Selective Antagonism of $GABA_A$ Receptor Subtypes: An In Vivo Approach to Exploring the Therapeutic and Side Effects of Benzodiazepine–Type Drugs

By James K. Rowlett, PhD, James M. Cook, PhD, Angela N. Duke, MA, and Donna M. Platt, PhD

FOCUS POINTS

- Benzodiazepines (BZs) are useful for the treatment of anxiety and sleep disorders, but their use is limited by undesirable side effects such as daytime drowsiness, loss of coordination, and liability for addiction.
- BZs act at multiple subtypes of the γ-aminobutyric acid type A ($GABA_A$) receptor, and recent evidence suggests that different behavioral effects of BZ-type drugs can be associated with subtypes of the $GABA_A$ receptor containing distinct α subunits.
- Pharmacologic antagonists, which bind to a receptor but have no action, can be useful for identifying the role of receptor subtypes in awake and behaving organisms.
- The development of subtype-selective antagonists for $GABA_A$ receptor, such as β-carboline-3-carboxylate-t-butyl ester, which targets the $GABA_A$ α1 receptor, can facilitate efforts to understand BZ action in nonhuman and human primates alike.
- Antagonists have proven useful as radiotracers for positron emission tomography and single positron emission computed tomography imaging, and have both experimental and clinical applications. Development of radiotracers based on subtype selective antagonists should provide more powerful experimental tools and may help refine radiotracers as diagnostic tools.

ABSTRACT

Benzodiazepines (BZs) are clinically used as anxiolytic, hypnotic, anticonvulsant, and antispasmodic drugs. Research using transgenic mouse models has suggested that the effects of BZs involve multiple subtypes of the γ-aminobutyric acid type A ($GABA_A$) receptor, identified by specific α subunits (α_1, α_2, α_3, α_5). This review discusses the experimental uses of β-carboline-3-carboxylate-t-butyl ester (βCCT), a drug that binds preferentially to the $GABA_A$ α1 subtype but exerts no action (ie, is a pharmacologic antagonist at the $GABA_A$ α1 subtype receptor). βCCT blocks the anxiolytic-like effects of BZs, although studies in primates suggests this antagonism may reflect multiple receptor populations. βCCT antagonized the ataxic but not muscle relaxant effects of BZs, a finding that implicates the $GABA_A$ α_1 subtype receptor in ataxia but not muscle relaxation. The potential clinical utility of βCCT is discussed, both in terms of treatment (ie, hepatic encephalopathy) and as a diagnostic imaging agent. Altogether, these results indicate that subtype-selective antagonists represent a useful approach to studying receptor mechanisms underlying the behavioral effects of BZ-type drugs.

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INTRODUCTION

Benzodiazepines (BZs) are used clinically to treat anxiety disorders and insomnia, and are used as muscle relaxants and antiseizure medications. The therapeutic use of BZs is constrained, however,

Dr. Rowlett is assistant professor of psychobiology in the Department of Psychiatry and the Division of Behavioral Biology at the New England Primate Research Center at Harvard Medical School in Southborough, Massachusetts. Dr. Cook is University Distinguished Professor in the Department of Chemistry at the University of Wisconsin-Milwaukee. Ms. Duke is a graduate student in the Neuroscience and Behavior Program at the University of Massachusetts-Amherst and Division of Behavioral Biology at the New England Primate Research Center at Harvard Medical School. Dr. Platt is instructor of psychobiology in the Department of Psychiatry and Division of Behavioral Biology at the New England Primate Research Center at Harvard Medical School.

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Please direct all correspondence to: James K. Rowlett, PhD, Harvard Medical School, New England Primate Research Center, One Pine Hill Drive, Box 9102, Southborough, MA, 01772; Tel: 508-624-8036, Fax: 508-624-8197; E-mail: james_rowlett@hms.harvard.edu.

by a number of unwanted side effects, including daytime drowsiness, impaired motor coordination, amnesia, and potential for abuse and dependence.[1-3] In recent years, the illicit diversion and subsequent abuse of prescription BZs has increased along with the popularity of these drugs in the "club drug" culture.[4,5] Because of the therapeutic benefit of BZs, however, development of novel BZs with reduced side effects is an important area of pharmaceutical research.

In the 40 years since the development of diazepam, tremendous strides have been made in understanding how BZs exert their diverse behavioral effects. Research in the 1970s[6] showed that BZs enhance the ability of the neurotransmitter γ-aminobutyric acid (GABA) to stimulate chloride ion flux through the GABA type A ($GABA_A$) receptor complex (ie, BZs are "positive allosteric modulators" of GABA). In the past two decades, it has become clear that the $GABA_A$ receptor can exist as multiple subtypes in the central nervous system (CNS), and that the diverse behavioral effects of BZ-type drugs may reflect the actions of these subtypes (see below). In turn, the existence of multiple subtypes of the $GABA_A$ receptor has given hope that new targets for improved therapeutic BZs may be developed. A thorough understanding of the relationship of multiple $GABA_A$ receptor subtypes and the behavioral pharmacology of BZ-type drugs is the foundation for development of new and improved BZ-type drugs.

MULTIPLE $GABA_A$ RECEPTOR SUBTYPES AND EFFECTS OF BZ LIGANDS

Molecular biological studies have shown that the $GABA_A$ receptor ionophore is a pentamer composed of at least three distinct subunits. Receptors sensitive to modulation by BZ ligands are those that contain α, β, and γ subunits.[3,7] Moreover, multiple subtypes of each of these subunits have been identified, raising the possibility of considerable $GABA_A$ receptor heterogeneity.[8] Based on a number of different findings, the existence of multiple BZ receptor subtypes has been proposed.[3,8,9] Early studies identified two main subtypes of BZ receptors, referred to as BZ1 and BZ2 receptors (also referred to as ω1 and ω2 receptors) based on anatomical and pharmacological findings. Recent studies,[3,8,9] however, have found that BZ1 and BZ2 receptors are associated with four different subtypes of the α subunit (Table 1). Conventional BZs characteristically bind to $GABA_A$ receptors containing α_1, α_2, α_3, and α_5 subunits, but are inactive at receptors containing α_4 and α_6 subunits. Of these receptor subtypes, $GABA_A$ receptors containing the α1 subunit ($GABA_A$ α_1 receptors) have a pharmacological profile characteristic of BZ1 receptors, whereas BZ2 receptors appear to comprise a heterogeneous population containing $GABA_A$ α_2, $GABA_A$ α_3, and $GABA_A$ α_5 receptors (Table 1).

Considerable effort has focused on the development of compounds that target specific BZ receptor subtypes. Some success has been achieved in synthesizing compounds that bind preferentially to $GABA_A$ α_1 receptors, including zolpidem, zaleplon, CL 218,872, and the recently developed indiplon.[10] Using a different approach, McKernan and colleagues[11] and Griebel and colleagues[12] described efforts to develop novel compounds that exhibit preferential agonist activity (ie, "functional selectivity") at $GABA_A$ receptor subtypes. For example, the compound L-838,417 has preferential agonist activity for $GABA_A$ receptors containing α_2, α_3, and α_5 subunits, but is an antagonist at $GABA_A$ α_1 receptors.[11,13] This review will focus on a third approach: the development of receptor subtype-preferring antagonists (ie, drugs that bind preferentially to a $GABA_A$ receptor subtype but lack agonist activity). We will describe the development of β-carboline-3-carboxylate-t-butyl ester (βCCT), a β-carboline compound that binds preferentially to the $GABA_A$

TABLE 1. **SUMMARY OF THE MAJOR $GABA_A$ RECEPTOR SUBTYPES IN THE CNS**[7]

Subtype	% Total $GABA_A$ Receptors	Anatomical Distribution	Previous Nomenclature
$GABA_A$ α_1	~50%	Ubiquitous	BZ1/ω_1
$GABA_A$ α_2	15% to 20%	Cortex, limbic system, spinal cord	BZ2/ω_2
$GABA_A$ α_3			
$GABA_A$ α_5	<5%	Hippocampus	

Adapted from McKernan RM, Whiting PJ. Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci*. 1996;19:139-143.

$GABA_A$=γ-aminobutyric acid type A; CNS=central nervous system %=percentage; BZ=benzodiazepine.

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α_1 subtype. Findings with this compound will be compared with other pharmacological approaches, as well as recent results obtained with transgenic mouse models. Although the value of a selective antagonist arguably is primarily as an experimental tool, we will conclude our review with potential clinical applications for this subtype-selective compound.

SUBTYPE-SELECTIVE GABA$_A$ ANTAGONISTS: βCCT

As allosteric modulators, BZ-type drugs act on chloride flux over a continuum from positive to negative modulation, with antagonists existing theoretically at a point on the continuum with zero intrinsic efficacy (Figure 1). Thus, an antagonist would bind to a receptor, exert no effect, and displace compounds with intrinsic efficacy from the receptor. The prototype BZ antagonist is flumazenil (also designated in the literature as Ro15-1788) for which intrinsic efficacy at all BZ-sensitive GABA$_A$ subtypes is relatively low but not zero. To date, no compound has been characterized that exhibits zero efficacy at all subtypes, raising the possibility that a compound labeled as an "antagonist" may indeed exhibit functional activity given the right circumstances.

Development and Characterization of βCCT

The synthesis and initial behavioral characterization of βCCT was first described by Shannon and colleagues.[14] In this study, βCCT did not induce seizures and was not proconvulsant; had no motor-impairing effects; and did not alter behavior in a model of anxiolysis. However, βCCT attenuated the anticonvulsant and anxiolytic-like effects of diazepam without altering the motor-impairing effects of this BZ, a pattern of findings believed at that time to reflect selective antagonism at BZ1 receptors. Although this interpretation has been called into question in recent years, research conducted with cloned GABA$_A$ receptors containing α_1, α_2, α_3, and α_5 subunits supported the idea that βCCT possesses BZ1 selectivity[15] (Figure 2A). βCCT is at least 20-fold selective for the GABA$_A$ α_1 receptor compared with the GABA$_A$ α_2 and GABA$_A$ α_3 receptors, with a >100-fold difference between GABA$_A$ α_1 and GABA$_A$ α_5 receptors, making this compound one of the most selective BZ-site ligands identified to date.[15,16] As additional evidence for the subtype selectivity of βCCT, Griebel and colleagues[17] showed that βCCT was more potent in blocking the GABA-potentiating effects of a BZ

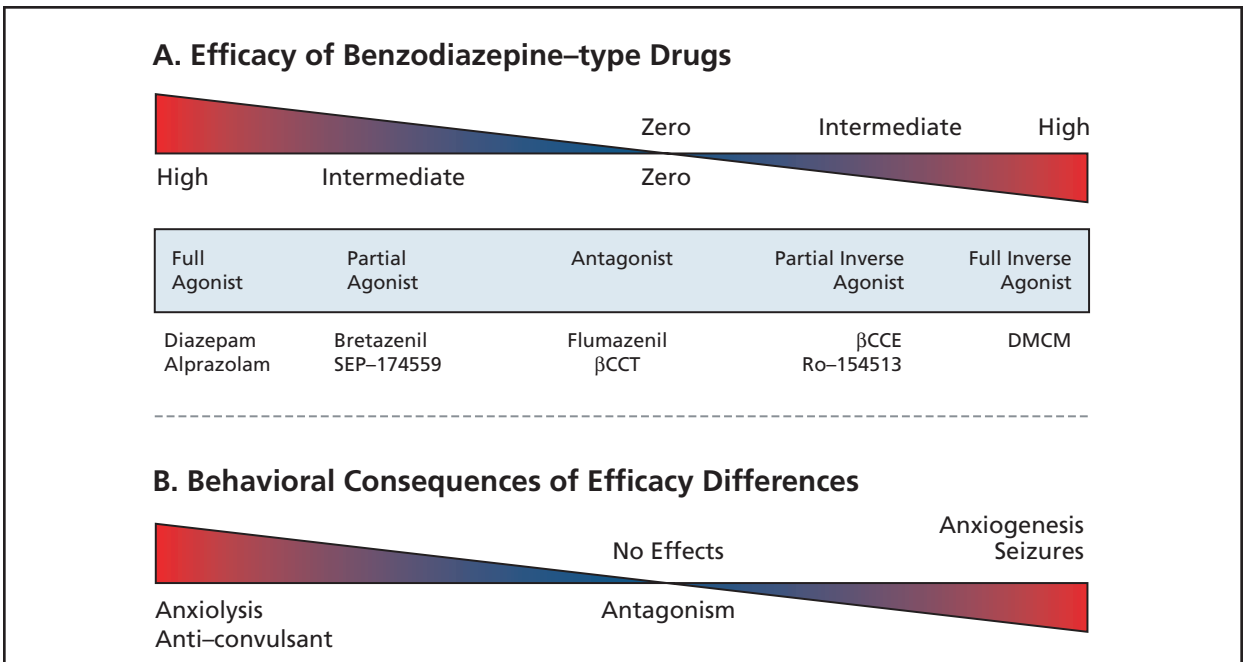

FIGURE 1. A. Continuum of intrinsic efficacy for benzodiazepine-type drugs, with representative compounds for each category. Intrinsic efficacy is based on results from studies in which the ability of the compound to positively or negatively modulate GABA-induced chloride flux was assessed. **B**. Behavioral consequences of variations in intrinsic efficacy for benzodiazepine-type drugs.

βCCT=β-carboline-3-carboxylate-t-butyl ester; βCCE=ethyl β-carboline-3-carboxylate; DMCM=methyl-6,7-dimethoxyl-4-ethyl-β-carboline-3-carboxylate; GABA=γ-aminobutyric acid.

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agonist at $GABA_A$ α_1 receptors than at either $GABA_A$ α_3 or $GABA_A$ α_5 receptors (Figure 2B; $GABA_A$ α_2 receptors were not evaluated).

To determine the extent to which the selectivity determined in cloned receptors in vitro translates to native receptors in vivo, we recently evaluated the ability of βCCT to displace binding of radiolabeled flumazenil in mice (Figure 2C). We compared binding in the cerebellum, which contains predominantly $GABA_A$ α_1 receptors, to binding in the spinal cord which has low levels of $GABA_A$ α_1 receptors but relatively high densities of $GABA_A$ α_2 receptors.[19,20] As shown in Figure 2C, the maximum inhibition of cerebellar BZ site binding by βCCT was ~80%, whereas maximum inhibition in the spinal cord was ~40% (A.N.D. and J.K.R., unpublished data, 2004). These findings are consistent with previous data obtained with a relatively high dose of βCCT (30 mg/kg) in mice.[17] Given βCCT's high affinity for $GABA_A$ α_1 receptors, combined with the fact that most of the $GABA_A$ receptors in the brain are $GABA_A$ α_1 receptors, these observations suggest that doses of βCCT that preferentially target the $GABA_A$ α_1 receptor can be identified. Along a similar vein, a previous study with zolpidem, which exhibits a moderate 10-fold selectivity for the $GABA_A$ α_1 receptor, suggests that this agonist's behavioral effects involve actions at $GABA_A$ α_1 receptors exclusively.[22] Thus, these observations suggest the possibility of βCCT as a useful tool for exploring the role of the $GABA_A$ α_1 receptor subtype in the behavioral effects of BZ type drugs. An important caveat to keep in mind, however, is that 20-fold selectivity for a receptor generally can be classified as modest selectivity, which likely translates to a narrow dose range of selective antagonism in behavioral studies. In this review, we use the phrase "preferring" rather than "selective" to describe βCCT's putative binding to $GABA_A$ α_1 receptors versus $GABA_A$ α_2 and/or $GABA_A$ α_3 receptors in order to presage this compound's modest selectivity.

As noted, Shannon and colleagues[14] showed that βCCT was devoid of several behavioral effects. This observation has been corroborated recently by evaluating this $GABA_A$ α_1-preferring antagonist's ability to modify GABA-mediated chloride flux in cloned receptors.[21] In fact, βCCT 's level of intrinsic efficacy is less at some receptor subtypes than the classical non-selective antagonist, flumazenil (Figure 2D). In addition, more recent behavioral studies suggest that under most circumstances, βCCT is a relatively

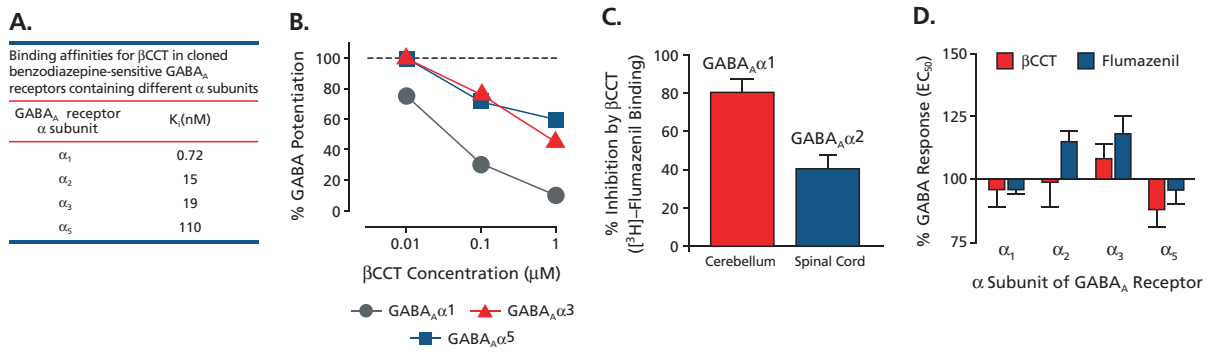

FIGURE 2. Neurochemical profile of βCCT. **A.** Binding affinities of βCCT in $GABA_A$ receptors containing different subtypes of the α subunit ($\alpha_x\beta_2/_3\gamma_2$).[15] These data show that βCCT is at least 20-fold selective for $GABA_A$ α_1 receptors compared with $GABA_A$ α_2 and $GABA_A$ α_3 receptors, and ~150-fold selective for $GABA_A$ α_1 receptors compared with $GABA_A$ α_5 receptors. **B.** Ability of βCCT to antagonize the potentiation by either zolpidem ($GABA_A$ α_1) or diazepam ($GABA_A$ α_3, $GABA_A$ α_5) of GABA-induced Cl–flux in cloned receptors. Effects of zolpidem or diazepam alone are represented by the dashed line at 100% GABA potentiation. These data show that βCCT is more potent in blocking GABA potentiation by a BZ agonist at $GABA_A$ α_1 receptors than either $GABA_A$ α_3 or $GABA_A$ α_5 receptors, consistent with its affinity differences.[17] **C.** In vivo binding of [3H]flumazenil following administration of 10 mg/kg of βCCT to BALB/c mice (N=8). Data are the mean±SEM of the percentage of inhibition of [3H]flumazenil binding by IP administration of βCCT. Thus, after peripheral administration to mice, βCCT occupied significantly more binding sites in the $GABA_A$ α_1 receptor-enriched cerebellum than in the spinal cord, which has predominantly $GABA_A$ α_2 receptors.[17] **D.** Intrinsic efficacy of βCCT and flumazenil determined by the ability of the drugs to modulate GABA-induced Cl–flux in cloned $GABA_A$ receptors ($\alpha_x\beta_2\gamma_2$). These findings suggest that βCCT has no appreciable intrinsic efficacy on its own.[21]

βCCT=β-carboline-3-carboxylate-t-butyl ester; %=percentage; GABA=γ-aminobutyric acid; $GABA_A$=γ-aminobutyric acid type A; Cl=chloride; BZ=benzodiazepine; BALB/c=a strain of mouse; SEM=standard error of mean; IP=intraperitoneal.

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"silent" antagonist,[17,23-25] although, in some cases, behavioral effects (eg, suppression of locomotor activity) have been shown at relatively high doses.[24,26]

Evidence that βCCT Blocks Behavior Selectively

In the report by Shannon and colleagues,[14] βCCT blocked the anxiolytic-like and anticonvulsant effects of diazepam but did not alter diazepam-induced impairments in a motor task. At the time, the anxiolytic and anticonvulsant effects of diazepam were proposed to be mediated by the BZ1 receptor,[27] and the results with βCCT were interpreted based on this viewpoint. About a decade later, several reports were published by researchers from Sanofi-Synthelabo Pharmaceuticals[17,23] that expanded the initial observations of Shannon and colleagues. That is, βCCT blocked the effects of diazepam and chlordiazepoxide in rodent models of the anxiolytic effects of drugs (elevated plus-maze, light-dark test).[17,23] βCCT also attenuated the anticonvulsant effects of diazepam. However, the attenuation was dependent on the agent used to trigger seizures.[17] Diazepam and other BZs suppress locomotor activity in rodents, an effect that was blocked by βCCT.[17] In contrast, the muscle relaxant effects of diazepam as well as the amnestic effects of chlordiazepoxide were not affected by βCCT.[17,23]

In a recent report, we sought to explore further the ability of βCCT to attenuate the anxiolytic effects of BZ agonists. Using a model in which mouse pups emit characteristic ultrasonic vocalizations in response to separation from their mother, the non-selective BZs diazepam and triazolam, as well as the $GABA_A$ α_1-selective agonist zolpidem, attenuated ultrasonic "distress" calls.[28] These anxiolytic-like effects could be abolished by pretreatment with flumazenil; however, βCCT blocked the reduction in distress calls induced by zolpidem only. These results clearly imply that anxiolytic-like effects of BZ agonists can occur by different mechanisms, which can be referred to as "βCCT-sensitive" and "βCCT-insensitive." The importance of these types of anxiolytic-like effects in rodents to anxiolysis in people has yet to be determined.

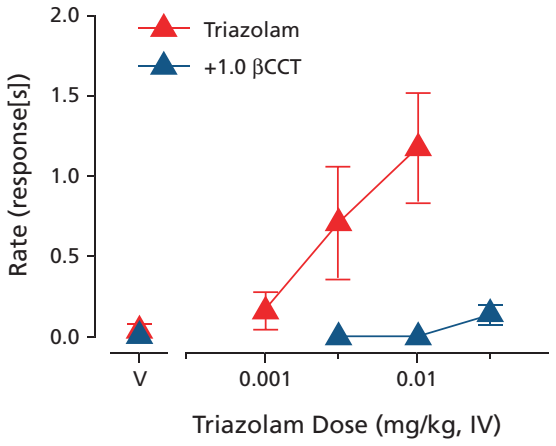

FIGURE 3. Anti-conflict effects of the non-selective BZ agonist triazolam, alone and following pretreatment with the $GABA_A$ α_1 receptor-preferring antagonist βCCT, in rhesus monkeys (N=3). Data are mean responses/second ± SEM in rhesus monkeys trained under a schedule of food delivery in which every eighteenth response produced a food pellet and every twentieth response resulted in delivery of shock (1–2 mA, 0.25 seconds). V represents responding following injections of drug vehicle (50% propylene glycol, 40% water, 10% ethanol). When triazolam was tested alone (red triangles), a dose-related increase in responding was observed (ie, anti-conflict effect) that was blocked by βCCT (blue triangles).

βCCT=β-carboline-3-carboxylate-t-butyl ester; IV=intravenous; BZ=benzodiazepine; $GABA_A$=γ-aminobutyric acid type A; SEM=standard error of mean.

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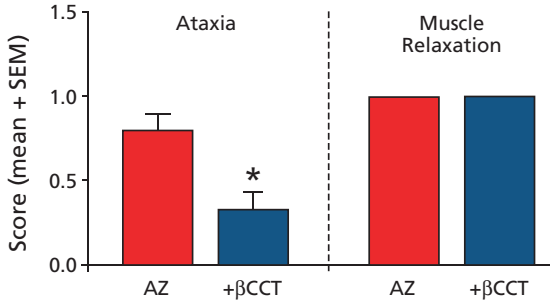

FIGURE 4. Ataxic and muscle relaxant effects of the non-selective BZ agonist alprazolam (AZ 0.3 mg/kg IM), alone and following pretreatment with 3.0 mg/kg βCCT IM, in squirrel monkeys (N=3). Ataxia was measured as the ability to balance on a horizontal pole (0=monkey was able to balance normally on the pole; 1=monkey was able to hold on to the pole but unable to maintain balance; 2=monkey could neither balance on nor hold on to the pole). Muscle relaxation was measured as the monkey's resistance to flexion of a hind limb, where a score of 0=normal resistance to flexion, 1=decreased resistance to flexion, and 2=flaccid. Measures of ataxia and muscle relaxation were obtained by trained observers unaware of the drugs under investigation. Analysis of the results showed that βCCT blocked the ataxic, but not muscle relaxant effects of alprazolam.

*P<.05 versus alprazolam alone, student's t-test.

SEM=standard error of mean; AZ=alprazolam; βCCT=β-carboline-3-carboxylate-t-butyl ester; BZ=benzodiazepine; IM=intramuscular.

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Comparison with Transgenic Mouse Studies and Results with New Agonists

As described in detail elsewhere in this issue, the past several years have seen a veritable leap in reports using transgenic mouse models to study $GABA_A$ receptor subtype function. In the initial reports by Rudolph and colleagues[29] and McKernan and colleagues,[11] mice with a point mutation that rendered the $GABA_A$ α_1 receptor insensitive to diazepam (α1His101R mice) were sensitive to the anxiolytic-like effects of diazepam, but showed a reduction in characteristic motor impairments. Subsequent studies with mice in which α_2 and α_3 subunits were rendered insensitive to diazepam have proposed that the anxiolytic effects of BZ agonists are mediated primarily via the $GABA_A$ α_2 subtype.[30] These findings have been largely supported by the development of functionally selective agonists, in which reduced or zero intrinsic efficacy at $GABA_A$ α_1 receptors results in a compound with anxiolytic-like effects but reduced motor impairments.[11,12,31] Altogether, these findings suggest that motor impairments (eg, ataxia, sedation), but not the anxiolytic-like effects of BZ-type drugs are mediated primarily by the $GABA_A$ α_1 subtype.

The preponderance of data obtained with transgenic mice and functionally selective agonists are concordant with previous findings with βCCT. Although not all motor impairments induced by BZ agonists are blocked by βCCT,[14] the models in which βCCT was ineffective may reflect some degree of muscle relaxation, an effect that likely does not involve $GABA_A$ α_1 receptors.[32] Perhaps the most striking finding with βCCT that contrasts with transgenic mouse data is the observation that βCCT attenuates the anxiolytic-like effects of BZ agonists in a variety of models. The reason for this discrepancy is not clear at present. βCCT exhibits the lowest degree of selectivity between $GABA_A$ α_1 and $GABA_A$ α_2 receptors (~20 fold); raising the possibility that blockade of anxiolytic-like effects is due to relatively high doses of βCCT binding to $GABA_A$ α_2 receptors. It is not apparent, however, why βCCT would antagonize one presumed $GABA_A$ α_2 subtype mediated effect—anxiolysis—and not another effect that involves $GABA_A$ α_2 receptors, namely muscle relaxation. Although speculative, a possible explanation may lie with the amount of receptor occupancy required for anxiolysis versus muscle relaxation. Anxiolytic-like effects in some procedures characteristically occur at lower levels of $GABA_A$ receptor occupancy than muscle relaxant effects.[33] Therefore, it may require less βCCT to block anxiolytic effects at the $GABA_A$ α_2 receptor than muscle relaxant effects. Evaluation of this possibility, as well as others (including the possibility that $GABA_A$ α_1 receptors are involved in anxiolysis in some as-yet uncharacterized fashion) awaits further investigation.

USE OF βCCT TO INVESTIGATE $GABA_A$ RECEPTOR MECHANISMS IN PRIMATES

An important component of the drug discovery process is investigation of a new therapeutic drug using relevant nonhuman primate models. The translation of basic research findings from rodent to primate models is based on the assumption that the mechanisms of drug action are similar (ie, conserved during evolution), and a key aspect of model development with primate species is to evaluate this assumption empirically. Investigations into the role of $GABA_A$ receptor subtype mechanisms in the therapeutic and side effects of BZ-type drugs using nonhuman primate models rely primarily on the availability of subtype-selective compounds in lieu of transgenic technologies in nonhuman primates.

Anxiolytic-Like Effects of BZ-Type Drugs and βCCT

Evidence for antagonism of anxiolytic-like effects by βCCT in a nonhuman primate species was first reported by Paronis and colleagues.[34] These investigators used a "conflict" model of the anxiolytic effects of BZs in squirrel monkeys. In a conflict model, subjects press a lever to receive food and once trained, a mild electric shock is occasionally delivered with a press of the lever. Drugs that have proven efficacious in treating anxiety in people increase responding suppressed by shock. That is, the drugs relieve the "conflict"—and presumably the anxiety—associated with being hungry but expecting punishment if the lever is pressed. For example, Figure 3 shows the effects of the non-selective BZ triazolam on responding suppressed by mild shock in rhesus monkeys (note that triazolam is marketed for treating sleep disorders. However, triazolam and other sleep aids, such as zolpidem and zaleplon, can have anxiolytic properties). In this preliminary study, the number of responses per second increased with the dose of triazolam, and this anti-conflict effect was abolished completely by pretreatment with βCCT. Therefore, as with the rodent anxiolytic tests, βCCT unexpectedly appears to be effective in reducing the anxiolysis induced by a BZ.

A more complete evaluation of βCCT antagonism, however, reveals that the blockade by this $GABA_A$ α_1 antagonist is far from straightforward. In the same study by Paronis and colleagues,[34] midazolam-induced increases in suppressed responding were antagonized by βCCT. The $GABA_A$ α_1 agonists zolpidem and zaleplon

also had anti-conflict effects that were attenuated at some doses by βCCT. However, βCCT also enhanced the anti-conflict effects of these agonists at higher doses of the agonists, a finding that did not occur with the BZ antagonist flumazenil. Collectively, these results suggest that in monkeys βCCT does not block the effects of BZ agonists in a manner consistent with simple antagonism. A possible reason for this complexity may be that βCCT blocks $GABA_A$ α_1 receptors specifically over a dose range that, when exceeded, results in blockade of other $GABA_A$ receptor subtypes.

Motor Side Effects of Benzodiazepines and βCCT

We recently have developed a procedure that allows investigation of the motor effects of drugs based on observable, naturalistic behaviors of squirrel monkeys.[24] When administered a non-selective BZ agonist, such as triazolam, or a subtype-preferring agonist, such as zolpidem, a profile of behavioral effects can be quantified from squirrel monkeys that has remarkable concordance with results from human studies. In this regard, triazolam and zolpidem increased observable measures of ataxia, decreased locomotor activity, and at the highest doses engendered a pronounced procumbent posture.[24] Conventional and subtype-preferring BZ agonists also induce muscle relaxation, measured by resistance to hind limb flexion (D.M.P. and J.K.R., unpublished data, 2004). These findings are similar to reports of motor incoordination, muscle relaxation, and daytime drowsiness frequently reported with BZ use in people.[35-37]

In the study by Platt and colleagues,[24] βCCT showed a profile of antagonism that clearly differed from that of flumazenil. In this regard, all behavioral effects of triazolam and zolpidem could be antagonized by flumazenil, whereas only a select number of behavioral effects could be blocked by βCCT. Ataxia was consistently blocked by βCCT. Neither the decreases in locomotor activity nor the increases in procumbent posture were altered by this $GABA_A$ α_1-preferring antagonist. Given that suppression of locomotor activity is frequently used as a measure of sedation in rodents, and that the procumbent posture appears to be a relatively straightforward measure of sedation, these results in monkeys appear to run counter to the idea that the $GABA_A$ α_1 receptor mediates the sedative effects of BZs.[11,29] However, the extent to which the observable locomotor activity measure used by Platt and colleagues[24] corresponds to rodent measures of locomotion, typically obtained via measuring the number of times a mouse or rat breaks an infrared photobeam in an experimental chamber is unknown. Other measures of sedation, such as rotorod (in which rodents are placed on a rotating drum and the latency to fall off the drum is measured), may involve ataxic as well as sedative effects of BZ-type drugs.[14] Thus, the discrepancy between rodent and squirrel monkey results may simply reflect differences across studies in the definitions of "sedation," "ataxia," and so on.

Recent findings with the observation procedure incorporating the hind-limb flexion test for muscle relaxation have also provided evidence for a unique profile of antagonism by βCCt (D.M.P. and J.K.R., unpublished data, 2004). As shown in Figure 4, the non-selective BZ alprazolam, a commonly used anxiolytic, induced ataxia and muscle relaxation. Interestingly, βCCT attenuated the ataxic effects but not muscle relaxation induced by alprazolam. A similar pattern of effects has been observed with triazolam and the non-selective BZ agonist chlordiazepoxide (D.M.P. and J.K.R., unpublished data, 2004). Thus, a relatively clear-cut finding from observational studies in squirrel monkeys is that the ataxic effects of BZ-type drugs are sensitive to βCCT, whereas the muscle relaxant effects of these drugs are not. These observations are entirely consistent with the idea that ataxia involves primarily the $GABA_A$ α_1 receptor while muscle relaxation likely involves $GABA_A$ α_2 and/or $GABA_A$ α_3 receptor subtypes.

SUMMARY OF PRECLINICAL DATA WITH βCCT: OF MICE AND MONKEYS

Studies published over the last several years[3,11,12,23-25] have provided important insights into the role of $GABA_A$ receptor subtypes in mediating the clinically relevant and side effects of BZ-type drugs. Results from rodent models employing transgenic technologies and newer selective pharmacological probes[11-13,22,29-32] have implicated the $GABA_A$ α_1 subtype in mediating the motor-impairing effects of BZs, whereas the anxiolytic and muscle relaxant effects of BZ-type drugs involve $GABA_A$ α_2 and $GABA_A$ α_3 receptor subtypes. Complementary studies in nonhuman primates have used subtype-preferring antagonists as an approach to understanding the role of $GABA_A$ receptor subtypes in the behavioral effects of BZs. In general, the hypotheses generated in studies with transgenic mice[3,9,11] have held up in experiments employing subtype-preferring antagonists and primate species. One notable exception to this observation is that anxiolytic-like effects in primates (and rodents) are sensitive to the $GABA_A$ α_1-preferring antagonist βCCT. This antagonism of the anxiolytic-like effects of BZ agonists in monkeys cannot be described as a simple competitive

antagonism, leaving open the possibility that this antagonist is working through multiple $GABA_A$ receptor subtypes. Another exception with primate data compared with the rodent work appears to be the observation that βCCT blocked some, but not all, measures of motor impairment in nonhuman primates, a phenomenon that may reflect differences in the measurement of sedation across species.

POTENTIAL CLINICAL APPLICATIONS FOR SUBTYPE-SELECTIVE $GABA_A$ RECEPTOR ANTAGONISTS

The conventional BZ antagonist flumazenil has been available clinically for a number of years for treatment of BZ overdose.[38] Numerous other clinical applications for flumazenil have been proposed, one of the most promising of which is the treatment of hepatic encephalopathy. In this regard, flumazenil consistently improves clinical signs and electroencephalographic measures associated with hepatic encephalopathy in patients with cirrhosis.[39] Flumazenil's efficacy in treating hepatic encephalopathy may reflect increased circulating levels of hemoglobin metabolites, which act as agonists at $GABA_A$ α_1 receptors and are subsequently blocked by flumazenil administration.[40] While the extent to which either BZ overdose or hepatic encephalopathy differentially involves the $GABA_A$ α_1 receptor is not yet known, these observations nevertheless suggest that βCCT's preferential binding affinity for this receptor might warrant consideration for clinical use.

In recent years, there has been growing interest in both the experimental and potential clinical application of radiotracers developed from flumazenil. A 11C-labelled form of flumazenil has been developed for positron emission tomography, whereas a related compound ([125I]iomazenil) has been developed for use with single positron emission tomography. Both radiotracers allow in vivo evaluation of BZ/$GABA_A$ binding capacity in humans in addition to experimental animals, with the advantage of the former being the ability to relate findings directly to a clinical syndrome. For example, $GABA_A$ receptor binding has consistently been found to be lower in patients with panic disorder[41] and altered in patients with certain forms of epilepsy.[42,43] With respect to the latter, use of [11C]flumazenil or [125I]iomazenil has been proposed as a potential diagnostic tool for identifying focal seizure points not detectable by conventional imaging methods.[42]

Both flumazenil and iomazenil are non-selective BZ antagonists; therefore, a radiotracer derived from βCCT or a similar $GABA_A$ α_1-preferring antagonist may have considerable potential as both an experimental and clinical diagnostic tool. Currently, no subtype-selective radiotracer is available, and a 11C-labelled form of zolpidem proved unsuitable for development due to pharmacokinetic issues.[44] As an experimental tool, a radiotracer based on βCCT should allow determination of doses in monkey that preferentially occupy $GABA_A$ α_1 receptor-rich brain areas. Conclusions could then be drawn regarding the extent to which $GABA_A$ α_1 receptors are involved in antagonism of a particular behavioral effect (eg, are the doses of βCCT that block anti-conflict effects the same as or higher than doses that occupy $GABA_A$ α_1 receptor-rich brain areas?). As a clinical tool, the ability to identify a receptor subtype might allow even greater precision in diagnosis of disease states involving specific components of the $GABA_A$ receptor system.[45]

CONCLUSION

Clinically available BZs engender a diverse array of therapeutic effects, such as anxiolysis, hypnosis, anticonvulsant effects, and myorelaxation. Understanding the receptor mechanisms underlying the clinical effects, as well as the unwanted side effects, of BZs might lead to promising new strategies for drug development. Subtype-selective antagonists of the GABAA receptor, such as βCCT, have proven useful as experimental tools for unraveling the roles of GABAA receptor subtypes in the therapeutic versu side effects of BZ-type drugs. Moreover, βCCT and other antagonists having subtype selectivity may have clinical utility in their own right, in particular as treatments for hepatic encephalopathy and as diagnostic imaging agents. CNS

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