several weeks in brain homogenates prepared from animals exposed chronically (Gallager et al., 1984; Hernandez et al., 1989). Similarly, chronic benzodiazepine treatment leads to a decrease in postsynaptic GABA sensitivity as measured by iontophoretic application of GABA in cell preparations (Crawley et al., 1982; Gallager et al., 1984). Moreover, these changes in receptor function are benzodiazepine-specific, as administration of the benzodiazepine antagonist flumazenil was able to block the uncoupling and reverse the subsensitivity (Gallager et al., 1984; Roca et al., 1990). Together, these findings indicate that chronic treatment with benzodiazepines reduces the function of GABA$_A$ receptors, in turn, requiring more agonist to achieve the desired result. Thus, these adaptations appear to be reasonable neuronal correlates of tolerance.

Prolonged exposure to benzodiazepines also may result in tolerance and/or dependence as a function of use-dependent changes in receptor subunit composition. Modifications in the expression of genes encoding various subunits of the GABA$_A$ receptor have been demonstrated in a number of studies. The most consistent changes that have been reported to date include down regulation of the α1, α5, and γ2 subunit mRNAs by approximately 30–50% (Follesa et al., 2001; Heninger et al., 1990; Holt et al., 1996, 1997; Impagnatiello et al., 1996; Longone et al., 1996; Wu et al., 1994b). While these studies either did not measure (Follesa et al., 2001; Heninger et al., 1990; Holt et al., 1997; Longone et al., 1996) or did not observe (Holt et al., 1996; Impagnatiello et al., 1996; Wu et al., 1994b) any benzodiazepine-induced changes in α2 or α3 subunit transcripts (or β subunits for that matter), most studies examined cortical areas which are typically more enriched with the α1GABA$_A$ receptor subtype (60% vs. 10–20%; for review, see Möhler, 2006). The one exception was reported by Holt et al. (1996), demonstrating a decrease in α3 subunit transcripts following 2 weeks of diazepam treatment.

Discontinuation of long-term treatment with diazepam resulted in a flumazenil-sensitive increase in both mRNA and protein levels of the α4 subunit (Follesa et al., 2001). Despite their lack of affinity for benzodiazepines and low expression levels throughout the brain (Pirker et al., 2000), these are significant findings in that the concomitant change in protein levels reflects de novo synthesis of α4GABA$_A$ receptors (Follesa et al., 2001), supporting the hypothesis that benzodiazepines induce a shift in GABA$_A$ receptor composition. Further, since these alterations often involve the α subunits which are

presumed to be responsible for conferring different benzodiazepine sensitivity and pharmacological effects (McKernan and Whiting, 1996; Pritchett et al., 1989; Rudolph et al., 2001), this $GABA_A$ receptor regulation could have a significant impact on behavior. Although the behavioral consequences of these alterations remain to be elucidated, especially in light of the differences observed across brain regions and with different treatment regimens (e.g., Ramsey-Williams and Carter, 1996), what has become apparent is that chronic treatment with and subsequent withdrawal from benzodiazepines produces not only different constellations of behaviors from one another, but also a different pattern of changes among the $GABA_A$ receptor subunits (e.g., Miller, 1991).

5.2. Benzodiazepine effects on neurotransmission within the reward circuitry

As a result of a large body of research undertaken over the past 50 years, much has been learned about the brain regions, connectivity, and neurochemistry involved in mediating the rewarding or pleasurable effects of drugs of abuse. The most critical component of the reward circuitry traditionally has been the mesolimbic dopamine system, which is comprised of cell bodies originating in the ventral tegmental area and projecting to and terminating in the nucleus accumbens and extended amygdala. However, plenty of evidence has suggested prominent roles for the ventral pallidum, hippocampus, hypothalamus, pedunculopontine nucleus, and prefrontal cortex in mediating the reinforcing effects of drugs of abuse (see reviews by Bardo, 1998; Kalivas and Volkow, 2005; Leshner and Koob, 1999).

Sufficient evidence has been provided here to assert that benzodiazepines are drugs of abuse. However, unlike most other drugs of abuse (e.g., Di Chiara and Imperato, 1988) benzodiazepine-type drugs do not simply increase extracelluar dopamine levels in the nucleus accumbens. Instead, benzodiazepine-site compounds have effects on accumbal dopamine that differ markedly depending on their intrinsic efficacy. For instance, extracellular dopamine levels are decreased by administration of the full benzodiazepine agonists diazepam, midazolam, or flurazepam (Finlay et al., 1992; Invernizzi et al., 1991; Murai et al., 1994; Zetterström and Fillenz, 1990), as well as by the partial agonist imidazenil (Motzo et al., 1997). In contrast, extracellular levels of dopamine are increased by administration of inverse agonists of the benzodiazepine-binding site on the $GABA_A$ receptor such as the anxiogenic β-carboline derivatives FG 7142 and β-CCE (McCullough and Salamone, 1992; Murai et al., 1994). These effects have been blocked by pretreatment with the benzodiazepine-binding site antagonist flumazenil (Murai et al., 1994), indicating that $GABA_A$ receptors contribute to this particular modulation of mesolimbic dopaminergic neurotransmission.

Based on the findings that both natural rewards and most drugs of abuse stimulate activity within the nucleus accumbens (see review by Carelli, 2002; but also see Salamone et al., 2007), it can be hypothesized that drugs of abuse must be biochemical homologues of some critical aspect of naturally rewarding stimuli. However, as the work with benzodiazepine-site agonists has demonstrated, stimulation of mesolimbic dopamine pathways cannot be the only factor that determines abuse and dependence liability. Inverse agonists especially are not known to be rewarding, but appear to be anxiogenic (Thiébot et al., 1988), and have been proposed to model core components of schizophrenia (Sarter et al., 2001) as well as stress (Motzo et al., 1997). Therefore, the abuse potential of benzodiazepine-type drugs must be a function of something other than stimulating dopamine release directly (Di Chiara et al., 1993; Finlay et al., 1992; Tsankova et al., 2007). This idea is supported by a compilation of studies suggesting that various drugs of abuse may activate the reward pathways differentially (Bardo, 1998). For example, although heroin is most certainly a drug of abuse, it appears to mediate its rewarding effects via a neural system separate from that of cocaine (Ettenberg et al., 1982).

Currently it is not clear if activation of the different anatomical structures and neurotransmitter systems ultimately converges on one output system to mediate the reinforcing effects of various drugs of abuse (Bardo, 1998). Specifically, it is unknown how these interactions engender benzodiazepine-induced abuse-related behaviors. Indeed, many of the neuroadaptations that contribute to the addictive processes following administration of drugs of abuse in general have been shown to occur in meso-cortico-limbic circuits involving not only dopamine, but GABA and glutamate (Baler and Volkow, 2006; Kalivas and Volkow, 2005). As discussed previously, there are a number of adaptations that occur at the level of the $GABA_A$ receptor (i.e., downregulation, allosteric uncoupling, subsensitivity, etc.) following administration of benzodiazepines. However, they do not appear to make an impact significant enough to account entirely for such complex behaviors as those associated with abuse potential (Pratt et al., 1998). Instead, non-GABAergic mechanisms must also contribute to the abuse and dependence liability of benzodiazepine-type drugs; accordingly, glutamatergic mechanisms are involved. For instance, the acquisition of a diazepam-induced conditioned place preference was attenuated by pretreatment with a glutamate receptor antagonist (Gray et al., 1999), suggesting that glutamate contributes to the rewarding or reinforcing effects of benzodiazepines.

With respect to tolerance and dependence, glutamate has been implicated in the hypothesis that in order to compensate for benzodiazepine-induced enhancement of inhibition, excitatory mechanisms become more sensitive. This sensitivity is manifested as over-activity upon withdrawal (Little et al., 1988; Stephens, 1995). Further support for glutamatergic mechanisms in these behaviors has been demonstrated by the disruption of the development of tolerance and dependence (Steppuhn and Turski, 1993) as well as the effects of withdrawal (Souza-Pinto et al., 2007) following administration of glutamate receptor antagonists. Moreover, both NMDA and AMPA receptors have been shown to be regulated following chronic benzodiazepine treatment. Specifically, cortical levels of the NR1 and NR2B, but not NR2A, subunits of the NMDA receptor (Tsuda et al., 1998) and the GluR1 subunit of the AMPA receptor were increased in diazepam-withdrawn rats compared to controls (Izzo et al., 2001). Similarly, in rats withdrawn from flurazepam, AMPA receptor-mediated miniature excitatory postsynaptic current amplitude was increased in hippocampal CA1 neurons (Van Sickle et al., 2004;

Xiang and Tietz, 2007). A 50% enhancement in AMPA receptor function was attributed to an increase in GluR1 protein trafficking from the endoplasmic reticulum and subsequent incorporation into membranes (Song et al., 2007), while NMDA receptor-mediated currents were reduced in this brain region (Van Sickle et al., 2004; Xiang and Tietz, 2007). In contrast to those studies, expression of the AMPA receptor subunits was decreased in the amygdala (GluR1 and GluR2) and limbic regions (GluR1; Allison and Pratt, 2006). Interestingly, the contribution of AMPA and NMDA receptor mechanisms may be regulated temporally such that each is involved at specific time points during the expression of withdrawal and development of tolerance, respectively (Izzo et al., 2001). Similar findings have been observed in long-term potentiation and kindling, which like the neuroadaptive processes associated with the consumption of drugs of abuse, are forms of synaptic plasticity (Baudry, 1986). However, whether or not the involvement of glutamate in the abuse and dependence liability of benzodiazepine-type drugs is similar to that observed with other drugs of abuse (e.g. psychostimulants), remains relatively unexplored.

5.3. Intracellular signaling molecule adaptations following benzodiazepine administration

In addition to benzodiazepine-induced receptor neuroadaptations, a recent study implemented microarray analysis to evaluate systematically the downstream signaling events following acute exposure to diazepam (Huopaniemi et al., 2004). Results demonstrated that in wild-type mice, diazepam reduced the transcripts of genes involved in regulating synaptic functions and plasticity, such as calcium/calmodulin-dependent kinase IIα (CaMKIIα; for review, see Soderling et al., 2001) and brain-derived neurotrophic factor (BDNF; for review, see Binder and Scarfman, 2004). Activation of CaMKIIα has been shown previously to be involved in the phosphorylation of the α1 subunit of the $GABA_A$ receptor, which subsequently regulated the binding of allosteric modulators to the receptor (Churn et al., 2002), and enhanced the inhibitory synaptic potential (Wang et al., 1995). Down regulation of CaMKIIα following exposure to diazepam, therefore, may contribute to the overall down regulation of the $GABA_A$ receptor and GABA sensitivity observed following prolonged exposure to benzodiazepines. Similarly, since BDNF has been shown to regulate the expression of cell surface $GABA_A$ receptors (Brünig et al., 2001; Mizoguchi et al. 2003), down regulation of BDNF may reduce $GABA_A$ receptor turnover. Although this study examined only an acute dose of diazepam (Huopaniemi et al., 2004), other evidence exists demonstrating that a single exposure to diazepam can have significant effects on $GABA_A$ receptor function (Holt et al., 1999).

Interestingly, the transcriptional regulation of those genes, as well as approximately 50 others, appears to be mediated by an α1$GABA_A$ receptor-dependent mechanism (Huopaniemi et al., 2004). Compared to wild-type mice, the observed changes in transcript levels following administration of diazepam were not exhibited in mice that were mutated in order to render the α1$GABA_A$ receptor insensitive to diazepam (Rudolph et al., 1999). These findings may have implications for the signaling events associated with the sedative actions of benzodiazepine-type drugs, since there is a body of evidence suggesting that α1$GABA_A$ receptors are responsible for mediating these effects (McKernan et al., 2000; Platt et al., 2002; Rudolph et al., 1999). Similarly, these signaling cascades may be involved in the abuse-related effects of benzodiazepines since α1$GABA_A$ receptors appear to be intricately involved in their reinforcing effects (Ator, 2005; Griffiths et al., 1992; Rowlett and Lelas, 2007; Rowlett et al., 2005). Indeed, both CamKIIα (e.g., Licata et al., 2004; Narita et al., 2004) and BDNF (e.g., Butovsky et al. 2005; Corominas et al., 2007) have been demonstrated to play prominent roles in the plasticity believed to underlie the addictive potential of drugs of abuse. Together, these results are just some examples of how intracellular events may function as the liaison between allosteric modulation of $GABA_A$ receptors by benzodiazepines and behavior—again, a relatively unexplored area of research.

6. Summary and conclusions

Of the diverse types of ligands that act at the $GABA_A$ receptor, the benzodiazepines and related drugs are unique in having widespread clinical use and the liability for abuse and dependence. Laboratory findings suggest that benzodiazepine-type drugs have reinforcing effects both in human and non-human subjects, and recent epidemiological data suggests that abuse of benzodiazepine-type drugs may be on the rise.

Recent research has begun to explore the role of $GABA_A$ receptor subtypes in the reinforcing effects of benzodiazepine-type drugs, and unlike other behavioral effects (e.g. motor coordination deficits) reinforcing effects are not easily attributed to a single receptor subtype. Perhaps the most firm conclusion at this point is that α1$GABA_A$ receptors are not necessary for self-administration of benzodiazepine-type compounds, although they might be sufficient. Research with more selective compounds that are full agonists for different subtypes clearly is needed to resolve some of the issues with our understanding of the reinforcing effects of benzodiazepine-type drugs.

In addition to reinforcing effects, it is well-documented that chronic exposure to benzodiazepines results in physical dependence, characterized by a withdrawal syndrome. Regarding receptor mechanisms, initial studies suggested that α1$GABA_A$ selective agonists are devoid of physical dependence liability, whereas the most recent findings in humans and non-human primates indicate that long-term use of these compounds can be associated with physical dependence. Moreover, studies examining benzodiazepine-induced changes in receptor composition primarily have demonstrated alterations in the α1 subunit. Accordingly, preliminary results suggest that compounds with selectivity for α2$GABA_A$, α3$GABA_A$, and/or α5$GABA_A$ receptors do not induce physical dependence, although these findings are complicated by the relatively low intrinsic efficacy of these ligands. As with reinforcing effects, systematic studies with selective compounds having relatively high intrinsic efficacy at particular subtypes should shed light on these important mechanistic issues.

In conclusion, the literature reviewed suggests that the abuse potential of benzodiazepine-type drugs is becoming an increasingly important issue to address on many levels. In the future, the epidemiology of benzodiazepine-type drug abuse should encourage empirical investigations regarding the behavioral phenomena associated with abuse potential, i.e. reinforcing effects, manifestations of tolerance and dependence. The development of new ligands should facilitate a better understanding of the $GABA_A$ receptor mechanisms underlying these behavioral effects. As new compounds become available, issues of cross-tolerance also need to be investigated. For example, it is not known the extent to which there is cross-tolerance between the new subtype-selective benzodiazepine ligands and conventional benzodiazepines (or alcohol for that matter) with respect to either the therapeutic or limiting effects of these drugs. Further, these pharmacological tools should be used to probe more comprehensively the cellular and molecular events that accompany the abuse-related effects associated with the administration of benzodiazepine-type drugs. Together, these investigations will help elucidate how benzodiazepine-type drugs exert their abuse and dependence liability, thus informing drug design strategies in order to develop safer and more effective anxiolytics and sleep-aids.

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