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Cognition-impairing effects of benzodiazepine-type drugs: Role of $GABA_A$ receptor subtypes in an executive function task in rhesus monkeys

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ABSTRACT

The present studies evaluated the role of α1 and α5 subunit-containing $GABA_A$ receptors (α1$GABA_A$ and α5$GABA_A$ receptors, respectively) in the ability of benzodiazepine (BZ)-type drugs to alter performance in the cognitive domain of executive function. Five adult female rhesus monkeys (ages of 9–17 years old) were trained on the object retrieval with detours (ORD) task of executive function. For the ORD task, the monkeys were required to retrieve food items from a clear box with one open end that was rotated to different positions along with varying placements of food. When the non-selective BZ triazolam and the α1$GABA_A$-preferring agonists zolpidem and zaleplon were evaluated in the ORD task, deficits in performance occurred at doses that did not increase the latency of monkeys to initiate responding and/or increase the percentage of reaches that were incorrect (i.e., reaches in which food was not obtained). Cognition-impairing effects of triazolam and zolpidem in ORD were blocked by the α1$GABA_A$-preferring antagonist, βCCT, whereas the α5$GABA_A$-preferring antagonist XLi-093 blocked the effects of triazolam but not zolpidem. While these findings suggest a role for both α1$GABA_A$ and α5$GABA_A$ receptor mechanisms, α1$GABA_A$ receptor mechanisms appear to be sufficient for impairments in executive function induced by BZ-type drugs.

1. Introduction

Drugs that act at the γ-aminobutyric acid type-A ($GABA_A$) receptor, such as the benzodiazepines (BZs) have been shown to alter learning and memory processes (Arolfo and Brioni, 1991; Buffett-Jerrott and Stewart, 2002). Cognitive deficits are a major impediment to the clinical use of BZ-type drugs as anxiolytics and hypnotics, especially in populations already suffering from neurocognitive disorders. At present, relatively little is known about the mechanisms underlying cognitive deficits induced by BZ-type drugs, or if there are any differences among the clinically available compounds.

$GABA_A$ receptors are heteropentameric chloride ion channels that are assembled in a typical stoichiometry of 2 α, 2 β, and 1 γ subunits, and conventional BZs bind specifically to $GABA_A$ receptors containing α1, α2, α3 or α5 subunits (α1$GABA_A$, α2$GABA_A$, α3$GABA_A$ or α5$GABA_A$ receptors, respectively; for review, see Rudolph and Knoflach, 2011). The α1$GABA_A$ receptor has been shown to account for approximately 60% of all $GABA_A$ receptors and is particularly dense on hippocampal and cortical interneurons (Fritschy et al., 1992; Gao and Fritschy, 1994; McKernan and Whiting, 1996; Rudolph and Knoflach, 2011). The α2$GABA_A$ and α3$GABA_A$ receptors represent a smaller percentage of the $GABA_A$ receptor population, with the α2$GABA_A$ receptor particularly dense in hippocampus, cortex, and basal ganglia, whereas the α3$GABA_A$ receptor is expressed in cortex and thalamus. In contrast, the α5$GABA_A$ receptor is found almost exclusively in hippocampus and deep layers of cortex (for review, see Rudolph and Knoflach, 2011).

BZs and related drugs have long been documented to disrupt memory in human and non-human subjects (e.g., Duka et al., 1996; Ghoneim and Mewaldt, 1975; McNaughton and Morris, 1987; for review, see Stewart, 2005). Broadly stated, in human subjects, administration of BZ-type drugs can result in a loss of the ability to form new memories (i.e., anterograde amnesia). Early reports in human subjects concluded that BZs blocked the acquisition of new information (e.g., Ghoneim et al., 1984a,b), and a number of clinically available BZ-type drugs have been shown to impair memories for facts that are explicitly stored and retrieved (explicit, or declarative, memory; cf. Mintzer et al., 1997; Mintzer and Griffiths, 1999). Although the exact cognitive processes underlying these types of memory deficits are unknown, it has been suggested that BZ-type drugs specifically impair memory for contextual information, i.e., information peripheral to an event, such as time and place in which an event is experienced (Brown and Brown, 1990; Duka et al., 1996; Mintzer and Griffiths, 1999).

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Relatively little information is available on the role that $GABA_A$ receptor subtypes plays in the ability of BZ-type drugs to impair memory processes. One finding from human laboratory studies is that the hypnotic BZ agonist zolpidem did not disrupt memory for spatial contextual information, in contrast to the non-selective BZ agonist triazolam (Mintzer and Griffiths, 1999). Zolpidem displays highest affinity for the $\alpha1GABA_A$ receptor subtype and does not bind appreciably to the $\alpha5GABA_A$ receptor (Hadingham et al., 1993); the latter of which are densely expressed in hippocampus (for reviews, see Rudolph and Knoflach, 2011; Sieghart and Sperk, 2002). Based on these observations, Mintzer and Griffiths (1999) suggested that zolpidem's apparent lack of impairment of a visual-cues based task (see also Balkin et al., 1992) is consistent with hippocampal regulation of spatial memory. Interestingly, studies in non-human animals generally suggest that $\alpha1GABA_A$-selective agonists are less effective than non-selective BZs in engendering memory deficits (Noguchi et al., 2002; Sanger et al., 1986). Moreover, some memory-impairing effects of BZs are not blocked by the $\alpha1GABA_A$-selective BZ antagonist, β-carboline-3-carboxylate-3-butyl-ester (βCCT; Belzung et al., 2000; Savic et al., 2008).

Although research in recent years has focused on the role of hippocampal $\alpha5GABA_A$ receptors in the memory-impairing effects of BZ-type drugs, as noted, BZ-sensitive $GABA_A$ receptors exist throughout the CNS (for reviews, see Rudolph and Knoflach, 2011; Sieghart and Sperk, 2002). Consistent with the relatively ubiquitous distribution of $GABA_A$ receptor subtypes throughout the brain, BZ-type drugs impair performance on tasks that likely involve regions other than hippocampal/temporal areas (e.g., attentional set-shifting, paired-associates learning; Coull et al., 1995, 1999).

A cognitive domain often associated with prefrontal cortical areas and not the hippocampus is executive function. This domain characteristically includes processes such as goal formation, planning, initiation, preservation and alteration of goal-directed behavior, problem solving, response inhibition, and cognitive flexibility (Kehagia et al., 2010). To our knowledge, no previous studies have evaluated systematically the effects of BZ-type drugs on executive function. However, Ballard et al. (2009) did demonstrate enhancement of performance in an executive function task in monkeys by administration of an $\alpha5GABA_A$-selective inverse agonist. To the extent that agonists act in an opposite fashion to inverse agonists, these data would suggest that "positive" intrinsic efficacy at the $\alpha5GABA_A$ receptor would result in impairments of executive function.

The object retrieval with detours (ORD) task in monkeys is often described as providing a measure of executive functioning (Ballard et al., 2009; Jentsch et al., 1999; Taylor et al., 1990). Lesion studies in monkeys have indicated the involvement of prefrontal cortex–striatal circuitry in mediating behavior in the ORD task, with no involvement of the $\alpha5GABA_A$ receptor-enriched hippocampus (e.g., Diamond et al., 1989; Dias et al., 1996). It is important to note, however, that $\alpha5GABA_A$ receptors are found in deep cortical layers, albeit at relatively low expression levels (Rudolph and Knoflach, 2011) in rodents, and importantly, the expression of $\alpha5GABA_A$ receptors in primate prefrontal cortex is unknown.

This experiment is part of a series of studies designed to evaluate the extent to which BZ-type drugs alter cognitive function via $\alpha1GABA_A$ and/or $\alpha5GABA_A$ receptor subtypes. Our overall hypotheses, based on the relative distributions of the two subtypes, are that (1) the $\alpha1GABA_A$ receptor is involved in cognitive impairments in tasks involving cortical regions; and (2) the $\alpha5GABA_A$ receptor plays a primary role in cognitive tasks that engage the hippocampus. For this report, we evaluated the role of $\alpha1GABA_A$ and $\alpha5GABA_A$ receptors in the ORD task of executive function described by Ballard et al. (2009), with the prediction that $\alpha1GABA_A$, but not $\alpha5GABA_A$ receptors, would mediate impairments in ORD performance. In contrast to this prediction, evidence was obtained for a role of both $\alpha1GABA_A$ and $\alpha5GABA_A$ receptors in the ORD task.

2. Methods

2.1. Animals

The subjects were five adult female rhesus monkeys (*Macaca mulatta*), age from 9 to 17 years (9, 11, 13, 17, and 17 years old) with no history of exposure to drug (except for occasional analgesics, anesthetics and/or sedatives for clinical exams/surgeries) or experimental compounds. The monkeys weighed between 6 and 9 kg during the course of the study. The monkeys were individually housed and maintained on a 12 hour lights on/12 hour lights off cycle, with water available ad libitum. All animals were maintained on 20–30 biscuits per day of commercially-available macaque food (LabDiet 5038), which allowed maximum allotment of food availability without a decrease in daily performance during experimental sessions, with the monkeys maintaining steady weights and body conditions as assessed by the clinical veterinary staff. Three times per week, the monkeys were given a small allotment of fruits and/or vegetables as part of the New England Primate Research Center's environmental enrichment program. The animals in this study were maintained in accordance with the guidelines of the Committee on Animals, Office of Research Subject Protection, of the Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (8th edition, 2011). Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

2.2. Surgery

The monkeys were prepared with chronic indwelling venous catheters (polyvinyl chloride, i.d.: 0.64 mm; o.d.: 1.35 mm) following the general surgical procedures described by Platt et al. (2012). The monkeys initially were anesthetized with 10–20 mg/kg i.m. injection of ketamine for transport to the surgical suite and preparation for the procedure. Throughout surgery, anesthesia was maintained by an isofluorane/oxygen mixture. Under aseptic conditions, a catheter was implanted in the femoral, brachial, or jugular vein and passed to the level of the right atrium. The distal end of the catheter was passed subcutaneously and exited in the mid-scapular region. The external end of the catheter was fed through a fitted jacket and tether system (Lomir Biomedical, Toronto, Canada) and attached to a fluid swivel mounted to the animal's cage. The exit site of the catheter was inspected routinely and the catheters were flushed two–three times per week with heparinized saline (150–200 U/ml). Physical exams were conducted that occasionally included contrast-dye infused into the catheter, followed by radiography to verify catheter patency and proper placement.

2.3. Overall design

The general design consisted of two phases: (1) determination of dose–response functions for the non-selective BZ triazolam and the $\alpha1GABA_A$ receptor-preferring drugs zolpidem and/or zaleplon, and if effects were evident, then (2) evaluation of blockade of a maximally-effective dose of triazolam and zolpidem (supplies of zaleplon were too limited for the antagonism studies) by the $\alpha1GABA_A$ receptor-preferring antagonist βCCT and the $\alpha5GABA_A$ receptor-preferring antagonist, XLi-093 (1,3-bis(8-ethynyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5*a*][1,4]benzodiazepine-3-carboxy)propyl diester; Li et al., 2003). All drugs/compounds were administered by the i.v. route, in order to make direct comparisons with other procedures in our laboratory that use this route (e.g., i.v. self-administration). In general, the animals were trained in the ORD task until performance reached a priori criteria (see below), and tests of individual drugs/compounds, their respective vehicles, or selected doses of agonists plus antagonists were conducted up to 3 times per week, with training days interspersed. A test session consisted of a 5-min pretreatment with one of a range of doses of agonist or vehicle (phase 1) or a dose of agonist (determined